

A.01.001

ROLE OF CoREST IN NEURONAL DIFFERENTIATION DURING THE DEVELOPMENT OF CEREBRAL CORTEX *IN VIVO*.

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CoREST protein is a transcriptional corepressor. It interacts directly with the carboxyl terminal domain of RE-1 silencing transcription factor (REST/NRSF), regulator of neuron-specific terminal differentiation genes containing a RE-1 motif. The *in vitro* evidence and biochemical data show that CoREST is present only in organisms with a nervous system, where recruits various chromatin modifiers such as HDAC1, HDAC2 and LDS1 for transcriptional silencing of neuronal genes, however, it has been difficult to establish its role *in vivo*. CoREST recruits to the RE-1 motif another silencing machinery, such as methyl DNA binding protein (MeCP2) and the H3K9 methyltransferases, G9a and SUV39H1. The recruitment of this molecular machinery could cause the spread of silencing through long chromosomal intervals containing neuronal genes, but they do not contain RE-1 motif, suggesting a relationship between higher levels of chromatin organization and gene expression patterns. By the other hand, CoREST can mediate gene silencing in the absence of REST/NRSF, it has been corroborated for the promoters of the genes BDNF and Calbindin. Thus, the persistence of CoREST even after REST departures may provide a platform for the dynamic assembly and disassembly of repressors complexes required during the course of neurogenesis. **Objectives:** Our goal is to demonstrate *in vivo* that the expression of CoREST is necessary for proper neural stem/progenitors cells differentiation during cortex development. **Methods:** Our experimental approach consists in the loss of function of CoREST in neural progenitors from the telencephalic ventricular zone of mouse embryos on E14.5 by *in utero* electroporation of plasmid vectors encoding eGFP and a shRNA directed against CoREST. The embryonic development continues normally until E18, stage where coronal sections are obtained from the electroporated brains to be analyzed by confocal microscopy. **Results:** Preliminary results show that the loss of function of CoREST is associated with significant defects in the migration of post-mitotic neurons from the sub-ventricular zone. **Conclusions:** The function of CoREST in a wild-type background is necessary for the differentiation and redistribution of neural progenitors cells during normal cortical development.

Palavras-Chave: CoREST, Neurogenesis, Cortex, *in utero* electroporation.

Apoio Financeiro: Conicyt, ACT 47, ICM-P04-068F.

A.01.002

Markers of photoreceptor death after light-induced damage

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Objectives: Degenerative retinal diseases are characterized by progressive and selective loss of photoreceptor cells that eventually cause blindness. In spite of the advances made in the characterization of retinal diseases, the cellular events that accompany the photoreceptor death are still not completely understood. One common mechanism of photoreceptor death is apoptosis. The aim of this study is to investigate the sequence of events associated with photoreceptor death using immunological and functional markers of early and late apoptotic events.

Methods: We investigated photoreceptor cell death induced by light-damage. Albino Sprague-Dawley (SD) rats were exposed to intense light levels for 24 hours. The animals were culled and the retina was dissected, separated from the rest of the eye tissue and incubated in a physiological buffer containing one or more combinations of molecules to visualize dying cells. To visualize increased channel permeability we employed agmatine (AGB), an organic cation that permeates through functional cationic channels. After the incubation agmatine was detected immunocytochemically with anti-AGB antibodies. Retinas were also incubated in propidium iodide (PI) which is endocytosed and excluded from viable cells, and only retained by dying cells. To detect changes in the cytoplasmic membrane composition we used annexin V antibodies that bind to phosphatidylserine residues externalised at late stages of the apoptotic process. After the incubation, the retina was fixed in a 4% paraformaldehyde/ 0.01% glutaraldehyde solution. Retinas were also analyzed with the Terminal dNTP transferase mediated dUTP nick end labelling technique (TUNEL).

Results: Analysis of the expression of the markers showed scattered PI labelling in the photoreceptor layer. TUNEL labelling was located in the outer nuclear layer. Annexin V labelled the outer segment of photoreceptors. AGB labelled all parts of the retina including photoreceptor cells. We did not observe co-localisation of PI-AGB or AGB-TUNEL. Quantification of the amount of labelling per unit area showed significantly increased AGB labelling compared to all other markers.

Conclusion: Light-damage induced photoreceptor cell death characterized by the expression of early and late conventional apoptotic markers. Increased AGB gating may reflect a different mechanism of photoreceptor death.

Palavras-Chave: retina, TUNEL, annexin V, agmatine, propidium iodide, cell death

Apoio Financeiro: New Zealand Visual Research Foundation

PROPIEDADES ELECTROFISIOLOGICAS DE NEURONAS QUE CONTACTAN EL CANAL CENTRAL

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Objetivo: Caracterizar funcionalmente las neuronas que contactan el canal central (CC) en la médula espinal de la rata. El rol de estas células no es claro. Por su ubicación y forma fueron asociadas a funciones neurosensoriales y/o neurosecretoras. Si bien se conocen algunas de sus características inmunohistoquímicas (J. Comp. Neurol. 457(2):159, 2003), es escasa la información sobre sus propiedades funcionales.

Métodos: Se realizaron registros electrofisiológicos de estas células en clampeo de corriente y voltaje obtenidos en rodajas de médula espinal de ratas neonatales (P0-P5). Se utilizó la técnica de *patch* en modo “*whole-cell*” incorporando en la pipeta biocitina y/o un fluoróforo para la visualización de las células registradas. Cuando fue posible, la técnica de inyección intracelular fue combinada con inmunocitoquímica.

Resultados: Se observaron diferentes patrones de respuesta ante una despolarización sostenida en neuronas que contactan el CC, destacándose tres grandes fenotipos electrofisiológicos: a) una subpoblación que presentó una respuesta lenta generada por canales de Ca^{2+} , b) células que descargaron una espiga única mediada por canales de Na^{+} y c) neuronas con capacidad de generar potenciales de acción de forma repetitiva. Estas dos últimas subpoblaciones exhibieron notables diferencias en la forma de la espiga (amplitud y duración), el pico máximo en la corriente de Na^{+} y la corriente de K^{+} de tipo I_A . Las células con descarga única adyacentes al CC expresaron HuC/D (marcador neuronal temprano) mientras que células con descarga repetitiva ubicadas en la periferia del CC expresaron NeuN (marcador de neurona madura).

Conclusiones: Las neuronas que contactan el CC en la médula espinal de ratas neonatales presentan diferentes estadios de diferenciación electrofisiológica, distinguiéndose en muchas de ellas fenotipos semejantes a los de neuronas inmaduras. Esta gran heterogeneidad sugiere que la zona que rodea al CC es una región plástica donde los fenómenos de diferenciación neuronal continuarían luego del nacimiento.

Palabras-Chave: patch clamp; neuronas inmaduras; canal central; médula espinal

NEUROBLASTOS EN LA MÉDULA ESPINAL DE LA RATA

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Objetivo: Caracterización de las células de la Gelatinosa Central (GC) de la médula espinal de la rata en los primeros días de vida postnatal (P0-P5).

La GC en la médula espinal comprende las células endimarias que tapizan el canal central (CC) y la sustancia gris próxima que lo rodea. En la tortuga, es un sitio de proliferación con células precursoras y neuronas inmaduras (J. Neurosci. 28(13):3298, 2008).

En los mamíferos, la neurogénesis postnatal en la médula espinal no ha sido claramente demostrada. No obstante, en un modelo de esclerosis múltiple se observó que la proliferación de las células endimarias generaría nuevas neuronas (Eur J Neurosci 23:394, 2006). Estos datos convierten a la GC en una región con potencial capacidad neurogénica luego del nacimiento. Por lo tanto, es relevante profundizar en el conocimiento de esta zona.

Métodos: Método de Golgi, técnicas inmunohistoquímicas, microscopía electrónica y registros electrofisiológicos de *patch clamp* en la modalidad “*whole cell*”.

Resultados: Se observó la presencia de células conectadas al CC que expresaron únicamente un marcador neuronal temprano (HuC/D) y células más distantes que fueron además inmunorreactivas para una proteína nuclear típica de neurona madura (NeuN). Registros electrofisiológicos en la GC confirmaron el carácter neuronal de estas células (HuC/D⁺-NeuN⁺) a través de su capacidad de generar potenciales de acción. Muchas de ellas co-expresaron PSA-NCAM (proteína relacionada con la migración y la plasticidad neuronal). Por otra parte, mediante inyecciones de BrdU durante la vida embrionaria (E7-E17) se comprobó que estos neuroblastos adyacentes al CC nacen durante este período.

Conclusiones: Durante los primeros días de vida postnatal existen células en la GC con características moleculares y funcionales de neuronas inmaduras. Estas células nacen en el período embrionario y permanecerían en esta región durante la vida postnatal como neuroblastos rezagados. La expresión de PSA-NCAM sugiere que poseen potencialidad para migrar e integrarse a los circuitos neuronales ya existentes.

Palavras-Chave: Neuroblastos; PSA-NCAM; epéndimo; médula espinal

Apoio Financeiro: PDT 135/63

A.01.005

SPHINGOSINE-1-PHOSPHATE PROMOTES THE PROLIFERATION AND DIFFERENTIATION OF RETINA PHOTORECEPTORS

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Objective: docosahexaenoic acid (DHA), the major polyunsaturated fatty acid in the retina, prevents apoptosis and promotes differentiation of photoreceptors. DHA decreases the levels of ceramide to promote photoreceptor survival (IOVS 47:1658, 2006) suggesting that sphingolipids might mediate other effects of DHA on photoreceptors. We now investigated whether sphingosine-1-phosphate (S1P) regulates proliferation and differentiation in photoreceptors.

Methods: Pure rat retina neurons in culture were supplemented with or without DHA or Glial Derived Neurotrophic Factor (GDNF) and/or S1P. The cultures were also treated with an inhibitor of Sphingosine Kinase (SK) to block S1P synthesis, or with Brefeldin A (BFA) which collapses the Golgi into the ER. Photoreceptor differentiation was determined by evaluating the amount of apical processes and opsin and peripherin expression. Proliferation was evaluated counting mitotic figures and BrdU uptake.

Results: photoreceptors in control cultures had few apical processes and opsin was distributed over the whole cell body and neurites. S1P or DHA enhanced the development of apical processes, increased opsin and peripherin expression and promoted their localization in apical processes. Inhibiting S1P synthesis blocked DHA-induced increase in photoreceptor differentiation and addition of S1P to these cultures restored photoreceptor differentiation. Treatment of DHA or S1P-supplemented cultures with Brefeldin A decreased the formation of apical processes, without affecting opsin or peripherin expression. Though S1P and DHA had similar effects on differentiation, they did not share their effect on proliferation. DHA induces the exit of photoreceptor progenitors from the cell cycle while GDNF promotes their proliferation. S1P treatment enhanced proliferation of photoreceptor progenitors, while inhibition of S1P synthesis blocked GDNF effect on proliferation.

Conclusions: These results suggest that S1P is a key mediator in photoreceptor proliferation and differentiation; GDNF and DHA might enhance its synthesis at different times in development to regulate the former the amount of photoreceptors and the latter their differentiation.

Palavras-Chave: Photoreceptors, sphingosine-1-phosphate, differentiation, proliferation.

Apoio Financeiro: Grants from FONCyT, CONICET, UNS, from Argentina

A.01.006

Estudio de la neurogénesis post-embionaria en cerebros adultos de peces del género *Austrolebias*.

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A diferencia de los mamíferos, los peces exhiben un enorme potencial para la regeneración neuronal y la neurogénesis adulta. La persistente proliferación celular en su sistema nervioso hace un modelo atractivo para estudiar los mecanismos celulares implicados en esos procesos. Las *Austrolebias* poseen regiones proliferativas potencialmente neurogénicas. Este trabajo tuvo como **objetivos**: 1) Identificar, topografiar y cuantificar las zonas proliferativas en cerebro adulto de *Austrolebias*. 2) Determinar la estirpe celular de las células recién generadas mediante marcadores específicos. **Materiales y Métodos**: Ejemplares adultos entre 2 y 8 meses de edad se inyectaron con dosis únicas de 5'-Bromodeoxiuridina (BrdU) 100 mg/kg ip. Pasadas 24 hs se anestesiaron y se perfundieron con Paraformaldehído 10% en Buffer Fosfato 0.1M. Los cerebros, se cortaron en serie, transversal y sagitalmente en Vibrátomo. Algunos se procesaron para Microscopía Electrónica de Transmisión (MET). Para la detección inmunocitoquímica de células proliferantes, se usó un anticuerpo monoclonal anti-BrdU (1:500) y un policlonal contra el antígeno nuclear de proliferación celular (PCNA, 1:100). Para determinar la estirpe de las células BrdU+, se realizaron dobles marcajes con marcadores específicos de neuronas (HuC), de neurotransmisores (5-HT) y de células gliales (S-100, GFAP). **Resultados**: La reconstrucción 3D, permitió topografiar algunas regiones proliferativas (bulbo olfatorio, tectum óptico, cerebelo y paredes ventriculares). Las zonas que mayor proliferación mostraron fueron; ventrículo del telencéfalo, tectum óptico y torus longitudinalis. Los dobles marcajes mostraron la presencia de células con estirpe neuronal y glial en algunas de las regiones exploradas. Por MET, se identificaron poblaciones celulares heterogéneas que podrían indicar diferentes estadios de diferenciación. Se encontraron mayoritariamente células con características gliales **Conclusiones**: Algunas regiones proliferativas mostraron heterogeneidad celular y capacidad neurogénica. Las similitudes constatadas, con reportes recientes en pez Zebra (*Neurosci. 146:679,2007*), validan nuestro modelo para estudiar la neurogénesis. **Palabras-Chave**: neurogénesis, peces, proliferación.

Apoio Financeiro: PEDECIBA

A.01.007

NeuroD1 protein in adult rat pineal gland

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NeuroD1 has been found to be a key determinant of the fate of specific endocrine and neuronal cells, including pancreatic β cells, cerebellar granule cells and retinal photoreceptors. Muñoz, Bailey, Rath, Shi, Morin, Coon, Møller and Klein (*J Neurochem* 102: 887-99, 2007) reported for the first time the expression pattern of NeuroD1 mRNA in the developing rat brain, including the pineal gland, and a list of genes affected in the neonatal pineal gland from BETA2/NeuroD1 KO mice. Gene expression analysis identified 127 transcripts down-regulated and 16 were up-regulated in the KO mice. The most dramatically down-regulated gene was *kinesin family member 5C* and the most up-regulated gene was *glutamic acid decarboxylase 1*. The gene that encodes the enzyme AA-NAT (aralkylamine *N*-acetyltransferase) also emerged as a potential NeuroD1 target gene. NeuroD1 mRNA levels in the adult rat pineal gland were found similar during the day and night, and did not appear to be directly influenced by sympathetic neural input. These results do not invalidate the hypothesis that the NeuroD1 protein might exhibit a rhythmic nature. Immunohistochemistry studies using a rabbit polyclonal anti-NeuroD1 antibody revealed that day and night pinealocytes express NeuroD1 protein; immunostaining was mainly seen at the cytoplasmic level. A second subpopulation of NeuroD1-positive cells was only observed in the nighttime. These cells, located in clusters in the heart of the gland and next to the stalk, exhibited diverse shape and numerous NeuroD1-positive cytoplasmic processes. A few cells with nuclear immunoreactivity were identified. Further studies using antibodies that recognize neuronal and glial markers, including GAP43 and GFAP, suggest that the nocturnal niche did not co-localize with mature neurons and GFAP-positive astrocytes. Intense S-antigen-immunoreactive cells were observed in the gland periphery. Further characterization is required to establish the precise lineage and source of these nocturnal NeuroD1-positive cells. These results are promising towards elucidation of the potential role of NeuroD1 in the pineal gland phenotype and therefore, in the melatonin rhythm.

Palavras-Chave: NeuroD1, pineal gland, development

Apoio Financeiro: CONICET-ARG, ANPCyT-ARG, NICHD-NIH-USA

A.01.008

The Effects of "Ecstasy" on Hippocampal Cell Proliferation and Survival in Adult Rats.

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3,4-Methylenedioxymethamphetamine (MDMA or Ecstasy) is a widely used illicit recreational drug that preferentially targets brain serotonergic neurons and can produce long-term deleterious effects on the serotonin system. MDMA abuse has been linked to cognitive impairments, which may, in part, be related to a disruption of adult hippocampal neurogenesis. Using Ki67 and bromodeoxyuridine (BrdU) immunohistochemistry, we examined the effects of binge administration of MDMA on cell proliferation and survival, respectively, in the dentate gyrus (DG) of adult rats. In addition, the relationship between MDMA-induced changes in cell proliferation/survival and loss of serotonin nerve endings in the brain, as assessed by immunostaining for the serotonin transporter (SERT), was determined. Rats received either one, two or five "binge" treatments of MDMA. Each "binge" consisted of three injections (6 mg/kg, i.p.) administered at 2½-h intervals. Treatments were applied on alternate days (double binge) or every fifth day (multiple binges). Our results indicate that only the multiple MDMA binges resulted in a significant, overall reduction (-15%) in cell proliferation. This effect was more prominent in the ventral (-21%) than in the dorsal (-8%) DG. Further regional analysis revealed decline (-53%) in cell proliferation specifically in the hilus, with no apparent change in the subgranular zone (SGZ). Rats exposed to multiple MDMA binges demonstrated significant decreases in SERT-IR axon density in both the DG (-35%) and cerebral cortex (-50%). The loss of serotonin nerve endings in both regions (DG and cortex) was correlated with the reduction in cell proliferation observed in both the ventral DG ($r = 0.577$, $p = 0.012$ and $r = 0.513$, $p = 0.029$, respectively) and the DG as a whole ($r = 0.600$, $p = 0.009$ and $r = 0.564$, $p = 0.014$, respectively), as well as the hilus ($r = 0.829$, $p < 0.0001$ and $r = 0.796$, $p < 0.0001$, respectively), but not the SGZ ($r = 0.115$, $p = 0.649$ and $r = 0.0094$, $p = 0.712$, respectively). By contrast, cell survival was not significantly altered after multiple MDMA binges. Although two MDMA binges resulted in a more severe loss of serotonin nerve endings in both the DG (-63%) and cortex (-68%), no change in proliferation was observed, suggesting that there is no simple linear relationship between serotonin depletion and neurogenesis. The single MDMA binge had no effect on brain serotonin or proliferation. Overall, these data suggest the possibility that chronic MDMA use in humans may compromise hippocampal adaptive plasticity and its associated functions. **Palavras-Chave:** bromodeoxyuridine (BrdU); Ki67; serotonin transporter (SERT); hippocampus; neurogenesis; serotonin neurotoxicity

Apoio Financeiro: NIMH (Grant MH 023433), CNPq (200574/2006-0), Fapesp (07/52445-5)

potential of early cortical progenitors revisited and role of shh signalling in cortical development

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During mouse cerebral cortex development, neuronal cells can be identified at much earlier stages than astrocytes or oligodendrocytes. However, it is still debated whether neuron- and glia-restricted progenitors would co-exist at early stages of corticogenesis with a delay in the differentiation of glial cells or whether glial cells would be generated from bipotent (neuron-glia) progenitor cells. In this work, we set up to re-examine the potential of cortical progenitors at different developmental stages *in vitro* and *in vivo*. Cell lineage studies of single progenitors by time lapse video microscopy and clonal analysis *in vitro* suggest that most cortical progenitors generate only neurons at early neurogenesis stages (being, therefore, depleted) whereas about 1/3 of progenitors generate neurons and then become glia-restricted progenitors at mid- to late-neurogenesis. This analyze was extended *in vivo* by using a combination of retroviral vectors expressing GFP and dsRed to infect a very low number of embryonic VZ progenitors *in vivo* and follow their progeny at postnatal stages. According to our *in vitro* data, we did not detect pure glial clones in the cerebral cortex after retroviral infection at early corticogenesis (E13), but these were the vast majority after infections at E16. We also noted that this switch from neuron to gliogenesis correlates with the expression of Gli1 and Gli2 in the dorsal telencephalon, suggesting that Shh signalling could play a role in this phenomenon. Indeed, we observed that addition of Shh to embryonic cortical cells *in vitro* increases significantly the generation of glial cells whereas blocking Shh signalling with cyclopamine decreases gliogenesis. Analysis of single cell clones indicate that Shh acts exclusively in the population of bipotent progenitors, as clones containing only neurons are not affect neither by Shh nor cyclopamine. Taken together, our data suggest the existence of two types of cortical progenitors at early corticogenesis: neuron-restricted and bipotent progenitors. The later give rise to the glia-restricted progenitors observed in the developing cortex at mid- to late-corticogenesis, a phenomenon controlled by Shh signalling.

Palavras-Chave: Cortical development, neurogenesis, gliogenesis, sonic hedgehog

Apoio Financeiro: DFG, EU, BMBF, FORNEUROCELL and Alexander von Humboldt

A.01.010

EXPRESSÃO DOS RECEPTORES VANILÓIDES DO TIPO 1 E 2 (TRPV1-2) DURANTE O DESENVOLVIMENTO DA RETINA DE RATOS

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Objetivo Avaliar a expressão de RNA e protéica dos receptores vanilóides do tipo 1 e 2 (TRPV1-2) durante o desenvolvimento da retina de ratos (*Rattus norvegicus*), bem como caracterizar os tipos celulares que os expressam.

Métodos Realizamos experimentos de reação de cadeia de polimerase em tempo real, de imunoistoquímica e de *immunoblotting* para determinar a presença dos receptores TRPV1 e TRPV2 na retina de ratos do estágio embrionário de 19 dias (E19) e em idades pós natais (P1, P5, P15 e P60).

Resultados Observamos que a expressão gênica e protéica do receptor TRPV1 mantém-se constante durante o período observado. Porém, nossos dados de imunoistoquímica indicam que esse receptor é expresso inicialmente nas porções mais externas da retina, incluindo a camada neuroblástica da retina, sendo que no animal adulto é encontrado principalmente nas porções mais internas da retina, localizado em vasos, em células da microglia e em neurônios. A expressão gênica e protéica de TRPV2 só foi encontrada em fases tardias do desenvolvimento retiniano, constituindo uma população de células amácrinas e ganglionares com longos processos na sublâmina *OFF* da camada plexiforme interna, e em processos celulares perivasculares nessa camada, além de finos processos na camada plexiforme externa. Não observamos co-localização dos receptores TRPV1 e TRPV2.

Conclusões A presença do receptor TRPV1 na camada neuroblástica retiniana pode ser indicativo de que esse receptor participe dos processos iniciais de divisão, diferenciação e/ou apoptose de células precursoras da retina. No animal adulto, a presença abrangente de TRPV1 em neurônios, células da microglia e vasos pode ser sugestiva de que esse receptor module funções que integrem a comunicação entre neurônios, vasos e glia. A localização perivascular de TRPV2 e vascular de TRPV1 podem ser sugestivas da participação do sistema endovanilóide na regulação da atividade vascular na retina de ratos adultos.

Palavras-Chave: Retina; receptores vanilóides; desenvolvimento; endovanilóides

Apoio Financeiro: FAPESP; CNPq

A.01.011

Papel das vias de SMADs e MAPK/PI-3K ativadas por TGF-beta1 na gliogênese no córtex cerebral

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O principal progenitor do córtex cerebral (Cc) em desenvolvimento é a célula de glia radial (GR), que origina neurônios e células gliais. Recentemente, descrevemos que o fator de crescimento transformante- β 1 (TGF- β 1) induz as células de GR a diferenciarem-se em astrócitos. A ativação desta via envolve a sua ligação ao receptor TGFRII, recrutamento de TGFRI, e fosforilação das proteínas SMADs 2/3. Evidências têm mostrado, no entanto, que outras vias podem ser ativadas em resposta a TGF- β 1, embora não se saiba seu papel na diferenciação astrocitária. O objetivo deste trabalho foi analisar a participação das vias de MAPK (proteína cinase ativada por mitógeno) e PI-3K (fostatidilinositol-3-cinase) na diferenciação astrocitária *in vitro* e caracterizar os membros da via de sinalização de TGF- β 1 no desenvolvimento do Cc de camundongos *in vivo*.

Culturas primárias de GR de Cc de camundongos com 14 dias embrionários (E14) foram mantidas por 24h em meio com 10% de soro fetal bovino, e por mais 24h em meio sem soro com: TGF- β 1 (10ng/mL) apenas ou em presença dos inibidores de TGFRI (SB431542, 10mM), PI-3K (LY294002, 5mM) e MAPK (PD98059, 50mM). A diferenciação celular foi analisada por imunocitoquímica para os marcadores: BLBP (proteína ligadora de lipídios do cérebro; GR), GFAP (proteína ácida fibrilar glial; astrócito) e nestina (precursor neural). Observamos um aumento de 35% no número de células GFAP+ em resposta a TGF- β 1 ($p < 0,04$), e este efeito foi revertido pelos por SB431542 e MAPK. O número de células nestina+/BLBP+ diminuiu em 30% em resposta a TGF- β 1 ($p < 0,02$), e este efeito foi revertido por SB431542 e LY294002. A análise *in vivo* por *Western Blotting* de extratos protéicos de Cc E14, E18 e P2 (recém-natos) revelou um aumento de 6X nos níveis de SMAD2 fosforilada a partir de E18. Além disso, os níveis de expressão de TGFRII analisados por RT-PCR, aumentaram em 50% a partir de E18.

Estes dados sugerem que TGF- β 1 atue via MAPK na geração de astrócitos e via PI-3K na manutenção do fenótipo progenitor da GR. O aumento de SMAD2 fosforilada e TGFRII a partir de E18, período de início da gliogênese, sugere um importante papel de TGF- β 1 na geração de astrócitos no Cc.

Palavras-Chave: glia radial, diferenciação astrocitária, TGF- β 1

Apoio Financeiro: FAPERJ, CNPq, CAPES

A.01.012

EL SISTEMA ENDOTELINERGICO EN CELULAS PRECURSORAS NEURALES

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Las moléculas endotelinérgicas incluyen 3 péptidos, ET-1, ET-2 y ET-3 y 2 receptores, ETR-A y ETR-B. Ya hemos mostrado que la Zona Subventricular (ZSV) de ratones contiene células ET+ y ETR-B+. Como estas células también expresan proteína glial fibrilar ácida (GFAP), es posible que las células endotelinérgicas de la ZSV sean células precursoras neurales (CPNs).

Para estudiar esta hipótesis, evaluamos los cambios de las células endotelinérgicas en condiciones que estimulan el crecimiento y migración de CPNs. Además, buscamos co-localización de células endotelinérgicas con marcadores conocidos de CPNs.

Ratones machos C57Bl/6 (6-8 semanas de edad) fueron devascularizados sobre la corteza sensitivo-motora. Los animales lesionados y los controles fueron sacrificados a los 5 días de la cirugía. La distribución de los marcadores se estudió con protocolos inmunoenzimáticos. Se observó doble y triple co-localización con microscopía confocal, para ET-1 o ETR-B con prominina-1 (PROM) y nestina (NES).

En controles, se encontraron células ET+ y ETR-B+ principalmente en ZSV, y menos en cuerpo calloso (CC) y cíngulo (cg). Luego de la lesión, hubo un aumento importante de células y prolongaciones endotelinérgicas en estas áreas. Además, aparecieron en la corriente migratoria rostral (CMR) y en la periferia de la lesión. Estos cambios en la expresión endotelinérgica fueron muy similares a los de marcadores de CPNs.

Los experimentos de co-localización demostraron que todas las células endotelinérgicas expresaban PROM, en lesionados y en controles. NES solo se observó en cerebros lesionados. En éstos, se encontró frecuentemente triple expresión de ET-1 o ETR-B con PROM y NES en ZSV y CMR. En la periferia de la lesión NES aumentó y PROM disminuyó, de manera que predominaban células ET/NES+. Mientras que en el área lesionada se observaron pocas células ET+ y predominaban las NES+.

Un mecanismo endotelinérgico autocrino de estimulación podría estar regulando las CPNs. Este sistema podría permanecer activo en estadios neuronales más maduros caracterizados por NES y luego desaparecer en estadios aun más maduros.

Se requieren más estudios para comprender el papel del sistema endotelinérgico en la neurogenesis del adulto.

Palavras-Chave: Neurogénesis, Endotelina, Isquemia

Apoio Financeiro: PICT 21399/2004 y Rectorado U. Austral

A.01.013

Interação entre Hormônios Tireoidianos e a Via de *Sonic Hedgehog* no Desenvolvimento de Astrócitos Cerebelares

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A astroglia é essencial para a ontogênese do cerebelo, evento que ocorre principalmente no período pós-natal. Dentre os fatores importantes para o desenvolvimento do cerebelo destacam-se: *Sonic hedgehog* (Shh), o mais potente mitógeno dos precursores de neurônios granulares e os hormônios tireoidianos (HTs: T₄ e T₃), importantes morfógenos para migração, proliferação e maturação cerebelar. No sistema nervoso central, os astrócitos representam a principal fonte de fatores de crescimento, muitos dos quais, sintetizados e liberados em resposta aos HTs. Neste trabalho, analisamos a cooperação dos HTs na síntese e via de sinalização de Shh em astrócitos cerebelares *in vitro*.

Para analisar a síntese de Shh, culturas secundárias de astrócitos cerebelares neonatais murinos foram tratadas com 1μM de T₄ por 3 dias. Ensaios de *Western blotting* e imunocitoquímica demonstraram que Shh é sintetizado por astrócitos. A ativação da via de Shh foi analisada por *Western blotting* e imunocitoquímica para o fator de transcrição Gli1, principal efetor desta via. Evidenciamos aumento de 2 vezes na translocação nuclear de Gli1 (característica da ativação da via de Shh) nos astrócitos tratados por T₄ (p=0.0001). Ensaios de imunocitoquímica para o marcador de proliferação KI67 e incorporação de timidina tritiada revelaram aumento de 10 vezes na proliferação de astrócitos em resposta a T₄ (p<0.0001). O tratamento das culturas cerebelares com meio condicionado proveniente de células QT6, linhagem comercial que produz Shh, induziu um aumento de 4 vezes na proliferação dos astrócitos (p<0,05).

Astrócitos cerebelares de camundongos neonatos são fonte de Shh *in vitro*. Estas células são capazes de responder a Shh, pois apresentam membros de sua via e aumentam sua taxa proliferativa em resposta a este fator. Aliado a isto, os HTs têm a capacidade de aumentar a proliferação astrocitária e induzir a translocação nuclear de Gli1. Em conjunto, nossos dados sugerem, de forma pioneira, um sinergismo entre HTs e Shh durante o desenvolvimento cerebelar.

Palavras-Chave: Cerebelo, Astrócitos, Sonic hedgehog, Hormônios tireoidianos

Apoio Financeiro: CNPq, CAPES, FAPERJ

CONNEXIN36, AN ESSENTIAL ELEMENT IN THE ROD PATHWAY, IS HIGHLY EXPRESSED IN AN ESSENTIALLY RODLESS RETINA.

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Objectives Electrical coupling provided by connexins (Cx) in gap junctions (GJ) plays important roles in both the developing and the mature retina. In mammalian nocturnal species, connexin36 (Cx36) is an essential element in the rod pathway, the retinal circuit specialized for night vision. Herein, we report the occurrence of Cx36 in a species (*Gallus gallus*) that phylogenetic development endowed an essentially rodless retina.

Methods Experiments were carried out with *Gallus gallus*, ranging from embryonic day 5 (E5) to postnatal day 15 (E5, E8, E12, E15, E18 and P15). The animals P15 were divided into 3 groups (Control, 3 hours, and 24 hours of dark adaptation). All animals were sacrificed in accordance to the NIH and the Institute of Biomedical Sciences guidelines. The retinas were collected and prepared for real-time PCR, Western blot and immunofluorescence.

Results In the adult retina Cx36 gene expression is very highly expressed in comparison to Cx43, Cx45 and Cx50. Compared to the mature retina (P15), relative low levels of Cx36 were found at E5 (7%, $P<0.01$) and E8 (18%, $P<0.01$). We examined Cx36 in the inner and outer plexiform layers. Cx36 was observed mainly in the off sublamina of the inner plexiform layer, rather than in the on sublamina described in the mammalian retina. Cx36 co-localizes with specific cell markers, assigning the presence of this protein in distinct amacrine cell populations. To investigate the involvement of Cx36 in visual processing, we examined Cx36 functional regulation in retinas from dark-adapted animals. Light deprivation upregulates Cx36 gene expression in the retina, resulting in an increased accumulation of the protein within and between cone synaptic terminals. When compared to the control, a tendency toward an increase in expression was observed after 3 hours of dark adaptation (43%) and after 24 hours (115% $P<0.05$).

Conclusions Regulated expression of Cx36 results in particular circuitry-related roles in the chick retina. Moreover, this study disclosed that Cx36 onto- and phylogenesis in the vertebrate retina simultaneously exhibit similarities and particularities.

Palavras-Chave: Gap junction, Connexin, Retina, Dark adaptation, Development

Apoio Financeiro: FAPESP and CNPq

A.01.015

INVESTIGATION ON THE INTERFERENCE OF PACAP (PITUITARY ADENYLYL CYCLASE ACTIVATING PEPTIDE) IN THE SHH (SONIC HEDGEHOG) SIGNALING PATHWAY: IMPACT IN THE CONTROL OF CELL PROLIFERATION IN THE RETINA.

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Objectives: The retina is made up of seven major cells classes: rods, cones, horizontal cells, bipolar cells, amacrine cells, ganglion cells and Müller glia. These cell types are generated from multipotent retinal progenitor cells in an evolutionary conserved birth order. Then, proliferation must be carefully regulated during retinal development in order to generate the correct proportion of retinal cell types and a normal visual signal processing.

Sonic Hedgehog (Shh) regulates cell proliferation in the retinal tissue through its receptor, Patched (Ptc). The primary mediators of the pathway in vertebrates are zinc finger transcription factors named Gli (Gli1, Gli2 and Gli3). Gli and Ptc are also targets of the pathway and are upregulated in Shh responding cells, serving as a convenient readout for the status of Shh pathway activation in tissues.

Shh-induced cell proliferation was shown to be antagonized by the intracellular messenger cyclic AMP (cAMP). We have previously shown that Pituitary Adenylyl Cyclase-Activating Polypeptide (PACAP) is an anti-mitogenic factor in developing retina and is able to induce de production of cAMP. In this study, we want to test if this anti-mitogenic effect is dependent on the regulation of Shh signaling pathway.

Methods: Total RNA was prepared from the retinas of rat at age P1 treated with PACAP for 3 hours. cDNA was synthesized and analyzed by Real Time PCR with primers for Shh, Ptc and Gli1.

Results and Conclusion: The treatment is able to reduce significantly the levels of Ptc and Gli1 when compared with the control. The level of Shh is maintained the same. The reduction of the levels of Ptc and Gli1 demonstrates that PACAP is able to reduce Shh signaling. To deepen these results, we will perform proliferation assays to show functionally the interaction between PACAP and Shh. Our results suggest that the interaction of these signals can be an important element to regulate the retinal progenitor proliferation in rats. **Palavras-Chave:** Shh, PACAP, retina, proliferation

Apoio Financeiro: FAPERJ, CNPq, FUJB

A.01.016

El secuestro del Factor Neurotrófico Derivado del Cerebro (BDNF) luego de status epilepticus previene la disminución temprana de su receptor y la inducción de muerte neuronal en hipocampo de ratas

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El Factor Neurotrófico Derivado del Cerebro (BDNF) ha sido involucrado en muchos aspectos de la fisiología del hipocampo adulto. La administración i.p. de pilocarpina induce status epilepticus (SE), que provoca muerte neuronal en el hipocampo y es un modelo de epilepsia del lóbulo temporal en humanos. En nuestro laboratorio recientemente demostramos que el SE induce una rápida disminución de TrkB en la región CA1 del hipocampo que precede a la muerte neuronal. Estos resultados sugieren el BDNF liberado luego de SE podría inducir la disminución de TrkB y que este fenómeno participe en el desarrollo de la muerte neuronal. Para corroborar esta hipótesis se administró unilateralmente anticuerpos contra BDNF en la región CA1 del hipocampo y se analizaron los niveles de TrkB 12 horas posterior al SE y la muerte neuronal 12 horas después. Los niveles de TrkB fueron determinados por Western blot y el daño neuronal por tinción con Fluoro Jade B (que marca neuronas en degeneración) y tinción de Nissl.

Se observó que el secuestro de BDNF previno la disminución del receptor TrkB en un $26\% \pm 14$ ($p=0,0476$) con respecto al lado no infundido. Este resultado indica que el BDNF liberado es capaz de producir la disminución de su receptor específico. El análisis del daño neuronal en animales infundidos 24 horas posterior al SE, mostró que la inhibición de BDNF llevó a un $63\% \pm 0,15$ ($p=0,0022$) la muerte neuronal cuando es infundido antes del SE y a un $40,3\% \pm 0,12$ ($p=0,001$) si la infusión fue realizada inmediatamente posterior al SE. Por otro lado, no se encontraron diferencias significativas en la región del hilus, lo cual indica la especificidad regional de los anticuerpos. Por el contrario la infusión de la proteína BDNF aumentó el daño en las regiones afectadas. Estos resultados sugieren que BDNF liberado luego de SE tiene un importante rol en la inducción de muerte en neuronas de la región CA1 hipocampal. **Palavras-Chave:** status epilepticus, BDNF, TrkB, p75ntr, pilocarpina

Apoio Financeiro: SECyT-UNC, CONICET, FONCyT.

A.01.017

EFEITO DO ETANOL SOBRE AS ATIVIDADES EPILEPTIFORMES SUSTENTADAS POR CONEXÕES NÃO SINÁPTICAS DA PROLE MACHO DE RATAS ALCOOLIZADAS

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Objetivo

Este trabalho tem por finalidade analisar através de experimentação eletrofisiológica as atividades epileptiformes sustentadas por conexões não sinápticas na camada granular do giro dentado e caracterizar a morfologia da camada granular do ápice do giro dentado no hipocampo da prole macho de ratas submetidas ao alcoolismo crônico.

Metodologia

Ratas Wistar com seis semanas de idade receberam dose diária de 1,5g/ kg em solução de 20% v/v de etanol via intraperitoneal, antes e durante o período gestacional e no decorrer da amamentação. A prole macho, proveniente deste grupo, foi dividida em dois grupos: álcool interrompido e álcool contínuo (mesma dosagem das mães) até a décima semana de vida. Após o período de alcoolização os animais foram sacrificados e seus hipocampus processados para o estudo eletrofisiológico e morfológico.

Resultados

As análises quantitativas dos resultados eletrofisiológicos demonstraram: alterações do componente DC (queda negativa da linha de base), da duração dos eventos, da amplitude dos populations spikes (PS's) e do número de eventos num mesmo espaço de tempo, quando comparados com os resultados do grupo controle. Análises morfológicas demonstraram alterações da densidade celular na região do hipocampo.

Conclusão

Os resultados obtidos demonstraram que o etanol, quando ministrado por tempo variado, na dosagem de 1,5g/kg em solução a 20% v/v altera a excitabilidade neuronal das conexões não-sinápticas e altera a densidade celular.

Palavras-Chave: Epilepsia, etanol, eletrofisiologia, conexões não-sinápticas e hipocampo.

Apoio Financeiro: FAPEMIG e CNPq

A.01.018

Anti-angiogenic Effects of Bevacizumab (Avastin®) at The Retina's Development in Newborn Rabbits

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Bevacizumab is a recombinant humanized monoclonal antibody that inhibits the vascular endothelial growth factor (VEGF), and it has been used for treatment of many eye's diseases involving neovascularization, as intravitreal injections [1, 2, 3]. VEGF, although it is not the only molecule involved in angiogenesis and neovasclogenesis processes, it has been shown as the major factor responsible for them, at both physiologic and pathologic conditions [4]. Most of the eye vascular disturbs, major cause of visual loss, are tightly linked to the neovascularization, which is, in the most of times, the result of VEGF overexpression [1]. Retinopathy of prematurity (ROP) is a proliferative disease of the retinal vasculature in premature infants, that cause severe visual loss and is the major cause of newborn blindness [5]. In this study, we investigated bevacizumab effects at the retinal development. For this, we injected bevacizumab in newborn New Zealand White rabbits' eyes (28 days old), this way: the left ones received the injection (experimental group) and the right ones did not (control group). After 7 days, the eyes were enucleated and prepared to be embedded in paraffin. Histological sections were made from them and they were stained with hematoxylin and eosin in order to analyze the retina at light microscopy [3]. The results observed have shown no evidence of cellular differentiation changes at the retinas of the control group's eyes; however, the experimental group's eyes have shown an arrest at cell migration at the ganglionar cell layer, where would occur their maturation into the retina's layers; we could also verify many vacuolizations in the ganglionar cell layer. In addition, the VEGF expression, analyzed by immunohistochemistry, has shown that both experimental and control groups' eyes were not affected. According to the morphological and immunohistochemical preliminary findings, we conclude that bevacizumab affects the retina development in this model. We suggested that bevacizumab could be arresting the normal cellular differentiation of the retina and consequently its maturation. Other experiments are underway to answer this question.

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- [5] D. Clark, K. Mandal, Earl. Hum. Develop. 84:95, 2008. **Palavras-Chave:** Angiogenesis, bevacizumab, cellular differentiation, retina, VEGF

Apoio Financeiro: FAPERJ

ALTERAÇÕES DOS NÍVEIS DA PROTEÍNA S100B NO LÍQUOR E BULBO OLFATÓRIO DE RATOS ADULTOS MANIPULADOS NO PERÍODO NEONATAL.

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Objetivo: O procedimento de manipulação neonatal envolve separações breves e repetidas da mãe. Em ratos, essa intervenção no período neonatal pode reduzir o número de neurônios encontrados em diferentes áreas do sistema nervoso central (Behav. Neurosci. 894-903, 2003). Elevados níveis de S100B podem ocasionar efeito neurotóxico resultando em morte celular (Int.J.Biochem&Cell.Biol. 637:68, 2001). Nosso objetivo foi investigar a expressão de S100B em fatias de bulbo olfatório e córtex frontal de animais não-manipulados e manipulados no período neonatal e a expressão extracelular desta proteína no líquido em ratos machos e fêmeas de 85 dias de idade.

Métodos: Os filhotes foram separados da mãe e manipulados por 1 minuto por dia durante os 10 primeiros dias de idade. Utilizamos 32 ratos Wistar com 85 dias de idade (machos e fêmeas em diestro) divididos em 2 grupos: não-manipulados (n=16) e manipulados do 1º ao 10º dia de vida por 1 min (n=16). Os animais foram anestesiados com tiopental sódico (ip, 50 mg/kg) para coleta das amostras de líquido por punção da cisterna magna. Foram coletadas fatias do bulbo olfatório e córtex frontal. A determinação da concentração de S100B foi obtida por ensaios imunoenzimáticos (método ELISA).

Resultados: A manipulação neonatal (média $\pm$ EPM; 5,341 $\pm$ 0,097; 2,563 $\pm$ 0,101) induziu aumento do nível da S100B no líquido comparado (ANOVA, seguida do teste de Newman-Keuls, $p < 0,05$) com o grupo não-manipulado em fêmeas e machos (2,050 $\pm$ 0,096; 1,388 $\pm$ 0,061).

No bulbo olfatório, as fêmeas manipuladas no período neonatal (5,326 $\pm$ 0,087) apresentaram maior nível de S100B comparado com as não-manipuladas (2,111 $\pm$ 0,104). Este efeito não foi observado nos machos manipulados comparados aos não-manipulados (2,611 $\pm$ 0,102; 2,400 $\pm$ 1,014). A manipulação neonatal não alterou os níveis de S100B no córtex frontal em ambos os sexos.

Conclusão: A manipulação neonatal induz alterações de longa duração no funcionamento das células gliais em ratos machos e fêmeas. As repercussões dos resultados obtidos podem estar relacionadas com as funções da proteína S100B na proliferação e sobrevivência de neurônios e glia no SNC.

Palavras-Chave: Manipulação Neonatal, S100B

Apoio Financeiro: CNPq, CAPES e FAPESP

A.01.020

Efeito do etanol na neurogênese de células GABAérgicas no telencéfalo ventral

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O desenvolvimento do sistema nervoso compreende uma série de eventos encadeados e interdependentes que se iniciam com a gênese das células nervosas e se prolongam até sua completa mielinização. O cérebro em desenvolvimento, tanto durante a fase embrionária quanto no período pós-natal imediato, é susceptível às influências de fatores externos ambientais, em particular, à intoxicação por álcool. Em casos de exposição pré-natal ao etanol, mudanças estruturais e metabólicas ocorrem e levam a alterações funcionais que podem alterar/reduzir a sobrevivência de indivíduos acometidos. Neste estudo investigamos quais são os efeitos provocados pelo etanol nas etapas de gênese e morte de células neurais *in vivo* nas eminências ganglionares, uma região do telencéfalo ventral que dá origem a maioria dos interneurônios corticais GABAérgicos. Para isso utilizamos camundongos aos 14 dias embrionários, cujas mães receberam etanol (2g/Kg) por via oral durante 4 dias (E11-E14) e analisamos a dinâmica de seu ciclo celular pela marcação com BrdU e seu padrão de morte celular pela marcação com vermelho neutro. Todas as análises foram feitas uma hora após a última gavagem e a dosagem de etanol sérica foi de 110mg/dL. Nossos resultados demonstram que o etanol é capaz de alterar o ciclo celular nas eminências ganglionares, modificando o número de células na fase S e a velocidade do ciclo celular, além de aumentar a morte celular. Estas alterações variaram de acordo com a região ântero-posterior estudada dentro da eminência ganglionar e afeta de forma diferente as eminências medial, lateral e caudal. Sugerimos que o uso de etanol, mesmo que por curto período durante a gestação, pode alterar a gênese de populações celulares durante o período analisado.

Palavras-Chave: eminência ganglionar, ciclo celular, GABA, álcool

Apoio Financeiro: FAPERJ, PRONEX

A.01.021

A Role for CK2 Upon Interkinetic Nuclear Migration in the Cell Cycle of Retinal Progenitor Cells

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During the cell cycle in the proliferative zone of the retina, similar to other parts of the central nervous system, the nucleus migrates back and forth through the neuroblastic layer (NBL), an event called interkinetic nuclear migration (INM). DNA is duplicated in the innermost margin, while mitosis occurs as the nucleus reaches the ventricular surface in each round of cell division. The G1 and G2 phases occur along the nuclear migration pathway. The relationships of INM with the cell cycle and the control mechanisms of interkinetic nuclear migration are not well understood. Casein kinase 2 (CK2) is a serine/threonine kinase required for many cellular events, such as the control of the cell cycle and differentiation. This tetrameric enzyme has a large number of known substrates, including cell cycle regulators and motor proteins, such as the heavy and light chains of the molecular motor dynein. We tested whether INM is controlled by CK2. Double immunolabeling showed that CK2 is expressed in BrdU-labeled retinoblasts. INM was examined after pulse-labeling proliferating cells with BrdU in the retina of newborn rats, and following only the nuclear movement that occurs during G2-phase, from the basal to the apical side of the elongated retinoblasts. The CK2 specific inhibitor 4,5,6,7-tetrabromobenzotriazole (TBB) inhibited the activity of rat retinal CK2, and blocked nuclear movement in a dose-dependent way. No apoptosis was detected, and the total numbers of BrdU nuclei remained constant following treatment with TBB. Nonetheless, TBB also blocked DNA synthesis, as shown by measuring the incorporation of [3H]-thymidine. TBB interrupts the mitosis process, as shown by counting nuclei labeled with phospho-histone H3 and blocks the nuclear migration itself. Furthermore, immunolabeling of retina explants for α -tubulin showed that TBB treatment caused microtubule disruption. Tubulin dimers or polymerized microtubules are subject to post-translational modifications, by tubulin tyrosin ligase and an acetyltransferase, respectively. Immunolabeling showed that TBB led to an increase of tyrosinated tubulin, suggesting that tubulin dimers are not incorporated into microtubules. On the other hand, acetylated tubulin labeling was drastically reduced in the presence of TBB, confirming that microtubules are not polymerized correctly. Phospho-serine western blots of either control or TBB-treated retina indicate that TBB selectively inhibits the phosphorylation of certain proteins, consistent with inhibition of CK2. Phosphoproteome techniques are currently performed to analyse which phosphoproteins may contribute for regulation of these events by CK2. The results show that CK2 activity is required both for the cell cycle and cytoskeleton dynamics, as well as for interkinetic nuclear migration in the developing retina. (CNPq, FAPERJ, PRONEX).

Palavras-Chave: Retina, Interkinetic Nuclear Migration, Cell Cycle, CK2

Apoio Financeiro: CNPq, FAPERJ, PRONEX

Distribution of Migrating GABAergic Interneurons in the Cerebral Cortex

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During central nervous system development, GABAergic interneurons that will occupy different cortical layers are originated in the ventral telencephalon, in proliferative zones of the medial, lateral and caudal ganglionic eminences. Little is known about the mechanisms responsible for the establishment of these cells across the latero-medial axis of the neocortex, after their migration from their zones of origin.

Objectives. The present study aimed to analyse a possible correlation between the specific sites and time of generation of these interneurons within the ganglionic eminences, and their final position in the developing cortex.

Methods. We used *in vitro* models of homotopic and heterotopic transplantations of small explants of transgenic mice expressing eGFP (*enhanced green fluorescent protein*) into the ventricular zone (VZ) of the medial ganglionic eminence (MGE) of Swiss mice brain slices at E14 (embryonic day 14) and E15. Slices were kept for 4 or 7 days in culture (DiV), after which the distribution of these cells in the latero-medial axis was analysed. Double transplantations were also performed, taking explants from E15 Swiss mice and labeling them with different dyes. In this case, explants were pre-labeled with *Cell Tracker Blue* and homotopically introduced into the VZ of the MGE. After 2DiV, they were removed and substituted by *Cell Tracker Orange* labeled explants from E17 mouse embryos.

Results. We observed that GABAergic precursors spread all over the cortex, independently of their sites of origin. We also observed that E15 GABAergic interneurons occupy the lateral portions of the neocortex (near the corticostriatal angle), while those generated later (E17) migrate faster and show a preference for the medial regions.

Conclusions. The results suggest that although migrating neurons display different speeds of movement towards the cortex, they do not settle in predetermined regions in any topographic organization. It is possible, therefore, that all GABAergic interneurons have the same potential for inserting into local cortical circuits, whatever their origin might be.

Palavras-Chave: Tangential Migration; Cortical Development; GABAergic Interneurons

Apoio Financeiro: CNPq, CAPES, FAPERJ, Ministério da Saúde.

PAPEL TRÓFICO DA OUABAÍNA NAS CÉLULAS GANGLIONARES DA RETINA DE RATOS.

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O objetivo deste trabalho foi analisar vias de sinalização envolvidas no efeito trófico da ouabaína (oua) sobre as células ganglionares (CG) da retina submetidas à axotomia.

Para marcação das CG, ratos neonatos receberam injeções de peroxidase (HRP) nos colículos superiores. Após 16h foram sacrificados, suas retinas dissecadas, dissociadas e plaqueadas ($1,0 \times 10^5$ céls/cm²) em meio de cultura completo (MCC). As culturas foram mantidas por 4h em MCC e a seguir algumas laminulas foram fixadas em aldeídos (100% da população de CG). As demais foram tratadas e mantidas por 48h quando então eram fixadas. A presença de HRP foi revelada por reação histoquímica utilizando-se tetrametilbenzidina como cromógeno. O n° de CG foi obtido por contagem em microscopia de campo claro. Os resultados mostram que o tratamento com ouabaína (oua) 3,0nM, aumenta a sobrevida das CG [CT48h=51,5% $\pm$ 3,9%; oua=99,2% $\pm$ 1,5%, n=9]. O efeito da oua foi abolido pelo inibidor do receptor do fator de crescimento epidermal, tirfostina 2,5mM [CT48h=58,7% $\pm$ 4,5%; tir=61,0% $\pm$ 8,4%; oua=99,9% $\pm$ 3,3%; oua+tir=56,5% $\pm$ 5,7%, n=7]. O inibidor da internalização de receptores cadaverina (cad) 9nM não aboliu o efeito da oua (CT48h=56,6% $\pm$ 0,6%; cad=22,5% $\pm$ 4,4%; oua=91,8% $\pm$ 7,3%; oua+cad=83,0% $\pm$ 6,7%, n=4). Entretanto, o tratamento com cad aboliu o efeito do fator do crescimento derivado do cérebro (BDNF) 50ng/mL na sobrevida das CG [CT48h=61,9% $\pm$ 2,5%; cad=40,5% $\pm$ 2,6%; BDNF=92,5% $\pm$ 6,8%; BDNF+cad=51,9% $\pm$ 2,8%, n=6]. O tratamento concomitante com oua 0,75nM e BDNF 10ng/mL, apresentou efeito sinérgico na sobrevida das CG [CT48h=47,7% $\pm$ 6,8%; oua=37,5% $\pm$ 2,1%; BDNF=44,1% $\pm$ 7,6%; BDNF+oua=78,6% $\pm$ 0,8%, n=4].

Em conjunto nossos resultados sugerem que o efeito trófico da ouabaína sobre as CG mantidas em cultura se dê através do receptor de EGF presente na membrana. Além disso, nossos dados indicam uma potenciação do efeito do BDNF induzido pela oua sugerindo uma ação sinérgica destes dois fatores.

Palavras-Chave: Ouabaína, Células ganglionares da retina, sobrevida, receptor de EGF

Apoio Financeiro: FAPERJ, CNPq e PRONEX-MCT

INVESTIGATION ON THE ROLE OF KRUPPEL LIKE FACTOR 4 IN THE CONTROL OF CELL PROLIFERATION IN THE RETINAL TISSUE

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Objectives: Deregulation of cell proliferation event may cause either a decrease and/or lack of cell types or dysplasia, and the consequent lost of adequate visual signal transduction. We are interested on the role of PACAP (Pituitary adenylate cyclase-activating polypeptide) in the developing retina and the molecular mechanisms involved. Our previous results showed that in this tissue, PACAP is a negative regulator of proliferation. Now, we are interested to study the hypothesis that KLF4 regulates this response to PACAP. KLF4 is a transcription factor that belongs to a family of zinc finger SP/KLF transcription factors and is involved in the control of proliferation through the regulation of the expression of cell cycle effectors in various tissues ([Eur. J. Cancer](#), 41:16, 2005).

Methods and Results: Using Real Time-PCR and Western-blot we demonstrated for the first time by that KLF4 is expressed in neonatal retina obtained from Lister-Hooded rats and its expression is increased after PACAP treatment for 1h. We also demonstrated by double immunofluorescence assay in confocal microscopy that after PACAP treatment KLF4 is expressed in retinal progenitors labeled with BrdU. Moreover, we confirmed that KLF4 is able to bind in SP1 motifs present in rat cyclin D1 promoter which is the cell cycle regulator that has its expression reduced after PACAP treatment.

Conclusions: Our data suggest that PACAP may modulate the proliferation of progenitor cells during retinal development through the regulation of cyclin D1 expression by KLF4 transcription factor. Finally, PACAP may be considered an extracellular cue with multiple roles in retinal development, such as cell cycle regulation, possibly acting at the transition from proliferation to neuronal differentiation.

Palavras-Chave: PACAP, proliferation, KLF4, retina

Apoio Financeiro: CAPES, CNPq, FUJB and FAPERJ.

N-TERMINAL c-FOS TYROSINE PHOSPHORYLATION REGULATES c-FOS/ER ASSOCIATION AND c-FOS-DEPENDENT PHOSPHOLIPID SYNTHESIS ACTIVATION

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c-Fos, a component of the AP-1 family of transcription factors, is expressed at very low levels in resting cells. However, its expression is rapidly up regulated in cells undergoing G0 to S phase transition leading to AP-1-dependent gene transcription responses. In addition, cytoplasmic c-Fos, detected by immunocytochemistry and western blot, is associated to the endoplasmic reticulum (ER) membranes and activates phospholipid synthesis during cell growth and differentiation. Herein, it is shown that in T98G cells, c-Fos/ER association and consequently phospholipid synthesis activation is regulated by the phosphorylated state of c-Fos tyrosine residues. The small amount of c-Fos present in quiescent T98G cells is tyrosine-phosphorylated and not ER-membrane bound. In growing cells, it is dephosphorylated, associated to ER membranes and promotes phospholipid synthesis activation. Impairing tyrosine dephosphorylation abrogates phospholipid synthesis activation and reduces proliferation rates to those of quiescent cells. Substitution of tyrosine residues 10, 30, 106 and 337 evidence tyr 10 and 30 as relevant for this regulatory phenomenon. It is concluded that phosphorylation of tyrosine residues 10 and 30 of c-Fos regulate the rate of synthesis of phospholipids by regulating c-Fos/ER association, (Oncogene, 2007 May 24;26(24):3551-8). At present, we are looking for the tyrosine-kinase and tyrosine-phosphatase of c-Fos, as a possible target to control cell growth.

Palavras-Chave: Cytoplasmic c-Fos, c-Fos-Tyrosine phosphorylation, Phospholipid Synthesis

Apoio Financeiro: James S McDonnell Foundation, FONCyT, CONICET and SeCyT, Universidad Nacional de Córdoba.

A.01.026

Phosphorylation of the Transcription Factor ATF-2 following optic nerve injury.

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In the retina the degeneration of ganglion cells can be blocked by an inhibitor of protein synthesis. This suggests that the expression of specific genes is necessary for the program of cell death. In this study, the behavior of both the transcription factor ATF-2 and of kinases that activate ATF-2, p38 and JNK, was investigated during retrograde degeneration of retinal ganglion cells induced by axotomy *in vitro* and by optic nerve crush *in vivo*.

Explants of the retina of neonatal rats were kept *in vitro* under different treatments after axotomy of the ganglion cells. ATF-2 was analyzed by immunohistochemistry. Antibodies to p-ATF-2 (Thr69/71), to p-p38 and to p-JNK were also used. The condensation of the chromatin is a characteristic of programmed cell death, and was detected using the fluorescent DNA marker Sytox Green. In *in vivo* experiments, optic nerves of 28 days old rats were unilaterally subjected to crush injury. Cross sections of retinal tissue were used for the detection of p-ATF-2 (Thr69/71). The sections of retinal tissue were analyzed by both fluorescence and confocal microscopy.

In the retina of neonatal rats, the ATF-2 protein is predominantly located in the nucleus of the ganglion cells. It was verified that a gradual loss of the immunoreactivity for ATF-2 occurs after axotomy of the ganglion cells. It was found that following axotomy of the ganglion cells, phosphorylation of ATF-2 occurred in the residues Thr69/71. The peak of phosphorylation of ATF-2 occurs within 3 hours after axotomy, and precedes the peak of cell death that occurs at 18 hours. Treatment with a JNK inhibitor completely blocked the phosphorylation of ATF-2 and almost completely the death of the ganglion cells. However, the treatment with an inhibitor of p38 did not change the phosphorylation of ATF-2 and reduced only slightly the rate of ganglion cell death.

In addition, the *in vivo* experiments showed that phosphorylation of ATF-2 occurred in the residues Thr69/71 in retinal ganglion cells following optic nerves crush.

The results showed that the phosphorylation of ATF-2 occurred in degenerating retinal ganglion cells *in vitro* and *in vivo* after optic nerve injury. **Palavras-Chave:** retinal ganglion cells, ATF-2, JNK, programmed cell death

Apoio Financeiro: Capes, CNPq, FAPERJ, PRONEX-MCT

A.01.027

Interleucina-4 aumenta o efeito proliferativo do Fator de Crescimento de Fibroblasto básico em células da retina de ratos neonatos mantidas em cultura.

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Objetivos: Neste trabalho investigamos o papel da interleucina-4 (IL-4) no efeito proliferativo do fator de crescimento de fibroblasto básico (FGFb) em células da retina de ratos neonatos mantidas em cultura.

Métodos: Células da retina de ratos Lister Hooded recém nascidos (P0-P2) foram dissociadas e plaqueadas ($1,25 \times 10^6$ céls) em placas de Petri (40mm) tratadas com poli-L-ornitina (50mg/mL). O FGFb e a IL-4 foram adicionados em diferentes períodos de tempo e em diferentes concentrações. A proliferação celular foi avaliada por incorporação de [³H]-timidina e a radioatividade das amostras foi medida em cintilador de fase líquida.

Resultados: O tratamento simultâneo com IL-4 (50U/mL) e FGFb (25ng/mL) por 48h, não alterou o efeito proliferativo do FGFb [ct=100%; FGFb=201,4±17,2; IL-4=96,35±7,06; FGFb+IL-4=212,8±24,2, (n=8)]. Porém, a pré-incubação com IL-4 (50U/mL) por 24h e posterior estimulação com FGFb (25ng/mL) por 48h induziu um aumento ainda maior no efeito proliferativo do FGFb [ct=100%; ct lavagem=91,43±3,7; FGFb=162,3±9,4; IL-4=85,79±4,9; FGFb/IL-4=164,8±24,6; IL-4/FGFb=257,03±41,3, (n=6)]. Esse efeito não foi inibido pelo tratamento com Brefeldina A (Bref) na concentração de 30ng/mL, [ct=100%; ct-lavagem=115,9±6,1; FGFb=201,6±14,6; IL-4=93,9±6,8; Bref=92,9±7,78; IL-4/FGFb=237,6±21,8; IL-4/Bref/FGFb=247,7±21 (n=12)]. Quando as culturas foram co-estimuladas com IL-4 (50 U/mL) e com FGFb (5ng/mL e 15ng/mL) o efeito proliferativo do FGFb também foi potencializado em 50 e 100% respectivamente [ct=100%; FGFb(5ng/mL)+IL-4(50U/mL)=141,5±17,3; FGFb(15ng/mL)+IL4 (50U/mL)=229,8±34,6 (n=9)]. Um aumento superior a 150% foi obtido com o tratamento com IL-4 nas concentrações de 6,25, 12,5 e 25U/mL juntamente com FGFb (25ng/mL) [ct=100%; FGFb(25ng/mL)+IL-4 (6,25U/mL)=199±8; FGFb(25ng/mL)+IL-4 (12,5U/mL)=249,7±14,4; FGFb(25ng/mL)+IL-4 (25U/mL)=294,7±15,3, (n=7)].

Conclusões: Nossos dados sugerem um importante papel da IL-4 no controle da responsividade das células da retina ao FGFb. Nossos resultados, obtidos com a brefeldina A, indicam que o efeito da IL-4 não seja mediado pelo aumento na inserção de receptores para o FGFb na membrana.

Palavras-Chave: Retina, FGFb, IL-4 e proliferação celular

Apoio Financeiro: FAPERJ, CAPES, CNPq, PRONEX-MCT.

CELL PROLIFERATION IN THE HIPPOCAMPUS OF DIABETIC RATS SUBMITTED TO NEONATAL HANDLING

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OBJECTIVES: Environmental changes in neonatal period affect the development of neural systems. Animals manipulated during the first two weeks of life present a reduction of the glucocorticoid secretion after the exposure to several stressors. Diabetes acts as a depression model and increases by itself the secretion of glucocorticoids. Our purpose in this study was to evaluate whether the neonatal handling acts as a neuroprotection against damages caused by diabetes to cell proliferation in the dentate gyrus of hippocampus from adult diabetic rats. **METHODS:** Wistar rats, male neonates, were divided into: handled- manipulated for 1 minute, daily, at alternate times, until the 14th postnatal day, and nonhandled: which did not receive any neonatal stimulation. On the 60th day, half the number of rats from each group was induced to diabetes (D) through the injection of 60 mg/kg streptozotocin, via intraperitoneal application. The other half, control (C), received only 1ml/kg saline (n = 6/group). Seven days after the diabetes induction, all rats were injected with 3 doses of BrdU (5-bromo-2'-deoxyuridine) on alternate days, which is a cell proliferation marker, via intraperitoneal application (dose= 100mg/kg). On the following day, anesthesia was performed (ketamine: xylazine= 50:10mg/kg) followed by encephalon perfusion. After the perfusion, the encephalon was removed, fixed and embedded in paraffin. The blocks were sectioned on a microtome (5 micrometers - 7 sections/animal) and the sections were incubated in specific antibodies for immunohistochemical analysis. Two independent investigators counted the BrdU-positive cells in the dentate gyrus in both right and left hemispheres. **RESULTS:** The mean of the BrdU-positive cell counting on the right side of the hippocampus were: Control nonhandled= 41.00, Diabetic nonhandled=29.75, Controlhandled=42.83 and Diabetic handled=31.20. The results on the left side were: Control nonhandled=36.83, Diabetic nonhandled=23.58, Control handled=37.91 and Diabetic handled=34.60. **CONCLUSIONS:** We concluded that in diabetes cases, the neonatal handling not revert the effects caused by the disease in the hippocampus of male rats.

Palavras-Chave: diabetes, dentate gyrus, neonatal handling

Apoio Financeiro: UFCSPA, CNPq

A.01.029

Mecanismos bioquímicos de regulación del ciclo celular mediados por GAP-43

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GAP-43 es una proteína específica del sistema nervioso y es el principal componente en la vía de señales intracelulares que integra las respuestas de la membrana plasmática y el citoesqueleto a señales extracelulares. La expresión de GAP-43 estaba acotada a células post mitóticas, pero resultados recientes demostraron que se induce su expresión en neuroblastos proliferantes y se requiere para la maduración de neuronas. Por otro lado se demostró que líneas celulares provenientes de gliomas pierden la expresión de GAP-43 y que su reexpresión resultó en la supresión del crecimiento por modulación de señales mitogénicas. Nuestro objetivo fue estudiar la participación de la sobre-expresión de GAP-43 en el control del ciclo celular. Demostramos que la sobre-expresión de GAP-43 en células NIH-3T3 produce un arresto del ciclo celular en la fase G0/G1 concomitantemente con una marcada disminución en los niveles de ciclinas D1 asociada a un aumento en el nivel de p27 en la etapa temprana de la fase G1, una caída en los niveles de ciclina E en la etapa tardía y una disminución en la actividad de las kinasas asociadas a las mismas, cdk2, 4 y 6. Estudiamos la participación de la vía de señalización mediada por ERK1/2 en este proceso y los resultados no mostraron una fosforilación diferencial de ERK1/2. Además el tratamiento con el inhibidor de las MEK, PD98059, no muestra reversión de los efectos observados en las células que sobre-expresan GAP-43. La vida media de un ARNm depende de secuencias específicas (ARE) en el mismo, generalmente localizadas en el 3'UTR y de las ARN binding proteins que se unen a dichas secuencias promoviendo su estabilización o degradación. Se ha demostrado que una de las ARE-binding protein (HuD) se une a la región 3'UTR de ciclina D1 promoviendo su estabilización. Estamos evaluando en células que sobre-expresan la región 3'UTR de GAP-43 y en aquellas que expresan la región codificante de GAP-43, si el control del ciclo celular se debe a un secuestro de la proteína HuD del ARNm de ciclina D1 desestabilizándolo y favoreciendo su degradación. De esta manera disminuirían los niveles de ciclina D1 con el consecuente arresto del ciclo celular observado.

Palavras-Chave: GAP-43; Ciclo Celular; HuD

Apoio Financeiro: Universidad de Buenos Aires 2004-2007 (Proyecto B032)

A.01.030

MORTE CELULAR INDUZIDA POR ATIVAÇÃO DE RECEPTORES P2X₇ EM CULTURAS MISTAS DE CÉLULAS DE RETINA DE EMBRIÃO DE GALINHA.

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ATP é um sinalizador extracelular com efeitos tanto em células neuronais quanto gliais, através de receptores P2Y ou P2X. Neste trabalho, investigamos o efeito de ATP sobre a morte celular em culturas mistas de células de retina de embrião de galinha. Células de retinas de embrião de galinha com 7 dias (E7), cultivadas por 2 dias (E7C2) e tratadas com ATP 3 mM por 3 h revelaram uma diminuição máxima de ~30% (n=3) na viabilidade celular avaliada pelo método do MTT que foi dose ($26,7 \pm 4,0\%$) e tempo ($24,0 \pm 7,0\%$) dependente ($p < 0.01$). Já a incubação das culturas com H₂O₂ 0.5 mM ($65,6 \pm 3,6\%$), por 5 h ($60,4 \pm 4,5\%$), diminuiu a viabilidade celular em ~70% (n=3) ($p < 0.05$). Morte celular induzida por ATP 3 mM foi observada apenas entre os estágios embrionários de E8-E14, sendo seu nível máximo observado em E9 e E10. Este efeito foi observado apenas em culturas mistas, mas não em culturas enriquecidas em neurônios ou em glia. ATP 3 mM induziu um aumento no número tanto de células marcadas para caspase-3 clivada quanto de células com condensação de cromatina, e no aparecimento de um padrão de fragmentação de DNA característico de apoptose nos extratos das culturas tratadas. Por outro lado, a liberação de LDH (lactato desidrogenase) induzida por ATP foi bastante reduzida ($12,2 \pm 2,8\%$) ($p > 0.05$), em contraste com H₂O₂ que induziu uma liberação significativa ($84,6 \pm 3,3\%$) ($p < 0.05$). Diminuição na viabilidade celular também foi observada incubando-se as culturas com 100 µM de BzATP, um agonista seletivo para receptores P2X₇, sugerindo que este seja o subtipo de receptor envolvido na morte celular induzida por ATP. Em acordo com esta hipótese, KN-62, um potente antagonista de receptores P2X₇ foi capaz de atenuar a diminuição na viabilidade celular induzida por ATP em nossas culturas. Além disto, tanto ATP quanto BzATP foram capazes de aumentar a permeabilidade do corante brometo de etídeo nas células de retina, fenômeno que foi bloqueado por BBG, outro antagonista específico para receptores P2X₇. Estes resultados sugerem que ATP seja capaz de induzir apoptose em uma população de células de retina de embrião de galinha em cultura através da ativação de receptores do tipo P2X₇. **Palavras-Chave:** ATP, P2X₇, apoptose, células de retina

Apoio Financeiro: CNPq, Faperj, Propp-UFF.

A.01.031

RNA interference targeting the purinergic receptor for studying neuronal differentiation *in vitro* in P19 mouse embryonal carcinoma

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Background. P2X receptors are a family of ligand-gated cation channels activated by extracellular ATP and comprise seven subtypes (P2X₁₋₇) involved in neurotransmission. Purinergic signaling has also been implicated in neuroprotection and early neuronal development.

Objective: The aim of this study was to target P2X₂ and P2X₇ receptor expression in P19 embryonal carcinoma and NH15-CA2 neuroblastoma x glioma double hybrid cells by small-interfering (si)-RNAs, respectively.

Methods. Real Time-PCR and immunocytochemical staining was used to examine P2X₂ and P2X₇ receptor gene and protein expression in the absence and presence of siRNA constructs.

Results. Following 24h of induction of RNA interference (RNAi) in the presence of siRNA-9 or siRNA-34, P2X₂ receptor gene-expression was reduced by 64% or 42%, respectively. In the case of the P2X₇ receptor (siRNA-2), the reduction of gene-expression was 88% in NH15-CA2 cells after 48h. Immunocytochemical staining revealed decreases of 70% or 55% in receptor protein-expression in the presence of siRNAs-9 or -34, and 90% in the presence of siRNA-2. Expression profiling of P2X₂ and P2X₇ receptors was performed during *in vitro* neuronal differentiation of P19 cells. P2X₂ receptor mRNA transcription increased during initial induction of the differentiation followed by a reduction of gene-expression until P19 cells became functional neurons. P2X₇ receptors were only present in late differentiation when glial cells dominated the cell culture.

Conclusion. Using RNAi for inhibiting gene expression of P2X₂ and P2X₇ receptors during differentiation of P19 cells, we will be able to study the participation of these receptors in a model of early and late neuronal development.

Palavras-Chave: RNA interference, Purinergic receptor, neuronal differentiation

Apoio Financeiro: FAPESP, CNPq and CAPES.

A.01.032

Effects of the neonatal handling on the apoptosis and cells proliferation in the Locus coeruleus.

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OBJECTIVES: Early-life events can exert profound long-lasting effects on the development of the neural system. The neonatal handling causes a reduction of the number of cells in the Locus coeruleus (LC). Present study aimed to examine the effects of the neonatal handling in the process of cellular proliferation and in the apoptotic process. **METHODS:** We used pregnant females (Wistar rats). The pups of these females were divided into: H (handled)- manipulated for 1 minute, daily, at alternate times, until the 10th postnatal day, and NH (nonhandled)- the pups not receive any neonatal stimulation. For the accomplishment of the immunohistochemistry for BrdU, on the 8th and 10th day, one of the pups of each box, was injected with BrdU (5-bromo-2'-deoxyuridine) which is a cell proliferation marker, via intraperitoneal application, at the dose of 100mg/kg. On the 11th day, anesthesia was performed (ketamine: xylazine = 50:10mg/kg) followed by encephalon perfusion. After the perfusion, the encephalon was removed, fixed and embedded in paraffin. The blocks were sectioned on a microtome (5 micrometers - 10 sections/animal) and the sections were incubated in specific antibodies for immunohistochemical analysis. Two independent investigators counted the BrdU-positive cells in both right and left hemispheres. For the accomplishment of the immunohistochemistry to verify the apoptotic cells we used the same groups (H and NH) and the same days old. The immunohistochemistry for the apoptag technic was made according protocol. **RESULTS:** Neonatal handling decreased the number of the BrdU-positive cell of the LC ($p < 0,0005$). H - right side: $30,60 \pm 17,99$; H - left side: $32,00 \pm 19,48$; NH - right side: $88,80 \pm 14,27$; NH - Left side: $88,20 \pm 17,64$. No significant differences ($p=0,44$) were detected on the apoptotic cells between the handled ($13,25 \pm 3,57$) and nonhandled ($17,75 \pm 4,17$) females rats. In conclusion, early-life stimulation not changes the apoptotic process in females rats at 11 days, but the handling reduces the number of proliferation cells and this can explain the reduction of the number of cells in the LC when the pups were stimulated in the neonatal period.

Palavras-Chave: neonatal handling, Locus coeruleus, apoptosis, cell proliferation

Apoio Financeiro: FAPESP, CNPq

Astrócitos tratados por esfingosina-1 fosfato induzem a diferenciação neuronal de progenitores neurais e células-tronco embrionárias

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Objetivos: A esfingosina-1-fosfato (S1P) é um lisofosfolípido envolvido em uma série de eventos biológicos como angiogênese e invasão tumoral. Suas ações no desenvolvimento do sistema nervoso central, no entanto, ainda não estão completamente elucidadas. Neste trabalho, analisamos o papel dos astrócitos como mediadores da ação de S1P na indução da diferenciação neuronal *in vitro*.

Métodos e Resultados: Astrócitos de córtex cerebral de camundongos neonatos foram cultivados em meio DMEM-F12 suplementado com 10% de soro fetal bovino e, após confluência, tratados por 4h com S1P [1uM]. Em seguida, precursores neurais de camundongos com 14 dias embrionários ou células-tronco embrionárias murinas (mES) pré-diferenciadas com ácido retinóico [2uM] foram cultivados sobre as monocamadas astrocitárias. Morfologia neuronal e crescimento neurítico foram analisados por imunocitoquímica para betaTubulinaIII (beta-TubIII). O cultivo sobre astrócitos tratados por S1P aumentou em 26% e 135% o número de células beta-TubIII geradas, respectivamente, a partir de precursores neurais e mES, sugerindo a participação deste lipídio na especificação neuronal. Análise morfolométrica dos neurônios derivados de precursores E14 revelou aumento no tamanho e complexidade neurítica daqueles cultivados sobre astrócitos tratados por S1P. O efeito indireto de S1P (via astrócitos) sobre a neuritogênese e indução neural foi mimetizado por meio condicionado de astrócitos tratados com S1P, sugerindo a participação de uma molécula solúvel secretada pelos astrócitos em resposta ao S1P. O crescimento neurítico pode ser modulado por diversas moléculas da matriz extracelular, como fibronectina e laminina. Ensaios de imunomarcagem revelaram um aumento de aproximadamente 60% e 80%, respectivamente nos níveis dessas proteínas em astrócitos tratados por S1P.

Conclusão: Nossos dados sugerem que astrócitos tratados por S1P induzam a diferenciação neuronal de progenitores neurais e células-tronco embrionárias, através da secreção de fatores solúveis e aumento dos níveis de laminina e fibronectina.

Palavras-Chave: lisofosfolídeos, precursores embrionários, células tronco embrionárias murinas, interação neurônio-glia

Apoio Financeiro: Ministério da Saúde, TWAS, FAPERJ, CNPq, CAPES

A.01.034

Chondroitin sulfate proteoglycans are involved in the regulation of tangential migration of cortical interneurons

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Objective:

The exact ratio of excitatory pyramidal neurons and inhibitory cortical interneurons and their precise connections ensure the correct function of the neocortex. Experimental and clinical studies show that the impaired differentiation and migration of interneurons, combined with environmental factors, can lead to autism, mental retardation, schizophrenia, epilepsy and other neurological diseases (Levitt et al., 2004). Most cortical interneurons are born in the medial ganglionic eminence (MGE) and migrate tangentially to the cortex. During the same developmental stages, the MGE also gives rise to interneurons that migrate radially into the striatum mantle zone. The segregation of these two populations of interneurons was shown to be mediated by semaphorin/neuropilin interactions. Neuropilin-1 positive cortical interneurons avoid the striatum mantle zone, where semaphorin-3A and -3F are expressed, while neuropilin negative striatal interneurons enter that region. Because chondroitin sulfate proteoglycans (CSPGs) are coexpressed with the semaphorins in the striatum mantle zone during the migration period, we examined their possible role in the guidance of cortical interneurons.

Methods:

Using the stripe-assay (Vielmetter et. Al 1999) differential substrate affinities of migratory cells can be investigated. We produced alternating lanes of CSPGs and control proteins (laminin/poly-L-lysine), to investigate selective neuronal migration of dissociated MGE-neurons. Further grafting experiments were applied, in which enhanced green fluorescent protein (EGFP)-labeled MGE explants were homotopically transplanted into wildtype slices to visualize and manipulate migration.

Results:

In a stripe assay, in which dissociated MGE-cells have the choice between CSPG containing and control stripes (laminin/poly-L-lysine), we found a repulsive effect of CSPG on cortical interneurons. Only 16% (SEM = 3.8) of the MGE-cells were growing on the CSPG lanes. In grafting experiments, most of the EGFP-labeled (fluorescent green) MGE avoid the striatal *anlage* under control conditions like *in vivo*, while chondroitinase-treated cultures showed greater migration of MGE-cells in the developing striatum.

Conclusion:

These data suggest that CSPGs contribute to the exclusion of migrating cortical interneurons from the striatal *anlage*.

References:

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Palavras-Chave: cortical interneurons, semaphorin3A, chondroitin sulfate proteoglycan, tangential migration, striatal *anlage*

Apoio Financeiro: Supported by CNPq, FAPERJ and Ministério da Saúde.

A.01.035

"Participation of Ryanodine Receptors and Iron on NMDA Receptor-induced Calcium Signals in Rat Hippocampal Neurons"

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INTRODUCTION: Excessive NMDA receptor (NMDAR) activation (excitotoxicity) triggers Ca^{2+} overload and oxidative stress, which may partially originate from iron (Fe) induced reactive oxygen species (ROS) production via the Fenton reaction. **OBJECTIVES:** We investigated redox-sensitive ryanodine receptor (RyR) activity and mitochondrial dynamics in response to excitotoxic levels of NMDA or Fe overload. **METHODS:** Ca^{2+} and ROS signals were determined by confocal microscopy with specific fluorescent probes in neuronal process of primary cell cultures from rat hippocampus. Cell viability was evaluated by MTT 24 h after stimulation, and mitochondrial dynamics by immuno fluorescence of cells transfected with a fluorescent protein targeted to mitochondria. **RESULTS:** Cultures stimulated for 5 min with 30-100 μM NMDA showed calcium signals; 1 h preincubation with 50 μM ryanodine to selectively inhibit RyR significantly decreased Ca^{+2} signals induced by addition of 50 μM NMDA for 5 min. 1 h preincubation with the Fe chelator deferoxamine (DFO, 500 μM) had similar effects. Cell viability decreased to 50.7 ± 7.9 % of controls with NMDA concentrations >30 μM . Immunofluorescence with anti-Map1b for neurons and anti-GFAP for glial cells, revealed greater glial survival compared with neurons after 50 μM NMDA addition. In the same assay, high levels of NMDA produced mitochondrial fragmentation, detected with anti-mHsp70 (a mitochondrial protein) and *in vivo* in neurons transfected with mitoGFP (a mitochondrial fluorescent protein). Fe overload induced similar responses. Fe-induced ROS signals were significantly decreased after 1 h preincubation with 50 μM MCI (a hydroxyl radical scavenger) or 4 h with 100 μM N-acetyl cysteine (an antioxidant). 1 h preincubation with 50 μM ryanodine significantly attenuated Ca^{+2} signals induced by Fe addition. **CONCLUSIONS:** Excitotoxic stimulation affects neuronal viability, and elicits Ca^{+2} and ROS signals that stimulate mitochondrial fragmentation. Our results suggest participation of RyR and Fe in the Ca^{+2} signals induced by NMDA activation, and crosstalk between ROS and RyR-mediated Ca^{+2} signals.

Palavras-Chave: calcium, excitotoxicity, ryanodine receptor, iron, NMDA receptor, mitochondria, oxidative stress.

Apoio Financeiro: FONDAP CEMC 15010006 y Proyecto ICM P05-001F

c-FOS ACTIVATES THE SYNTHESIS OF PROTEINS IN DIFFERENTIATING PC12 CELLS AND IN VITRO ENDOPLASMIC RETICULUM TRANSLATION

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We have found that c-Fos, in addition to its activity as a member of the AP-1 family of transcription factors, associates to the endoplasmic reticulum (ER) and activates the synthesis of phospholipids in events that require membrane biogenesis. However, membrane biogenesis not only requires lipids, it also requires other components such as proteins. Consequently, we examined if c-Fos also activates protein synthesis by an AP-1 independent mechanism.

Cultured PC12 cells induced to differentiate by feeding with nerve growth factor (NGF) showed an increased labelling of proteins with ³⁵S-methionine; this activation was abolished upon blocking of c-Fos expression; culturing cells with a peptide that specifically blocks the nuclear import of AP-1-c-Fos shows that AP-1 is required to trigger neuronal differentiation whereas cytoplasmic c-Fos is necessary for differentiation to continue. Neuronal differentiation positively correlates with protein synthesis activation.

To establish if the activation of protein synthesis by c-Fos is a direct effect of this protein on the ER we performed *in vitro* translation. Increasing concentrations of recombinant c-Fos were incubated with the rabbit reticulocyte system in the presence of canine pancreatic microsomal membranes and a signal sequence mRNA. These results show activated protein biosynthesis in the ER that are dependent on the concentration of c-Fos.

To analyse if c-Fos associates to the ER membranes to activate protein synthesis, membranes were pre-incubated with c-Fos, washed and translation measured *in vitro* in the pelleted fraction. The results show that c-Fos associates to the ER and activates protein synthesis. Similar results were found with a synthetic peptide of the basic domain of c-Fos (aa 139-160).

This is the first evidence that c-Fos can activate, in addition to the metabolism of lipids, protein metabolism. Since c-Fos protein is highly regulated, the regulation of its expression can result in an interesting mechanism for controlling the genesis of the components that the cell requires according to its physiological status that is, during growth, proliferation, differentiation, etc.

Palavras-Chave: c-Fos, neuronal differentiation, protein biosynthesis activation and ER protein synthesis

Apoio Financeiro: FONCyt, CONICET, SECyT, Agencia Córdoba Ciencia S.E. and James S. McDonnell Foundation

AVALIAÇÃO DA SUSCEPTIBILIDADE À ISQUEMIA *IN VITRO* EM CAMUNDONGOS KNOCK-DOWN PARA TRANSPORTADOR VESICULAR DE ACETILCOLINA

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Objetivo: Verificar a susceptibilidade de camundongos machos knock-down Homozigotos (KD^{HOM}) para Transportador Vesicular de Acetilcolina (VACHT) à isquemia cerebral *in vitro* em relação a animais selvagens. **Metodologia:** Foram utilizados camundongos machos (25 e 30g). As fatias de hipocampo (300µm) foram incubadas em câmaras de perfusão de tecido com meio ACSF contendo glicose e oxigenadas (mistura carbogênica contendo 95% de O₂ e 5% de CO₂) à temperatura controlada de 34,5 °C por um período de 90 minutos. Em seguida, as fatias submetidas à isquemia foram privadas de glicose/oxigênio através da perfusão com ACSF sem glicose e aeradas com uma mistura gasosa contendo 95% de nitrogênio e 5% de dióxido de carbono, durante 10 minutos. Após este período de isquemia, as fatias foram mantidas em reperfusão com ACSF contendo glicose e oxigênio por mais 4 horas. As fatias controle foram submetidas ao mesmo procedimento, no entanto não foram privadas de oxigênio e glicose. Após este período, as fatias foram marcadas com Etídio Homodímero, marcador de morte celular, e analisadas no microscópio de fluorescência. A quantificação da morte celular foi realizada através do programa ImageJ (NIH,USA). **Resultados:** A isquemia ocasionou aumento da morte celular nas fatias de animais KD^{HOM} VACHT de 21,2±7,3% para 55,9±11,2% (p<0,05) e nas fatias de animais selvagens de 27,6±11,0% para 65,2±3,9% (p<0,05). Quando comparados os dois grupos entre si, em relação ao efeito da isquemia, notamos que o aumento percentual médio de morte celular não demonstra diferença significativa entre fatias de animais KD^{HOM} VACHT (60.2±13.2%) e selvagem (58.6±16.5%), p>0,05. **Conclusão:** Camundongos KD^{HOM} VACHT não apresentam maior susceptibilidade à isquemia quando comparados com selvagens.

Palavras-Chave: Isquemia in vitro, morte celular, acetilcolina

Apoio Financeiro: Apoio financeiro: CNPq, CAPES, FAPEMIG, PRPq-UFMG

A.01.038

Rol de los nucleótidos extracelulares en la proliferación celular de la retina adulta de pez cebra

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La retina adulta de peces teleósteos es un modelo adecuado para estudiar neurogénesis ya que crece durante toda la vida del animal adicionando nuevas células, y se regenera morfológica y funcionalmente luego de un daño. Las neuronas y células gliales se originan a partir de células germinales retinianas, pero se desconocen los mecanismos que regulan la proliferación y diferenciación.

Objetivo: Evaluar la capacidad regenerativa de la retina de pez cebra y el rol del ATP/ADP extracelular en la regulación de este proceso luego de una lesión citotóxica.

Métodos: Se lesionó mediante inyección intraocular (IIO) de ouabaina. A diferentes días post-inyección (dpi) se determinó histológicamente la regeneración retiniana. Utilizando bromodeoxiuridina se determinó la activación de células germinales de la retina. Por inmunohistoquímica de fluorescencia se evaluaron cambios en la localización de ENTPDasa2 (ectoenzima que hidroliza ATP extracelular a ADP) y de SV2 (marcador de vesículas presinápticas). Se examinó luego si los nucleótidos extracelulares ATP y ADP regulan la proliferación celular. Se realizaron IIO de Apirasa (hidroliza nucleótidos di y trifosfato) y Hexoquinasa (hidroliza preferencialmente ATP) en retinas lesionadas, y de ADPβS y ATPγS en retinas no lesionadas.

Resultados: La lesión produjo degeneración y desorganización de las capas internas 2dpi, con reorganización a 30dpi. Además, produjo activación mitótica con un máximo a los 5-7dpi.

Se observaron cambios en la localización de E-NTPDasa2 y su posterior reubicación a 30dpi, mientras que SV2 no exhibió cambios.

La IIO de Apirasa provocó disminución significativa de la proliferación ($P < 0.05$), no así la de Hexoquinasa. Además, se observó un aumento significativo en la proliferación por IIO de ADPβS ($P < 0.05$) pero no de ATPγS.

Conclusión: Estos resultados sugieren que el ADP extracelular regula la proliferación en la retina adulta de pez cebra.

Palavras-Chave: ouabaina, neurogénesis, proliferación, retina, ATP, ADP.

Apoio Financeiro: ANPCyT, CONICET

A.01.039

Ontogenetic expression of connexins in the rabbit retina

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Adjacent cells are commonly coupled by gap junctions (GJ) formed by two hexameric arrays of a multigene family of integral membrane proteins called connexins (Cx). In particular, in the retina, connexins play an important role as essential circuit element in the visual processing as well as an independent functional role during developmental stages. Although its remarkable importance, the knowledge regarding Cx expression is incipient and arising from nocturnal mammalian species whose visual system is adapted to scotopic vision. Consequently, crepuscular habits in *Oryctolagus cuniculus* allowed to describe the connexins expression in mesopic visual systems, as a new comparative model. In the present study, Cx36, Cx43 and Cx45 expression during the New Zealand – White rabbit retinal histogenesis were determined by a combination of qualitative (immunohistochemistry) and quantitative (real time PCR) methods. In the inner plexiform layer (IPL), Cx36 immunoreactivity appeared at postnatal day 10 (P10) whereas Cx43 staining was weakly observed at P10 and towards. In the transition area of neuroblastic layer (NBL) and pigmented epithelium (PE) Cx43 and Cx45 staining was first observed at embryonic day 20 (E20). In the NFL, Cx43 staining was observed from postnatal day 3 (P3) to adult age. These findings show distinct temporal and spatial patterns revealing the possible role of Cx in histogenesis of the rabbit retina, allowing comparisons with other species.

Palavras-Chave: Retina, development, connexins, rabbit.

Apoio Financeiro: FAPESP

A.01.040

Gliose reativa induzida por lesão da monocamada de células de retina de galinha em cultura.

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Após lesão, células de Müller, principal célula glial da retina, inciam a gliose reativa, que foi caracterizada através da proliferação celular, alterações morfológicas e mudanças na expressão de genes (*Nature Neurosci.* 3(9),2000). Neste trabalho, investigamos a proliferação celular e as mudanças morfológicas induzidas por lesão da monocamada celular em culturas de retinas de embrião de galinha. Cultura de células de retinas de embriões com 8 dias (E8), cultivadas na densidade de 10^7 células/placa por 1 (E8C1) ou 7 dias (E8C7), foram riscadas com o auxílio de uma ponteira de micropipeta, gerando uma região desprovida de células (área de lesão da monocamada). Em culturas riscadas em E8C1, um aumento no preenchimento da área descoberta pela lesão foi detectado entre o 1º e o 8º dia pós-lesão (área livre de células: 0=100; 1= 64.2 ± 5.0 %; 2= 56.5 ± 6.5 %; 6= 45.2 ± 3.4 %; 8= 25.9 ± 3.3 %). Entretanto, em culturas mais diferenciadas (E8C7), recobrimento significativo da área de lesão foi detectado somente a partir do 3º dia pós-lesão (área livre de células: 0=100; 1= 89.8 ± 3.6 %; 2= 79.7 ± 3.6 %; 3= 45.9 ± 4.4 %; 7= 19.6 ± 4.5 %). Culturas riscadas em E8C1 apresentaram nos 3 primeiros dias pós-lesão uma intensa neuritogênese, com inúmeras conexões entre as bordas da região lesada, assim como extensas áreas recobertas por células com morfologia neuronal e glial. Já as áreas provenientes de culturas riscadas em E8C7 não apresentaram neurônios ou processos. Somente células com morfologia glial foram observadas nestas regiões. Ensaio de imunofluorescência para BrdU incorporado por 48h após a lesão revelaram que todas as células encontradas na área de lesão eram positivas para este marcador. Estes dados sugerem que culturas de retina de embrião de galinha de diferentes estágios de maturação “in vitro” respondem de maneira diferencial à lesão da monocamada celular. Enquanto culturas em estágios mais avançados respondem com proliferação e hipertrofia glial, culturas em estágios precoces do desenvolvimento respondem com intensa neuritogênese acompanhada pelo recobrimento da área de lesão tanto por células gliais quanto por neuroblastos.

Palavras-Chave: Retina, Lesão, Gliose reativa, Proliferação

Apoio Financeiro: CNPq, Faperj, Propp-UFF

A.01.041

Señalización GABAérgica en el canal central de la médula espinal de la tortuga

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La región que rodea al canal central (CC) de la médula espinal de tortugas juveniles posee células que proliferan activamente y neuronas inmaduras. En esta región hay un plexo bien desarrollado de terminales que contienen ácido γ -aminobutírico (GABA). Es posible que, al igual que lo descrito en otros sistemas, la señalización GABAérgica forme parte de los mecanismos que regulan la neurogénesis alrededor del CC.

Objetivo: estudiar las respuestas al GABA de las células que contactan el CC.

Métodos: La técnica de "patch" en modo "whole-cell" (fijación de corriente o voltaje) se utilizó para registrar células en contacto con el CC visualizadas con óptica Nomarski. El GABA se aplicó localmente por presión y las drogas por perfusión. En algunos experimentos se utilizó la técnica de "patch" perforado con gramicidina. En modo "cell-attach", el potencial de membrana se estimó del potencial de reversión de las corrientes de K^+ dependientes del voltaje.

Resultados: En células de tipo precursor, el GABA generó pequeñas corrientes entrantes en un rango de potencial de entre -120 mV a +20 mV. En neuronas inmaduras, las respuestas al GABA fueron mediadas por corrientes reversibles y rápidas que se bloquearon con un antagonista específico de los receptores GABA_A. En los registros obtenidos con gramicidina, se observaron variaciones en el potencial de reversión de las corrientes. Esto hizo que en algunas células el GABA actuara como una señal inhibitoria mientras que en otras produjera despolarizaciones capaces de llegar al umbral de descarga. El hecho de que el potencial de membrana medido en modo "cell-attach" fuese generalmente más hiperpolarizado indicaría que el GABA actuaría despolarizando a la mayoría de las células estudiadas.

Conclusiones: Se encontraron dos tipos de respuestas al GABA: 1) en células de tipo precursor, respuestas que podrían ser mediadas por transportadores y 2) en células de estirpe neuronal, respuestas mediadas por receptores GABA_A. En estas últimas, la heterogeneidad de sus efectos fisiológicos apoya nuestra hipótesis de que existen rodeando al CC células en distintos grados de maduración neuronal.

Palabras-Chave: GABA, médula espinal, neurogénesis

Apoio Financeiro: National Institutes of Neurological Disorders and Stroke, National Institutes of Health; Grant number: NS048255

The Expression of VMP1, a Novel Mammalian Trans-Membrane Protein, is Related to Basal Autophagy in the Rat Brain

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Autophagy is a degradation process of cytoplasmic contents and it is characterized by sequestration of bulk cytoplasm and organelles in double-membrane vesicles called autophagosomes. Recently we characterized the Vacuole Membrane Protein 1 (VMP1) as a new autophagy-related protein that localizes in the membrane of autophagosome. We have also shown that VMP1 triggers autophagic process in mammalian cells. Activation of autophagy preferentially occurs under stress conditions such as starvation and alterations of this process have been associated with a variety of pathological conditions such as neurodegenerative disorders. **Objectives:** To investigate whether VMP1 is expressed in normal rat brain and if this expression is related to basal brain autophagy. **Methods:** Male Sprague–Dawley rats weighing 200-250g were used. Animals were sacrificed; the brain was removed and immediately processed for total RNA isolation. Another group of rats were transcardially perfused with PBS-4% Paraformaldehyde. Brains were removed, embedded in paraffin and cut in serial sections. **Results:** RT-PCR assay in total brain RNA showed that VMP1mRNA is expressed in rat brain. On sagital brain sections we identify the brain area that expresses VMP1 transcript by *in situ* hybridization and VMP1 protein by immunofluorescence. We found high signal of VMP1 in cerebral cortex, hippocampus and cerebellum. We also analyzed LC3 expression as a marker of autophagosome and we found a remarkable colocalization of VMP1 and LC3 in the dentate gyrus of hippocampus and in purkinje layer of cerebellum. These findings suggest that VMP1 expression is involved in brain basal autophagy **Conclusions:** Our results demonstrated that the new autophagy-related protein VMP1 is expressed in the cerebral cortex, hippocampus and cerebellum of rats and this expression is related to autophagy in physiological conditions.

Palavras-Chave: Autophagy, Hippocampus, Cerebellum, VMP1

A.01.043

Ação trófica de uma fosfolipase A₂ isolada do veneno da serpente *Lachesis muta* sobre células ganglionares da retina de ratos em cultura.

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Objetivo: O objetivo deste trabalho foi analisar a atividade trófica de uma fosfolipase A₂ (PLA₂) isolada do veneno da serpente *Lachesis muta* em células ganglionares (CG) da retina submetidas ao processo de axotomia. Investigamos também vias de sinalização envolvidas no processo.

Métodos: Para identificação das CG, ratos neonatos receberam injeções de peroxidase (HRP) nos colículos superiores. Após 16h, foram sacrificados, suas retinas dissecadas, dissociadas e plaqueadas (1,0x10⁵ céls/cm²) em meio de cultura completo (MCC). As culturas foram mantidas por 4h em MCC e a seguir algumas laminulas foram fixadas em aldeídos (100% da população de CG). As demais foram tratadas e mantidas por 48h em cultura sendo neste período fixadas. A presença de HRP foi revelada por reação histoquímica utilizando-se tetrametilbenzidina como cromógeno. O n° de CG foi obtido por contagem em microscopia de campo claro.

Resultados: Os resultados mostraram que o tratamento com PLA₂ aumentou a sobrevivência das CG de forma dose-dependente, sendo a melhor concentração 2,5µg/mL [CT4h=100%; CT48h=53,6% ±4,0%; PLA₂=84,4% ±2,5%, n=7]. O efeito da PLA₂ (2,5µg/mL) foi abolido pelo inibidor da proteína cinase C (PKC), cloreto de queleretina (CC) 1,25µM [CT4h=100%; CT48h=52,1% ±0,3%; PLA₂=90,8% ±1,9%; CC=51,2% ±3,9%; PLA₂+CC=46,3% ±2,8%, n=7]. O inibidor da PKC delta, rottlerina (rot) 4µM aboliu o efeito da PLA₂ (CT4h=100%; CT48h=56,1% ±2,5%; PLA₂=93,0% ±3,1%; rot=59,4% ±1,4%; PLA₂+rot=44,5% ±4,5%, n=6). Observamos também o envolvimento da via da c-jun cinase (JNK), já que o seu inibidor (jJNK 1µM) bloqueou a ação da PLA₂ na sobrevivência das CG [CT4h=100%; CT48h=48,5% ±1,2%; PLA₂=94,0% ±4,1%; jJNK=44,7% ±2,1%; PLA₂+jJNK=41,9% ±1,7%, n=6].

Conclusões: Nossos resultados demonstraram que o tratamento com a PLA₂ resgata CG da morte celular, através da ativação das vias da PKC delta e da JNK.

Palavras-Chave: Fosfolipase A₂, PKC, JNK e sobrevivência neuronal

Apoio Financeiro: FAPERJ, CNPq e PRONEX-MCT.

HYPOXIC CELL DEATH SHARES A COMMON EXECUTION PATHWAY WITH NATURALLY OCCURRING CELL DEATH

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The aim of the present work was to compare mechanisms involved in the execution of the naturally occurring cell death (NOCD) and in the hypoxic cell death (HxCD). Also, we have tested the influence of the fibers of the retinal ganglion cells (RGC) on the HxCD that occurs in the optic tectum (OT).

In previous works we have found that TUNEL+ nuclei, active caspase-3 and nuclear pyknosis colocalize for both control (C) and hypoxic (Hx) (8% [O₂], 60 min) embryos of embryonic day (ED) 12. However, only at 6 h post-hypoxia (p-hx) a significant increase in cell death rate was observed (Neuroscience, 142,645. 2006).

In the present study, a co-immunofluorescence anti-cytochrome C and anti-active caspase-9 was performed in C and Hx OT. Both treatments present colocalization of cytoplasmatic cytochrome C and active caspase-9, observed with confocal microscopy and nuclear pyknosis was detected with Hoechst stain. In Hx embryos the increase in the presence of both markers was observed at 4 and 6 h p-hx.

Electron microscopy was performed to analyze differences in cell degeneration phenotype for C and Hx embryos. Both treatments showed figures clearly apoptotic, with dense circular-shaped nuclear material, absence of a regular nuclear membrane and absence of organelles .

To test the influence of the RGC fibers on the HxCD, the right eye of ED4 embryos was removed and then left to develop until ED12 where quantification of TUNEL+ nuclei of entire OT sections was performed in C and Hx embryos. We have found no significant differences between contralateral (without fibers) and ipsilateral (with fibers) OT in neither of the treatments (CC:233 ±25 vs. CI:255 ±36; HxC:416 ±55 vs. HxI:388 ±56; p>0,05 for both comparisons). However, Hx OT had a significant increase in TUNEL+ nuclei compared to C for both sides (same values as above: CC vs. HxC, p<0,05; CI vs. HxI, p<0,05).

Colocalization and electron microscopy results suggest that the same execution pathway of cell death is shared by both the NOCD and the HxCD, namely the intrinsic mitochondrial pathway. Enucleation experiments show that retinal fibers are not involved in HxCD (i.e. via excitotoxicity or neurotrophic factors). **Palavras-Chave:** Hypoxia, Apoptosis, Development, Chick, Optic Tectum

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Analysis of CoREST-complex expression and its role in heat shock response in differentiated PC12 cells

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The mammalian CoREST-complex is a chromatin-modifying transcriptional co-repressor complex that exhibits both histone deacetylase and histone demethylase activity mediated by HDAC1/2 and LSD1, respectively. CoREST-complex was initially identified in association with the repressor for element-1 silencing transcription factor (REST)/neuronal restrictive silencing factor (NRSF), thus, its biological role has been linked to neural gene expression. Recently, we have reported that CoREST is a transcriptional co-repressor of the heat shock response, a cell survival mechanism activated by many stressful stimuli. Strikingly, the heat shock response, characterized by the induction of heat shock proteins expression, is impaired in neuronal cells and the molecular mechanism responsible for this phenomenon is unknown.

Objective: 1) To analyze the expression and subcellular localization of CoREST, HDAC1 and LSD1 2) to determine the functional role of CoREST-complex in the regulation of the heat shock response during PC12 differentiation induced by NGF treatment. **Methods & Results:** Western-blots and immunofluorescent assays show that the localization of CoREST-complex components does not change, although their expression decrease during NGF treatment. By performing gene reporter assays, it was corroborated the role of CoREST as co-repressor of the heat shock response in PC12 cells. Decreasing CoREST levels by using shRNA induced an increase of Hsp70 levels under heat shock treatment.

Conclusion: the expression of mammalian CoREST-complex components decreases in PC12 neuronal differentiation model, and we have approached a feasible molecular mechanism responsible for the impaired heat shock response in neuronal cells.

Palavras-Chave: Heat shock, CoREST, Hsp70, PC12, HDAC, LSD1

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Differential Sensitivity of Retinal Cells to Endoplasmic Reticulum Stress–Induced Cell Death

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Recently, retinal degeneration that occur during retinitis pigmentosa was associated with endoplasmic reticulum (ER) stress and activation of unfolded protein response (UPR). The molecular mechanisms that lead to cell death induced by ER stress have not been entirely elucidated yet. We previously described that thapsigargin, an inhibitor of the calcium ATPase of the ER, classically described as an inductor of ER stress, selectively leads to photoreceptors cell death during development of retinal tissue (Chiarini, L.B. et al, 2003, J. Neurosci. Res. 74: 875). We showed that photoreceptor cell death induced by thapsigargin is dependent on both caspases and ERK activation. In this study we tested whether degeneration of photoreceptors following treatment with thapsigargin is associated with UPR activation. We also tested the sensitivity of retinal cells to ER-stress inductor Brefeldin-A (BFA), an inhibitor of ER/Golgi transport. In addition, in this work we tested the participation of caspases and ERK pathway during ER stress–induced cell death after treatment with BFA.

Retinal explants were maintained *in vitro* for 24 hours in the presence or absence of BFA. Cell death was detected either as condensed profiles stained with neutral red or with TUNEL technique. BAF (Boc-D-fmk), an inhibitor of caspases, and U0126, an inhibitor of MEK, were used to test the involvement of caspases and ERK in the ER stress-induced cell death, respectively.

We found that BFA induces retinal cell death. However, photoreceptors were not susceptible to cell death induced by BFA. Western blot for CHOP/GADD153, a transcription factor associated with programmed cell death induced by ER-stress, indicated that both thapsigargin and BFA increased the levels of this transcription factor. Moreover, the cell death induced by BFA was blocked by salubrinal, an inhibitor of ER-stress cell death. In addition, we found that BFA-induced cell death was blocked by the pan-caspases inhibitor, BAF, and by ERK pathway inhibitor. We concluded that there is a differential sensitivity of retinal cells types to ER-stress-induced cell death.

Palavras-Chave: Retina; Programmed cell death; Unfolded protein response; Endoplasmic reticulum Stress; UPR; Neurodegeneration

Apoio Financeiro: FAPERJ, CNPQ, PRONEX, PIBIC/UFRJ

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Suplementação com óleo de peixe em ratos Wistar da geração F1, é capaz de alterar a morfologia hipocampal?

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Objetivos: Ácidos graxos poliinsaturados n-3 (AGPIS n-3) têm sido mencionados como reguladores de processos de proliferação e diferenciação células, podendo atuar sobre vias de transdução de sinal e expressão gênica, alterando características morfofisiológicas das células. No sistema nervoso central têm sido correlacionados com neuritogênese e crescimento axonal *in vitro*, bem como neurogênese no adulto, sugerindo o efeito destes AGPIS sobre o desenvolvimento e maturação de neurônios. Neste trabalho investigamos o efeito da suplementação com óleo de peixe, composto marinho rico em ácidos graxos n-3, sobre a morfologia e ultraestrutura do hipocampo de ratos.

Métodos: Ratas Wistar foram divididas em: controle (C), suplementadas com óleo de peixe (OP) ou gordura de coco (GC), na dose de 1g/kg. Estas ratas foram acasaladas e receberam suplementação durante a gestação e amamentação. Após desmame, machos das proles obtidas receberam diariamente a mesma suplementação de suas genitoras até completarem 90 dias, sendo então obtidas amostras cerebrais para análise morfológica.

Resultados: estereologia do hipocampo dos animais revelou que a suplementação com óleo de peixe promoveu aumento no número de células na região CA₁ (C - 39,2±2,57; OP- 52,9±1,2; GC- 41,2±1,4) e das células granulares do giro dentado (C - 6,5±0,52; OP- 11,2±0,08; GC- 6,6±0,26). Também foi possível identificar aumento de projeções axonais na via perfurante que comunica CA₁ e giro dentado. A análise de microscopia eletrônica mostrou existir aumento expressivo de fibras mielinizadas no encéfalo dos animais do grupo OP, quando comparados ao controle e aos suplementados com gordura de coco.

Conclusão: a suplementação com óleo de peixe foi capaz de induzir aumento no número de células em duas áreas do hipocampo, bem como na comunicação entre estas áreas. Além disso, foi hábil em alterar o padrão de mielinização axonal destes animais, quando comparados com os demais grupos. Nossos dados sugerem que o óleo de peixe tem ação sobre a proliferação e diferenciação celular no hipocampo.

Palavras-Chave: óleo de peixe, suplementação, hipocampo, morfologia

Apoio Financeiro: CNPq, Fundação Herbárium de Pesquisa e Saude

PUTRESCINE SYNTHESIS INHIBITION EFFECT OVER POSTNATAL SUBVENTRICULAR ZONE PROGENITORS *IN VIVO*

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Our group showed that GABA synthesis in the subventricular zone (SVZ) is partially due to its conversion from putrescine. We then asked if putrescine synthesis inhibition *in vivo* would affect GABA levels in the rat postnatal SVZ. We also analyzed events known to be regulated by GABA.

We inhibited putrescine synthesis through intraperitoneal (IP) injections of difluoromethylornithine (DFMO), an irreversible inactivator of putrescine synthetic enzyme, in postnatal rats from the seventh postnatal day (P6) during 9 days. We evaluated the effect of the treatment over putrescine synthesis in the brain measuring the quantity of putrescine in the cerebellum. To do this, we fluorescently labeled aminated compounds in the tissue through dansylation and separated putrescine utilizing a thin layer chromatography. We observed that treated animals have a 78% reduction in putrescine levels. To analyze the drug effect over the proliferation of SVZ cells we injected (IP) 5-Bromo-2'-deoxyuridine (BrdU) 10 days after the first DFMO injection and 1 hour before sacrifice. We quantified two regions, around the lateral ventricles (posterior SVZ) and its rostral projection until the olfactory bulb (OB; anterior SVZ). The data show no difference between the number of BrdU cells in control and treated animals (posterior SVZ, control=155.7 ± 10.96; DFMO=215.9 ± 40.02; anterior SVZ, control=34.33 ± 4.997; DFMO=38.19 ± 3.593). To observe the effect of the treatment over GABA synthesis we performed immunohistochemistry to detect GABA and GAD. After treatment, SVZ neuroblasts still express GABA. However, the inhibition of putrescine synthesis leads to the stimulation of GAD expression by SVZ cells, which is not seen in control animals.

We observed that DFMO treatment does not lead to a decrease in GABA expression and in the number of BrdU positive cells. As the treatment leads to an early expression of GAD, we suggest that the maintenance of GABA levels is due to the substitution of the synthetic pathway being utilized. We propose a model where one of the putrescine derived GABA functions would be to maintain neuroblasts uncommitted to the GABAergic phenotype.

Palavras-Chave: Neurogênese; Bulbo olfatório; GAD; difluoromethylornithine; GABA

Apoio Financeiro: PIBIC/UFRJ, CNPQ, FAPERJ, PRONEX

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Radial glia is involved in gap junction-mediated cell coupling in the postnatal subventricular zone.

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Objectives: Radial glial cells (RG) are multipotent precursors present in neural embryogenesis characterized by a bipolar radial morphology, spanning the whole cerebral wall. After birth these cells populate the subventricular zone (SVZ), an important postnatal neurogenic region. We have shown previously that early postnatal SVZ cells display extensive gap junctional coupling. In this study we will analyze the specific contribution of RG cells to cell coupling in the SVZ.

Methods: To target RG as sources for dye coupling *in situ*, we have developed a new technique that involves cell loading through processes that reach the pial surface, using a mix of gap junction permeant (lucifer yellow, LY) and non-permeant (rhodamine-conjugated dextran 3KDa, RD) fluorochromes. Briefly, the pial surfaces of rat brains with 1, 4 and 6 postnatal days were scraped and exposed to the fluorochromes, allowing intracellular loading through the ruptured cell processes. Brains were then processed to be analyzed under epifluorescence. Retrograde labeling of these cells with carbocyanines placed in the pial surface confirms their RG identity based on morphology.

Results: Directly loaded cells (LY+RD+) were restricted to pial loading surface region. Coupled cell profiles (LY+RD-) were distributed in a bimodal manner, with coupled chains in the dorsal surface of the SVZ and that aligning the ventricle, leaving the SVZ core relatively free. This pattern was unchanged throughout the ages examined. Coupled cells were exclusively labeled by glial markers, with complete exclusion of neuronal/neuroblast markers. Inhibition of gap junctional coupling with intraventricular injection of carbenoxolone (100µM) or high [CaCl₂] (5mM) reduced the proportion of LY+RD- cells.

Conclusions: By specific dye loading through the pia we have demonstrated the direct participation of RG in cell coupling within the SVZ. Its spatial distribution and apparent homocellular nature suggest that RG-mediated cell coupling leads to the establishment of a functional syncytium that may be involved in regional specialization of the postnatal SVZ.

Palavras-Chave: SVZ, radial glia, gap junctions

Apoio Financeiro: CAPES, CNPq, FAPERJ, FUJB, PADCT, PRONEX

ENDOPLASMIC RETICULUM STRESS-INDUCED CELL DEATH INVOLVES BOTH AUTOPHAGY AND THE ERK PATHWAY IN RETINAL TISSUE

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Objectives: Endoplasmic reticulum (ER) stress has been associated with cell death in various degenerative pathologies in which accumulation of mutated proteins occur, including retinitis pigmentosa. However, the mechanisms involved in ER stress-induced cell death have not been fully established. Recently, autophagy was shown to be linked to ER stress. ER stress-induced autophagy has been linked to both protection from and induction of cell death. Previously, we showed that tunicamycin (TN), an inhibitor of N-glycosylation, also described as inducer of ER stress, triggers cell death in the retina. Here we studied the role of autophagy in TN-induced cell death in retinal tissue, as well as the participation of the ERK pathway.

Methods: Retinal explants from 6 day-old rats were maintained for 24 hours *in vitro* with TN (1µg/mL), in the presence or not of either salubrinal (1-10µM), an inhibitor of ER stress-induced cell death, 3-methyladenine (3MA, 1-10mM), an inhibitor of autophagy, or BAF (100mM), a general inhibitor of caspases. U0126 (10µM) and PD98059 (30µM), inhibitors of the ERK pathway, were used to analyze the participation of ERK in TN-induced cell death. Programmed cell death was evaluated by counting condensed profiles stained with neutral red or by TUNEL technique. Protein content and phosphorylation were detected by *western blot*.

Results and conclusions: We found that TN increased the level of CHOP/GADD153, a transcription factor implicated in cell death induced by ER stress. Salubrinal partially blocked TN-induced cell death. Inhibition of caspase activity with BAF blocked TN-induced cell death. We verified an increase in the amount of phospho-ERK after TN treatment and inhibitors of the ERK pathway (U0126 and PD98059) partially blocked TN-induced cell death. Finally, we observed that treatment with 3MA prevented cell death in this model. Our data show that TN-induced cell death in retinal tissue involves induction of ER stress and the participation of both autophagy as well as the ERK pathway.

Palavras-Chave: autophagy; endoplasmic reticulum stress; cell death; retina; tunicamycin.

Apoio Financeiro: CNPq, FAPERJ.

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GTPases Rho e seus efetores durante o desenvolvimento da retina de camundongos rd1

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Objetivos: Estudos anteriores do laboratório mostraram que a degeneração induzida por luz promove um aumento na expressão e na atividade de Rac1, uma GTPase da família Rho, nos fotorreceptores em apoptose (1). No presente estudo, investigamos a possível relação entre as GTPases RhoA, Rac1 e Cdc42 e seus principais efetores (RhoA: ROCK I e II; Rac1 e Cdc42: PAKs 3, 4 e 5) na degeneração hereditária de fotorreceptores apresentada pelo camundongo rd1.

Material e Métodos: Os olhos de camundongos rd1 (C3H) e selvagem (C57BL/6) com idade pós-natal P1, P5, P10, P15 e P60 foram coletados e processados para imunofluorescência com os anticorpos contra RhoA, Rac1 e Cdc42 (1:50, Sta. Cruz) e PCR em tempo real para determinar a expressão gênica dos efetores ROCK I, ROCK II, PAKs 3, 4 e 5. A apoptose foi verificada através do método de TUNEL.

Resultados: RhoA, Rac1 e Cdc42 foram observadas em neurônios e células de Müller durante o desenvolvimento dos camundongos. De modo geral, a distribuição das GTPases Rho foi similar durante o desenvolvimento, exceto por Rac1, cujo aumento de expressão em núcleos de fotorreceptores em P10 coincidiu com o aumento da expressão dos seus efetores PAK3 (5.74 vezes, $P < 0,05$) e PAK4 (8.46 vezes, $P < 0,01$) e com a degeneração dessas células em animais rd1. Embora a distribuição de RhoA não tenha alterado, seus efetores ROCK I (7.26 vezes, $P < 0,01$) e ROCK II (7.89 vezes, $P < 0,01$) mostraram um aumento de expressão em P5 e P10, respectivamente quando comparado com o C57BL/6. Estudos de dupla marcação mostraram que os fotorreceptores Rac1-positivos eram também TUNEL-positivos.

Conclusões: Esses resultados sugerem que as GTPases Rho são importantes durante o desenvolvimento da retina de camundongos rd1 e que essas vias de sinalização podem estar potencialmente envolvidas na degeneração de fotorreceptores. É possível que o aumento na expressão dos efetores PAK3, PAK4 e ROCK II durante a degeneração funcione como mecanismo anti- e/ou pró-apoptótico dessas vias.

Referência: 1- Invest.Ophthalmol.Vis.Sci. 47:1193-200, 2006

Palavras-Chave: degeneração, fotorreceptores, GTPases

Apoio Financeiro: FAPESP, CNPq.

CALPAIN INTERFERE ON RESPONSE OF CALCIUM AND ON TRANSLOCATION OF BAX IN ASTROCYTES OF RATS.

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AIM: Calpain is a cytosolic calcium-dependent protease. After the activation of glutamate receptors calpain may promote the cell death (1) as well as the cell survival (2). Previous data has shown that in astrocytes of rat, glutamate was able to increase the intracellular calcium and to promote cell death. The aim of this work was to study the effect of glutamate on intracellular calcium and on translocation of pro-apoptotic protein Bax, in the presence or absence of inhibitor of calpain.

METHODS: Primary cortical astrocytes cultures were prepared from the cerebral cortices of newborn rats Wistar, cultivated per 8 days. For measurement of intracellular calcium (Ca^{2+}_i), the cells were loaded with fura-2 and stimulated with glutamate (100 μM) in absence or presence of the inhibitor of calpain, ALLN (N-acetyl-L-leucil-L-leucil-L-norleucinal, 50 μM , 30 min). For analyses of Bax translocation the cells were transfected with GFP-Bax and stimulated with glutamate (100 μM), in absence or presence of ALLN. These measurements were realized in high-resolution digital microscope coupled to a cooled CCD camera in real time. Statistical analysis: The statistical significance control and treated was determined by student's *t* test with $p < 0,05$.

RESULTS AND CONCLUSION: Glutamate increased the Ca^{2+}_i in 33.25% in relation to basal calcium and this effect was inhibited in 81.08% in the presence of ALLN. These results suggest that the receptors stimulation by glutamate can be affected by calpain or that calpain participates of the transduction of this receptor stimulation. An interaction between the different types of glutamate receptors could explain these effects (1). The increase of Ca^{2+}_i induced by glutamate did not induce the translocation of Bax, in the present work conditions, although calcium induces Bax translocation in some experimental models. However, in the presence of ALLN, the Bax translocation was observed. More experiments must be done to clarify these results.

REFERENCES: 1-Neuron, 53:399, 2007; 2 - J Biol Chem. 280:21588, 2005.

Palavras-Chave: glutamate; calcium; calpain; astrocytes

Apoio Financeiro: CNPq e FAPESP

The MAPK/ERK pathway is involved in oligodendroglial differentiation *in vitro*: effects on NG2 expression and cell morphology

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Goals: Oligodendroglial cells treated with an ERK pathway inhibitor present a decrease in the number of differentiated cells, based in 2'3'cyclic nucleotide 3'phosphodiesterase (CNPase) immunoreaction and cell morphology. CNPase was also present in a group of rounded cells, which could not be classified in any of the defined stages of oligodendroglial differentiation. ERK pathway influences the CNPase distribution and its relationship with cytoskeleton. Here, we studied the expression of NG2, a chondroitin sulfate proteoglycan present in oligodendroglia in early stages of differentiation, in order to verify whether changes observed in oligodendroglia treated with the MEK inhibitor are caused by a delay in differentiation or an effect in cytoskeleton rearrangement.

Methods: Cultures from cerebral hemispheres of neonate rats were maintained for 3 days in medium with 10% FCS, and after that, in defined medium for oligodendrocytes (N2B3). After 5 days, the MEK inhibitor PD098059 – PD – (10µM) was added for 30min, 24h or 48h. Cells were then fixed and immunoreacted using anti-NG2 and anti-CNPase antibodies. The number of NG2+ cells was submitted to repeated measures ANOVA. *TREATMENT* (CONTROL or PD) was considered the between-subjects factor and *TIME* (30min, 24h, 48h) was considered the within-subjects factor.

Results: A *TREATMENT X TIME* interaction ($F=5.0$; $df=2$; $p=0.039$) was observed. Post Hoc analysis indicated that control cultures presented a reduction in the number of NG2+ cells ($F=7.28$; $df=2$; $p=0.046$) that was not observed in PD cultures ($F=1.03$; $df=2$; $p=0.434$). Regarding morphology, control cultures displayed multibranched oligodendrocytes while, in PD cultures, cells displayed less extensive processes. The CNPase+ rounded cells observed in PD cultures did not present NG2, suggesting that the morphological changes are also due to cytoskeleton rearrangement.

Conclusions: The present data corroborate the findings indicating that ERK plays a role in oligodendroglial differentiation. Our findings also showed an effect of the ERK pathway in the elongation of oligodendroglial processes, with possible consequences for the formation of the myelin sheath.

Palavras-Chave: oligodendroglial differentiation; MAPK/ERK pathway; NG2; CNPase

Apoio Financeiro: CNPq, FAPERJ, SR-2/UERJ

LYSOPHOSPHATIDIC ACID INCREASES PROGENITOR CELLS IN THE CEREBRAL CORTEX DURING POSTNATAL DEVELOPMENT *IN VIVO*

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Objectives: During postnatal development, neural progenitors in the subventricular zone (SVZ) give rise to glia and neuroblasts. Glial cells migrate radially out of the SVZ into the overlying white matter and cerebral cortex to develop into astrocytes and oligodendrocytes. Lysophosphatidic acid (LPA) is a signaling molecule that is generated by phosphodiesterase-1a/autotaxin, which is expressed predominantly in the central nervous system and is found in the cerebral spinal fluid (CSF). *In vitro* studies have shown that LPA promotes transient process retraction of migratory neural cells. Therefore LPA could act as a non-permissive or even repulsive extracellular cue for glial cells migrating radially out of the SVZ. The aim of this study was to investigate the role of LPA in glial cell migration from SVZ to the cerebral cortex *in vivo*.

Methods: To assess the *in vivo* functional role of LPA on cell migration, intraventricular infusion of 5 mL of 1 mM LPA or bovine serum albumin (0,004%) was performed in 4-day-old rats, following by an intraperitoneal injection of bromo-2-deoxy-5-uridine (BrdU; 60 mg/Kg). 48h later, animals were sacrificed, fixed and 12µm coronal cryosections were processed for immunohistochemical identification of BrdU. BrdU-positive (BrdU⁺) cells were quantified in the SVZ (arbitrarily divided in dorsal, lateral and medial regions). At least three sections per animal (3 animals per condition) were analyzed within the middle-third of the cortex.

Results: LPA showed tendency for reduction of BrdU labeled cells in the lateral SVZ (12%, p<0,06) and significantly reduced BrdU labeling in dorsal SVZ (38,8%, p<0,05). Decrease of BrdU⁺ cells in the SVZ was followed by increase of cell labeling in the cortex (58,4%, p<0,01), but no changes were observed in the white matter. Surprisingly, LPA increased (~50%, p<0,05) the number of BrdU⁺ cells in the medial SVZ which is considered to have low proliferative activity.

Conclusion: These results suggest that increased levels of LPA in the CSF may accelerate glial migration to the cerebral cortex, the principal cell type translocating from the SVZ at this age.

Palavras-Chave: Lysophosphatidic acid, glia migration, postnatal development, SVZ, córtex

Apoio Financeiro: PRONEX-FAPERJ, CAPES, NIH-FIRCA

EFFECTS OF PROTEIN RESTRICTION ON ADULT RAT NEUROGENESIS

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Introduction and objectives: Neurogenesis persists on adulthood of mammals in the subventricular zone (SVZ) of lateral ventricles (LVs) and in the hippocampal dentate gyrus. It has been reported that dietary restriction is able to modulate neurogenesis. Insults during critical periods of the development can cause significant changes in structures and body functions that affect adult life (metabolic programming). Our group has already demonstrated that protein restriction during gestation and lactation increases the SVZ stem cells/progenitors proliferation in 21 days-old rats and decreases the SVZ neurogenesis in 90 postnatal days-old (P90) rats. This study aims to observe whether protein restriction affected cells in SVZ are the slow cycle ones (B cells and/or endymary cells).

Materials and methods: After mating female Wistar rats were divided into two groups: Control group (CG) that receive a 20% protein diet and protein restriction group (PRG) with 8% protein diet during gestation (21 days) and lactation (21 days). At 21 days of age, offsprings received a control diet until P90. For neurogenesis investigation rats received 4 injections of 5-bromodeoxyuridine (BrdU) in P90 and were perfused with paraphormaldehyde 4% at P120. The encephalon were cryosectioned and processed for imunohistochemistry (anti-BrdU). BrdU+ cells in the SVZ of the lateral, medial and dorsal walls of LVs were counted using optic microscope equipped with fluorescence optics.

Results: The average number of BrdU+ cells/mm of lateral wall of SVZ was significantly lower in PRG (3.51 ± 0.40 ; n=3) than CG (7.01 ± 0.29 ; n=3). The average number of BrdU+ cells/mm of medial wall of the SVZ was also significantly lower in PRG (0.57 ± 0.05 ; n=3) than CG (1.27 ± 0.21 ; n=3). Both groups have a similar number of BrdU+ cells/mm in dorsal wall of the SVZ.

Conclusion: Protein restriction reduced the number of slow cycle cells (B cells and/or endymary cells) around the lateral ventricles on adult rats under metabolic programming. This reduction on neural stem cells pool could cause olfactory deficits and/or decrease capacity for cerebral microinjuries regeneration.

Palavras-Chave: Protein restriction; Malnutrition, Neurogenesis; SVZ

Apoio Financeiro: CAPES; FAPERJ; CNPq

PARTICIPAÇÃO DA VIA PI3K/AKT/TOR NO EFEITO PROLIFERATIVO DE NUCLEOTÍDEOS EM CÉLULAS DE RETINA EM CULTURA

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ATP e ADP são capazes de induzir a proliferação de células de retina de galinha em cultura, através da ativação de PKC e MAP cinases¹. Atualmente, a via PI3K/Akt/TOR tem sido apontada como importante mediadora de eventos proliferativos durante o desenvolvimento. Neste trabalho evidenciamos a participação desta via na proliferação celular induzida por nucleotídeos em culturas de células de retina embrionária de galinha.

Culturas de embriões com 7 dias mantidas por 24h (E7C1) na presença dos nucleotídeos ATP ou ADP juntamente com o inibidor da PI3K, LY 294002, apresentaram incorporação de [³H]-timidina ($ATP = 147 \pm 7,9\%$; $Ly+ATP = 73 \pm 27,85\%$, $n=3$) e expressão de Ciclina D1 ($ADP = 13.853 \pm 735,8$; $Ly+ADP = 9.651 \pm 408,5$, $n=4$) bastante reduzidas quando comparadas a culturas tratadas apenas com os agonistas purinérgicos, o que indica a participação da via PI3K/Akt neste efeito proliferativo. Em ensaios de imunoblot, observou-se que ATP e ADP induziram a fosforilação da proteína Akt em culturas. A estimulação foi tempo dependente, apresentando efeito máximo em 5 min de incubação e diminuindo nos tempos subsequentes. Rapamicina, inibidor do complexo TOR bloqueou o aumento da incorporação de [³H]-timidina induzido por ATP, sugerindo a participação deste complexo no efeito mitogênico deste nucleotídeo ($ATP = 154,5 \pm 7,0\%$, $ATP+Rapa = 105,1 \pm 7,98\%$, $n=3$). A análise da marcação por imunofluorescência para o fator 4EBP-1 fosforilado (p4EBP1), um dos principais alvos do complexo TOR, revelou células positivas em culturas de E7C1. Um discreto aumento no número de células marcadas para p4EBP1 nas culturas tratadas com ADP por 5 min foi observado em relação as culturas controle. A marcação por imunofluorescência para p4EBP-1 mostrou que células marcadas apresentavam um perfil de marcação nuclear (DAPI) característico de células em mitose, tanto em culturas quanto em retinas intactas de embriões de 8 dias. Esses resultados sugerem que a sinalização mitogênica induzida por nucleotídeos em retina embrionária de galinha é mediada por PI3K/Akt, TOR e p4EBP1. Referências:

¹ Intl. J. Dev. Neurosci. 20:21, 2002.

Palavras-Chave: ATP; proliferação; PI3K/Akt; TOR

Apoio Financeiro: CNPq, CAPES, FAPERJ, Propp-UFF

EFFEITO DIFERENCIAL DAS IL-2 E IL-4 NA SOBREVIDA DE CÉLULAS GANGLIONARES DA RETINA E EXPRESSÃO DE HEPARAN SULFATO *IN VITRO*.

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Objetivos: Durante o desenvolvimento do sistema nervoso a proliferação, sobrevivência e diferenciação de células neurais são alteradas pela presença distinta temporal e espacialmente de citocinas (*e.g.* neurotrofinas e interleucinas), e moléculas da matriz extracelular (*e.g.* glicosaminoglicanos). Nossos objetivos foram verificar: (1) o potencial neurotrófico das interleucina (IL)-2 e IL-4 na sobrevivência de células ganglionares (RGC); (2) a relação entre a sobrevivência das RGC induzida por IL-2 e IL-4 e a expressão de heparan sulfato (HS).

Métodos: Explantes (1mm²) de retina de ratos neonatos da linhagem Lister Hooded foram mantidos em meio de cultura completo (BME + 5% soro fetal bovino + 20mg/mL glutamina + antibióticos) por dois ou cinco dias *in vitro* (DIV) na presença ou ausência de IL-2 (50U/mL) ou IL-4 (5U/mL), heparitinase (enzima que degrada HS) e inibidores de específicos de vias de sinalização. Secções (10µm) dos explantes de retina foram utilizadas para se avaliar o efeito das ILs na sobrevivência de RGC. Foram contados o número total de células presentes na camada de RGC após coloração do tecido com vermelho neutro e o número específico de RGC marcadas retrogradamente com rodamina dextran (injeção nos colículos superiores) em microscopia de fluorescência. A expressão de HS foi verificada por imunohistoquímica.

Resultados: O tratamento com IL-2 e IL-4 por 2 DIV bloqueou 100% da morte de RGC. Após 5 DIV, o bloqueio foi de aproximadamente 60% (IL-2) e 80% (IL-4) na morte das RGC. Ambas ILs aumentaram significativamente a expressão de HS, mas em padrões temporais distintos. A expressão de HS induzida por IL-2 foi máxima em 2DIV, enquanto que aquela induzida pela IL-4 foi máxima em 5 DIV. O Pré-tratamento com heparitinase bloqueou completamente o efeito apenas da IL-2 na sobrevivência de RGC após 5 DIV.

Conclusão: Nossos resultados sugerem que a sobrevivência de RGC após 5 DIV induzida por IL-2, mas não por IL-4, é dependente da presença inicial de HS no tecido retiniano e que estas ILs são capazes de modular a expressão de HS *in vitro*.

Palavras-Chave: Retina, células ganglionares, interleucinas, heparan sulfato, sobrevivência

Apoio Financeiro: CNPq, FAPERJ e CAPES.

A.01.058

Atividade neuroprotetora do composto FrPbAII isolado da peçonha da aranha *Parawixia bistriata* em ratos submetidos a um modelo de glaucoma experimental agudo.

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Objetivos: Verificar o efeito neuroprotetor da FrPbAII injetada via intra-vítreo (i.v) em ratos Wistar submetidos ao glaucoma agudo.

Material e Método: Utilizou-se ratos Wistar, injetados por via i.v de salina, FrPbAII (0,03; 0,075 e 0,15 µg/µL), ácido nipecótico (NIPEC; 12µg/5µL), FrPbAII+NIPEC; FrPbAII+bicuculina; FrPbAII+picrotoxina e FrPbAII+imidazol. Após 15 min foi induzida a isquemia retiniana. Metade dos ratos foi submetida à isquemia por 45 min, enquanto que o restante teve a pressão intra-ocular reduzida aos níveis normais por 15 min. Em seguida os ratos foram eutanasiados.

Resultados: A análise quantitativa mostrou diferenças significativas entre os tratamentos com relação à densidade de células para os animais isquêmicos: camada nuclear externa (CNE) [F(9,29)=18,64; p=0,0001]; camada nuclear interna (CNI) [F(9,29)=10,96; p=0,0001] e camada de células ganglionares (CCG) [F(9,29)=46,80; p=0,0001], e para os animais isquêmicos com reperfusão: CNE [F(9,29)=28,05; p=0,0001]; CNI [F(9,29)=27,16; p=0,0001] e CCG [F(9,29)=46,80; p=0,0001]. Os ratos tratados com a FrPbAII (0,15 µg/µL) e NIPEC tiveram proteção das células das camadas CNE e CNI, enquanto a CCG dos ratos tratados com FrPbAII (0,15 e 0,075 µg/µL) e NIPEC apresentou maior densidade de células sobreviventes. A análise histopatológica das retinas isquêmicas com e sem reperfusão revelou lesões do tipo vacuolização citoplasmática, núcleos picnóticos e desorganização das camadas. Com relação à densidade de células das retinas isquêmicas com reperfusão, observou-se diferenças significativas entre os tratamentos da CNE, CNI e CCG quanto a vacuolização citoplasmática e núcleos picnóticos.

Conclusão: A FrPbAII protegeu as células das três camadas retinianas, enquanto o NIPEC protegeu as camadas CNE e CCG. Além disso, a associação de doses menores da FrPbAII (0,03 µg/µL) e NIPEC (6 µg/µL) não inibe a perda celular em nenhuma das camadas, sugerindo competição entre os compostos. Além disso, os pré tratamentos com antagonistas GABAérgicos bicuculina, picrotoxina e imidazol inibe a ação neuroprotetora da FrPbAII nas três camadas.

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Palavras-Chave: Glaucoma, *Parawixia bistriata*, GABA, FrPbAII, ácido nipecótico

Apoio Financeiro: Cnpq, FAPESP

A.01.059

Efeitos da ingestão da semente de linhaça (*Linum usitatissimum*) desde o período intra-uterino sobre a massa cerebral pós natal e início da vida adulta

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Objetivo: A semente de linhaça é uma oleaginosa rica em ácido graxo alfa-linolênico, precursor do ácido docosahexaenóico essencial para o desenvolvimento do cérebro. Nosso objetivo foi avaliar os efeitos da semente como fonte exclusiva de lipídeos e fibras na dieta materna, durante a pré-concepção, gestação e lactação na massa cerebral dos filhotes ao nascer e aos 52 dias de vida. **Métodos:** Foram utilizados ratos Wistar fêmeas, obtidas após o desmame que foram divididos em 3 grupos conforme a ração: Grupo Controle (GC) e Grupo Linhaça (GL), recebendo ração *ad libitum* e Grupo Caseína Modificada (GCM) recebendo ração no sistema de *Pair feeding* com o GL. As fêmeas acasalaram aos 90 dias e após o parto, as ninhadas foram ajustadas para 6 filhotes que foram aleitados e, após o desmame, receberam a mesma ração das mães até os 52 dias quando foram sacrificados para avaliação da massa cerebral assim como o excedente às ninhadas ao nascer. Todos tiveram a massa corporal aferida durante todo o período. **Resultados:** A massa corporal dos filhotes ao nascer foi inferior no GL ($5,00 \pm 0,33$ g) em relação ao GC ($5,45 \pm 0,33$ g) e GCM ($5,45 \pm 0,33$ g). No entanto, a massa cerebral dos animais do GL ($0,26 \pm 0,02$ g) foi superior ao GC ($0,18 \pm 0,01$ g) e GCM ($0,19 \pm 0,01$ g). A massa cerebral relativa do GC ($3,38 \pm 0,11$ g) e do GCM ($3,57 \pm 0,07$ g) foram inferiores ao do GL ($5,14 \pm 0,34$ g). Aos 52 dias, o GL ($122,5 \pm 7,76$ g) teve sua massa corporal equivalente ao GCM ($12,38 \pm 7,73$ g), mas ambas inferiores ao GC ($185,25 \pm 11,36$ g). Porém, a massa cerebral do GL ($1,72 \pm 0,09$ g) foi igual ao GC ($1,98 \pm 0,40$ g) e superiores ao GCM ($1,61 \pm 0,03$ g). Portanto, a massa cerebral relativa do GL ($1,41 \pm 0,08$ g) foi semelhante ao GCM ($1,34 \pm 0,07$ g), mas inferior ao GC ($1,09 \pm 0,23$ g). **Conclusão:** O consumo de semente de linhaça do período intra-uterino aos 52 dias confere maior massa cerebral no início da vida provavelmente devido a maior incorporação dos AG da ração, no entanto, o maior teor lipídico do GCM também provocou um maior crescimento cerebral com relação ao GC.

Palavras-Chave: ácidos graxos, DHA, crescimento cerebral, dieta

A.01.060

A ingestão de semente de linhaça (*linum usitatissimum*) durante a pré-concepção, gestação e lactação por ratas favorece a incorporação de ácidos graxos omega-3 no leite materno e no cérebro da prole e altera seu crescimento

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Objetivo: A composição de ácidos graxos da dieta materna tem sido diretamente associada ao desenvolvimento do sistema nervoso do feto e do recém-nato. O ácido graxo da série n-3 mais importante para o cérebro é o ácido docosahexaenóico (DHA) derivado do ácido alfa-linolênico (ALA), que está presente na semente da linhaça. Nosso objetivo foi avaliar a ação da semente de linhaça como fonte exclusiva de lipídeos e fibras na dieta materna, durante a pré-concepção, gestação e lactação na distribuição percentual de ácidos graxos cerebrais e no crescimento da prole. **Métodos:** Foram utilizados ratos Wistar fêmeas, obtidas após o desmame (21 dias) que foram divididos em três grupos conforme a ração: Grupo Controle (GC) e Grupo Linhaça (GL), recebendo ração *ad libitum* e Grupo Caseína Modificada (GCM) recebendo ração no sistema de *Pair feeding* com o GL. As fêmeas acasalaram aos 90 dias e após o parto, as ninhadas foram ajustadas para 6 filhotes sendo excedente sacrificado para análises de ácidos graxos cerebrais por cromatografia gasosa. Todos tiveram a massa corporal monitorada durante o período experimental. **Resultados:** Aos 21 dias, o GL apresentou crescimento inferior ($38,25 \pm 5,71$ g) ao GC ($46,92 \pm 6,73$ g) e equivalente ao GCM ($39,67 \pm 3,79$ g) provavelmente em função da presença de fatores antinutricionais e do desequilíbrio entre os ácidos graxos das séries n-3 e n-6 da semente, que inibiu a síntese de ácido araquidônico (AA). O perfil de AG cerebrais mostrou eficiência na conversão do ALA da ração em DHA no GL ($14,33 \pm 0,25$ g) em relação ao GC ($10,41 \pm 0,06$ g) e GCM ($10,85 \pm 0,53$ g). **Conclusão:** A adição de semente de linhaça na dieta materna levou à maior incorporação de ácidos graxos da série n-3 no cérebro dos filhotes no entanto a síntese de AA foi prejudicada provocando déficit no crescimento **Palavras-Chave:** leite, omega-3, ácidos graxos cerebrais, dieta

DISTRIBUIÇÃO DE NEURÔNIOS IMUNOREATIVOS À TRIPTOFANO HIDROXILASE NO TRONCO ENCEFÁLICO E DIENCÉFALO DE POMBOS (*Columba livia*).

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Embora o sistema serotoninérgico de aves tenha sido descrito através de imuno-histoquímica (IHQ) para serotonina (5-HT), a distribuição de neurônios contendo triptofano hidroxilase (TPH, 1.14.16.4, enzima limitante na síntese de 5-HT e melatonina) ainda não foi investigada em aves. Neste trabalho descrevemos a distribuição da TPH no tronco encefálico e diencéfalo de pombos. Cinco pombos adultos foram perfundidos e cortes (40 µm) do tronco encefálico e diencéfalo foram ensaiados por IHQ para revelação da TPH. No bulbo, pericários imunoreativos (TPHir-p) concentram-se na linha média em 2 subnúcleos (ventral e dorsal) e no n. do trato solitário (subnúcleos LDp, mSp e tela coróideia). TPHir-p intensamente marcados povoam a região dorsolateral ao fascículo longitudinal medial (zpFLM) e o 1/3 rostral da formação reticular parvocelular ventrolateral ao longo do bulbo e da ponte. Nesta última, os TPHir-p concentram-se na zpFLM, locus ceruleus (LoC) e n. subceruleus dorsal (SCd), com um pequeno número na rafe ventral. Poucos TPHir-p são observados espalhados pela formação reticular magnocelular pontomesencefálica. No mesencéfalo aumenta a densidade de TPHir-p na zpFLM, LoC, SCd e área tegmental ventral. Medialmente, grande número de TPHir-p são notados no n. linearis caudalis e n. annularis. No hipotálamo TPHir-p concentram-se no n. periventricular hipotalâmico, n. medial hipotalâmico posterior e estrato celular interno. O n. pré-mamilar (PMM) contém grande quantidade de TPHir-p densamente marcados. No tálamo há TPHir-p no n. dorsomedial anterior talâmico e n. superficial parvocelular. Fibras contendo varicosidades são visualizadas no epêndima do assoalho ventricular e aquedutal e concentram-se na camada ependimária da face ventromedial do bulbo e da ponte. No diencéfalo são visualizadas no epêndima e PMM. Conclui-se que corpos celulares contendo TPH são localizados em toda a rafe e em núcleos do tronco encefálico e diencéfalo. Grupos de TPHir-p são encontrados no tronco encefálico em regiões imunoreativas à 5-HT (5-HTir); no diencéfalo, TPHir-p são encontrados em diversos núcleos não 5-HTir, sugerindo que estejam relacionados, por exemplo, com a síntese de melatonina.

Palavras-Chave: Triptofano hidroxilase, tronco encefálico, diencéfalo, melatonina, pombos

Apoio Financeiro: CAPES

A.02.002

A constant ratio between total neuronal and glial mass in mammalian brains: Consequences for the Scaling of average neuronal cell mass

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The glia/neuron ratio is widely believed to increase with brain size. We have shown, however, that this ratio only increases when average neuronal cell size increases with brain size, such as in rodents (Herculano-Houzel et al., 2006), but remains approximately constant when average neuronal cell size does not increase with brain size, as is the case with primates (Herculano-Houzel et al., 2007). Instead, and despite the manifest lack of a universal correlation between the glia/neuron ratio and brain size, we show that the ratio between total neuronal and total non-neuronal mass in the brain remains approximately constant across species and brain structures.

The development of the isotropic fractionator made feasible the precise measurement of total numbers of neuronal and non-neuronal cells in the brain, or any part thereof. In particular, we have shown that, for eight species of rodents and eleven species of primates, the mass of various brain structures scale as similar power laws of the numbers of glia in the structures, which results in an approximately constant density of glia per unit mass across all structures and species. The residual variation in glial number, on the other hand, is not correlated with structure mass. This strongly suggests that the average mass of glial cell is essentially invariant across species and structures.

Assuming therefore that average glial cell size is invariant, here we show that the ratio between total neuronal and glial mass (defined as the product of the number of cells by the average cell size) remains the same, not only across brain structures, but also across species of primates and rodents alike (in practice, one should expect deviations commensurate with the variance of the glial density ratio). This in turn has significant consequences for the modeling of brain structure, as one may assume the existence of a neuronal and glial unit quantum as the fundamental building block of all brain matter.

Also, this invariance allows us to calculate the ratio between the average neuron mass in different species and between different structures. We show that the average neuron mass follows a power law of total brain or structure mass, varying by as much as a factor of 8 in rodent brains, but remaining approximately constant in primates.

Palavras-Chave: Neuron/glia ratio, comparative neuroanatomy, scaling laws, isotropic fractionator

Apoio Financeiro: FAPERJ, CNPq

A.02.003

Shared cellular scaling rules for rodent and primate olfactory bulb

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Comparative studies aim at identifying the rules relating the size of the brain and its structures to the numbers of neuronal and non-neuronal cells that compose them. We have demonstrated that while the size of the primate brain, excluding the olfactory bulb, is a linear function of its number of neurons (PNAS 104(9):3562, 2007), the rodent brain grows in size with the number of neurons raised to the power of 1.7, which indicates that average neuronal size increases with number of neurons (PNAS 103(32):12138, 2006). The difference suggests that the cerebral cortex, cerebellum and remaining areas of primate and rodent brains are built according to cellular scaling rules that have diverged from their common ancestor.

Since the olfactory bulb is considered to be an evolutionarily old structure, preceding the appearance of a well-developed mammalian cerebral cortex, we set out to investigate whether the scaling rules for the olfactory bulb differ between rodents and primates or are conserved across these orders.

We applied the isotropic fractionator (J Neurosci 25(10):2518, 2005) to examine the cellular composition of the olfactory bulbs of a total of 65 animals of 7 rodent species, 6 primate species and one species of the order scandentia varying in bulb size from 0,023g in the hamster to 0,651g in the capybara and from 0,008g in the marmoset to 0,100g in the galago.

Although primate olfactory bulbs differ from rodent bulbs in that their mass does not increase with brain mass (raised to the power of 0,8 in rodents; $p=0,002$), we show that olfactory bulb mass is a function of its number of neurons regardless of brain size or order, increasing across rodents, primates and the scandentia species with the number of neurons raised to the power of 1.177 (rodents only, 1.129, $p=0,0067$; primates only, 0.962, $p=0,027$; all animals, $p<0,0001$) and with the number of non-neurons raised to the power of 1.070 (rodents, 1.050, $p=0,0005$; primates, 0.980, $p=0,0213$; all animals, $p<0,0001$).

The shared scaling rules suggest that, despite the evolutionary divergence between rodents and primates in the scaling of the cerebral cortex and cerebellum, the olfactory bulb scales with a conserved pattern that is consistent with its being an evolutionarily older brain structure whose construction rules have remained unchanged in mammalian evolution at least since the divergence between the rodent and primate lineages.

Palavras-Chave: evolution, cortex, comparative neuroanatomy, allometry

Apoio Financeiro: CNPQ e Faperj

A.02.004

Cellular scaling rules for an extended number of primate species

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What are the rules relating the size of the brain and its structures to the number of cells that compose them and their average sizes? We have shown previously that the cerebral cortex, cerebellum and the remaining brain structures increase in size as a linear function of their numbers of neurons and non-neuronal cells across six species of primates (Herculano-Houzel et al., 2007). Here we investigate whether the same rules apply to other species of primates or depend on the particular species sampled. Total number of cells and neurons in the cerebral cortex, cerebellum, and remaining areas of the brain were determined with the Isotropic Fractionator (Herculano-Houzel and Lent, 2005) in adult mouse lemur, Goeldi's marmoset, long-tailed monkey, bonnet monkey and baboon. The numbers obtained were compared to the values expected from the cellular scaling rules found for the previous sample of six primate species. Values for all 11 primate species were then combined to examine whether the scaling rules for the extended sample are similar to those determined previously.

We find that the cellular composition of each of the present five species departs from the expected for structure mass typically by at most $\pm 20\%$, within the same range of variation found for the six species from which the cellular scaling rules were obtained. This finding suggests that the cellular composition of the brain structures of the current five species conforms to the scaling rules identified previously for a different set of species.

In line with this observation, we find that the updated power functions for the extended sample are similar to those determined earlier. The cerebral cortex, cerebellum and the remaining structures scale linearly as a function of their numbers of cells, neurons, and non-neuronal cells. This conformity strongly suggests that, in contrast to the allometric rules relating body and brain size, which are very sensitive to the particular species sampled, the cellular scaling rules we have identified apply to primates in general, and not only to particular subgroups of primate species.

Palavras-Chave: number of neurons ; brain size ; allometry ; evolution ; scaling

Apoio Financeiro: FAPERJ Jovem Cientista do Estado (SHH); CNPQ-Edital Universal (SHH)

Coordinated scaling of primate hippocampus and cerebral cortex

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The hippocampus is widely believed to serve an associative function with the neocortex, to which it is densely connected. These two structures have been shown to scale in mass coordinately, as hippocampal mass increases across primate species as a function of neocortical mass raised to the power of 0.61 (Stephan, Bauchot and Andy, 1970). We have shown previously that the primate cerebral cortex increase in size as a linear function of its number of neurons (Herculano-Houzel et al., 2007). Here we investigate how the number of neurons in the primate hippocampus scales with the number of neurons in the remainder of the cortex.

Total number of neurons in the hippocampus and remainder of the cortex (hereafter called “cortex”) were determined with the Isotropic Fractionator (Herculano-Houzel and Lent, 2005) for 30 individuals of eight primate species (*Microcebus* sp., *Galago moholi*, *Callithrix jacchus*, *Otolemur garnettii*, *Aotus trivirgatus*, *Macaca mulatta* and *Papio* sp.). Power functions describing relationships between absolute mass, relative mass and numbers of cells were then computed for hippocampus and cortex and across the two of them.

We find that hippocampal mass varies as a function of the number of neurons in the structure raised to the power of 1.239, and increases linearly with its number of non-neuronal cells. Cortical mass varies coordinately with hippocampal mass, increasing as a function of hippocampal mass raised to the power of 2.020. Although the hippocampal neuronal scaling rules seem to depart slightly from linearity, the number of cortical neurons accompanies the number of neurons in the hippocampus raised to the power of 1.969, similar to the mass relationship between these structures. In line with the faster increase in the number of neurons in the cortex than in the hippocampus, the relative number of cells in this structure compared to the cortex decreases from 51% to 1% with increasing brain size in our sample.

The finding that both cortical mass and number of neurons are related to hippocampal mass and number of neurons by an exponent of 2.0 suggests a combinatorial relationship in which pairwise associations made by N hippocampal neurons are stored in a network of cortical neurons that grows in number of neurons and mass with N^2 . **Palavras-Chave:** hippocampus ; cortex ; allometry ; brain scaling ; number of neurons

Apoio Financeiro: FAPERJ Jovem Cientista do Estado (SHH); CNPQ-Edital Universal (SHH)

AVALIAÇÃO DA SAZONALIDADE SOBRE A LIPOPEROXIDAÇÃO NO SISTEMA NERVOSO CENTRAL DE *Rana catesbeiana*

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Objetivos: As rãs são excelentes modelos experimentais em estudos fisiológicos e farmacológicos. Nestes animais, o metabolismo da glicose sofre modificações nas diferentes estações do ano. A atividade de algumas enzimas antioxidantes também é sazonalmente alterada. Com o intuito de trazer subsídios ao entendimento dos efeitos das diferentes estações do ano sobre o metabolismo oxidativo de rãs, este estudo apresenta o perfil de lipoperoxidação da medula espinal e do encéfalo de rãs coletadas nas estações do inverno, primavera e verão. **Métodos e Resultados:** Foram utilizadas 10 rãs *Rana catesbeiana*, adultas, machos, que foram sacrificados por decapitação nas estações do inverno, primavera e verão para a obtenção do encéfalo e da medula espinal. Após homogeneização em solução de KCl 1,15%, estes tecidos foram processados de acordo com a técnica para quimiluminescência descrita por Gonzalez-Flecha (Free Radic Biol. Med. 10:93,1991). A determinação das proteínas foi feita pelo método de Lowry. Os dados obtidos foram analisados por ANOVA de uma via. A lipoperoxidação no verão foi significativamente menor do que aquela obtida no inverno, tanto no encéfalo como na medula espinal. Os valores encontrados nas três estações foram os seguintes (expressos em cps/mg de proteína - média±erro padrão): inverno: [encéfalo (1926,57±388,96); medula espinal (1466,832±152,4773)]; primavera: [encéfalo (2739,51±314,35); medula espinal (2302,36±519,46)]; verão: [encéfalo (832,95±234,44); medula espinal: (219,23±22,79)]. **Conclusão:** Os valores mais baixos de lipoperoxidação do tecido nervoso de rãs foram encontrados durante o verão, enquanto os maiores ocorreram durante a estação da primavera. Neste período as rãs estão emergindo de um estado de quiescência, começam a repovoar suas reservas energéticas e a se prepararem para a reprodução, a qual ocorre no verão. É possível que o baixo valor de lipoperoxidação obtido no verão seja resultado de uma maior proteção antioxidante, já que está ocorrendo maior atividade metabólica. Assim, estudos complementares estão sendo realizados para o esclarecimento desta hipótese.

Palavras-Chave: lipoperoxidação; medula espinal; encéfalo; rã

Apoio Financeiro: FAPERGS, CNPq

Efeitos ingestivos de injeções locais de agonistas e antagonistas de receptores NMDA e AMPA-Kainato no n. Teniae da amígdala (TnA) de pombos (*Columba livia*).

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Introdução: Mecanismos motivacionais ligados ao comportamento alimentar parecem ser controlados por núcleos do complexo amigdalóide (CA) em mamíferos. Aferentes glutamatérgicos e projeções dos núcleos central e basolateral do CA para o hipotálamo lateral parecem exercer papel fundamental sobre aspectos motivacionais da ingestão de alimentos. Em aves, a região provavelmente homóloga ao núcleo medial do CA de mamíferos é o TnA. **Objetivo:** Investigar o envolvimento de receptores NMDA e AMPA-kainato no núcleo TnA no controle do comportamento ingestivo em pombos. **Material e Métodos:** Foram utilizados pombos adultos (300-400 g) portando cânulas-guia implantadas cronicamente 1 ou 2 mm acima do TnA (N=6 para cada tratamento), com água e alimento *ad libitum*, e tratados com ácido N-methyl-D-aspartico (NMDA, 1 nmol), ácido kaínico (KA, agonista de receptores AMPA-kainato, 0,5 nmol), MK-801 (doses de 1,8 e 6,0 nmol – antagonista de receptor NMDA), DNQX (0,79 e 2,8 nmol – antagonista de receptor AMPA-kainato) ou líquido artificial (200 nl); em estudos anteriores, as doses usadas foram efetivas em alterar o comportamento ingestivo de pombos quando injetadas no hipotálamo lateral. Durante 1h após a injeção foram registradas a latência para o primeiro episódio, a frequência e a duração de comportamentos ingestivos (água e alimento) e não- ingestivos (auto-limpeza, locomoção, imobilidade alerta, exploração do ambiente e sono). A quantidade de alimento e água ingeridos foi registrada 1, 2, 3 e 24 h após a injeção dos agonistas. **Resultados:** As injeções de NMDA e KA no núcleo TnA, não alteraram a ingestão de comida e água na 1^a, 2^a, 3^a e 24 horas após a administração quando comparada ao controle. Da mesma forma os comportamentos não ingestivos não sofreram modificação após a injeção de NMDA e KA. As injeções de MK-801 e DNQX nas diferentes doses também não modificaram o comportamento ingestivo de alimento e água na 1^a, 2^a, 3^a e 24 horas após a injeção. **Conclusão:** Estes resultados sugerem que circuitos glutamatérgicos do TnA não participam do controle de comportamentos ingestivos de alimento e água em pombos. **Apoio Financeiro:** FAPESC

Palavras-Chave: Glutamate, Feeding behavior, Amygdalid complex, avian

Apoio Financeiro: FAPESC

EFEITOS HIPERFÁGICOS E DIPSOGÊNICOS DO BLOQUEIO DE RECEPTORES 5-HT₁₋₂ E DA ATIVAÇÃO DE RECEPTORES 5-HT_{1d} NO N. TAENIAE DA AMÍGDALA (TnA) DE POMBOS (*Columba livia*).

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Introdução: Em mamíferos, a injeção local de metergolina (MET, antagonista 5-HT₁₋₂) no n. posterior medial do complexo amigdalóide (CA) provoca hiperfagia, sugerindo que Circuitos serotoninérgicos participam de mecanismos tônicos de controle da ingestão de alimentos nesta região. Em aves, a região provavelmente homóloga ao núcleo medial do CA de mamíferos é o TnA; neste trabalho, examinamos mas seu envolvimento no controle de comportamentos ingestivos. **Material e Métodos:** Foram utilizados pombos adultos (300-400 g) portando cânulas-guia implantadas cronicamente 1 ou 2 mm acima do TnA (N=6-9 por tratamento), com água e alimento ad libitum, e tratados com MET (6 ou 20 nmol), GR46611 (GR, um agonista 5HT_{1D}, 2 ou 6 nmol) ou veículo (ac. ascórbico 1%, 200 nl). Durante 1h após a injeção, foram registradas a latência para o primeiro episódio, a frequência e a duração de comportamentos ingestivos (água e alimento) e não ingestivos (auto-limpeza, locomoção, imobilidade alerta, e sono). Uma, 2, 3 e 24 h após a injeção foram quantificados alimento e água ingeridos. **Resultados:** Injeções de MET e GR nas doses mais altas (20 e 6 nmol, respectivamente) no TnA aumentaram a ingestão de alimentos na 1ª h de registro e no total acumulado em 3 h, mas não em 24 h após o tratamento. A hiperfagia induzida por essas drogas foi acompanhada por uma redução na latência para o primeiro episódio de comer (sem modificação na duração dos episódios). A ingestão de água aumentou após injeções de MET (20 nmol) e GR (6 nmol) na 1ª, 2ª e 3ª hs. Os efeitos dipsogênicos da MET e GR no TnA persistiram na ingestão acumulada em 24 h, e foram associados a maior frequência de beber. **Conclusão:** Estes dados indicam que circuitos serotoninérgicos no TnA atuam de forma tonicamente inibitória sobre comportamentos ingestivos em pombos, característica esta partilhada semelhante ao observado em mamíferos. Este controle pode contar com a participação de receptores 5HT_{1D}, cuja ativação em ratos tem mostrado efeitos opostos aos aqui observados em pombos **Palavras-Chave:** Serotonin, feeding, drinking, amygdaloid complex, avian

Apoio Financeiro: FAPESC

A.02.009

Thalamocortical connections of areas 1 and 2 in the New World cebus monkey (*Cebus apella*)

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Although dexterity is one of the landmarks of primate evolution the neural substrate of such trait remains unclear. A number of studies in Old World monkeys indicate the involvement of the parietal cortex (PC) in generating visual, somatosensory, and proprioceptive references required for the execution of visual guided movements and complex forelimb maneuvers. Amongst the New World monkeys the only identified species able to execute sophisticated hand manipulations is the *Cebus apella*.

Details of the *Cebus* PC are hitherto unknown. A cortical region named area 2, exclusively known in humans and Old World monkeys, was recently described in the *Cebus*¹. Nevertheless no information is available regarding the thalamic inputs to this area. The goals of the present study are (1) identify the thalamic relays to specific subregions of the *Cebus* PC and (2) determine the relative input contribution of each thalamic nucleus to these subregions.

Three different retrograde neurotracers were injected into the PC of a *Cebus apella* monkey, fluoro emerald (FE) into the area 1, and fluoro ruby (FR, rostral injection) and diamidino yellow (DY, caudal injection) and into the area 2 and. Following two weeks of a recovery period to allow for transport of the neuroanatomical tracers, the animal was transcardially perfused and the brain processed for histological analysis. Alternated sections were prepared for cytochrome oxidase (CO), Nissl, and fluorescence analysis. Retrograde labeled thalamic neurons found upon microscopy survey were identified and classified according to each neurotracer. Slices reacted to CO and Nissl yielded the identification of different thalamic nuclei. Overlapping CO and fluorescence slices allowed the localization of labeled neurons in each thalamic nucleus.

313 cells were identified in 14 coronal thalamic sections. Upon neurotracer injections into area 2 (DY and FR), 40% of cells labeled with DY and 36% of those labeled with FR were found in the anterior pulvinar nucleus (Pa). 36% of cells labeled with DY and 34% with FR were found in the ventral posterior superior nucleus (VPS), 1% of cells labeled with DY and 12% of cells labeled with FR were found in the ventral posterior lateral nucleus (VPL). 10% of the FR labeled cells were found in the ventral posterior inferior nucleus (VPI). The remaining labeled cells were uniformly distributed in the central nucleus (Cent), central lateral nucleus (Cel), pulvinar lateral nucleus (PuL), and lateral posterior nucleus (Lap). The injection in area 1, revealed 54% of cells in Pa, 18% in VPI and 14% in VPL. The remaining labeled cells were distributed amongst VPS, Cent and Cel.

The pattern of thalamo-cortical connections found in this study is similar to previous descriptions in other primate species and provides the anatomical basis for a comprehensive study of physiology, behavior and comparative anatomy under an evolutionary perspective.

1. Padberg J, Franca JG, Cooke DF, Soares GMJ, Rosa MGP, Fiorani M, Gattass R, Krutitzer L (2007). Parallel Evolution of Cortical Areas Involved in Skilled Hand Use. *J. of Neurosci.* 27(38):10106 –10115.

Palavras-Chave: Parietal cortex, *Cebus apella*, thalamus

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A.02.010

Cellular scaling rules for eight species from the order Rodentia

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Abstract:

What are the rules relating the size of the brain and its structures to the number of cells that compose them and their average sizes? We have shown previously that the cerebral cortex, cerebellum and the remaining brain structures increase in size as power functions of their numbers of neurons across six species of rodents (Herculano-Houzel et al., PNAS 2006), which suggests that the average neuronal size in these structures increases. Here we investigate whether the cellular composition of the brain of two additional rodent species, the Amazonian ground-dwelling spiny rat, casiragua (*Proechimys guyannensis*) and the North American grey squirrel (*Sciureus carolinensis*), conforms to the rules derived from the study of six rodent species.

The total number of cells and neurons in the cerebral cortex, cerebellum, and remaining areas of the brain were determined with the Isotropic Fractionator (Herculano-Houzel and Lent, 2005) in three adult squirrels and two adult casiraguas (average brain mass, 5.548 and 2.078 g, respectively). The numbers obtained were compared to the values predicted from the cellular scaling rules found for the previous sample of six rodent species. Values for all eight rodent species were then combined to examine whether the scaling rules for the extended sample are similar to those determined previously.

We find that the cellular composition of the casiragua is within the same range of variation found for the six species from which the cellular scaling rules were obtained, varying by ± 10 -20% from the values predicted from structure mass, similar to all rodent species examined previously. The squirrel, however, has consistently over 30% more neuronal and non-neuronal cells in every structure than expected for their mass in a rodent.

The updated power functions derived from data from all eight rodent species are similar to those based on the original six species, falling in the same 95% confidence intervals. This consistency strongly suggests that the cellular scaling rules we have identified in rodents are less variable than allometric rules relating body and brain size.

Palavras-Chave: allometry ; comparative neuroanatomy ; brain size ; number of neurons ; isotropic fractionator

Apoio Financeiro: FAPERJ- CNPQ

A.03.001

Rat bone marrow mesenchymal stem cells do not die or express neural markers in response to factors secreted by organotypic cultures of rat hippocampus submitted to oxygen and glucose deprivation

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Objectives: Mesenchymal stem cells (MSCs) derived from bone marrow stroma give rise to a variety of mesenchymal cell types, such as osteocytes, chondrocytes and adipocytes. Recent studies suggest that these cells can also transdifferentiate into cells from the neural-ectodermal lineage and because of this they could be promising for using as treatment in neurodegenerative diseases. Some differentiation protocols are very aggressive and transdifferentiation of MSC is believed by some authors to be only an artifact. The aim of this study was to investigate whether conditioned medium derived from rat organotypic hippocampal cultures with or without injury was capable to induce a neuro-glial phenotype in rat MSCs.

Methods: Rat organotypic cultures of hippocampus were submitted or not to 60 minutes of OGD and MSCs were exposed to these conditioned media for 24 or 72 hours, having no contact with the brain tissue cells. The MSCs in these experiments were seeded in a density of 30.000 cells/well and used between the 10th and 25th passages. Propidium iodide uptake and western blotting to detect caspase 3, nestin, neurofilament 68kDa, GLAST, GLT1 and b-actin were used to determine if MSC were dying or starting to express neural makers in these conditions.

Results: Propidium iodide staining (n=4; p>0,05) and caspase 3 imunoblots (n=3; p>0,05) showed that MSCs are not dying in response to the factors secreted by the hippocampus, even when the tissue is exposed to oxygen and glucose deprivation, a lesion model that mimics an ischemic event. Also, no expression of Nestin, Neurofilament 68kDa, GLT-1 and GLAST was observed in MSCs after a 72 hours-exposure to hippocampal factors with or without lesion (n=4; p>0,05).

Conclusion: Taken together, our results suggest that neuro-glial transdifferentiation in these conditions is not occurring in MSCs. **Palavras-Chave:** mesenchymal stem cells; neural markers; organotypic hippocampal culture

Apoio Financeiro: CNPq and Propesq/UFRGS

A.03.002

Experimental technique for real time monitoring of bone marrow stem cells in a rat model of Huntington's disease.

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Huntington's disease (HD) is a fatal neurodegenerative disorder. Cellular therapy using bone marrow stem cells (BMSCs) is promising in this pathology. It improves motor performance in quinolinic acid-induced rat model of HD, but little is known at the cellular level. In addition, the need to track transplanted cells in a noninvasive manner is one limitation for the clinic application of BMSCs. Here we designed an experimental technique to label BMSCs with superparamagnetic iron nanoparticles (SPIO) for posterior magnetic resonance (MR) in a rat model of HD.

Rats were injected with 2µl of quinolinic acid (QUIN) into the right striatum and saline into the left striatum. To detect cellular death in the striatum, rats were perfused 48h after QUIN injections and Fluoro-Jade-C staining was performed. The mononuclear fraction was purified by ficoll gradient and electroporated with SPIO (2mg Fe/mL). To determine cellular viability of electroporated cells, flow cytometry with propidium iodide (PI) was performed. BMSCs were injected by intra-striatal or intravenous surgery 7d after QUIN injections. The incorporation of SPIO by cells was evaluated with prussian blue reaction for iron detection and immunohistochemistry with anti-dextran.

Fluoro-Jade-C showed a well-delimited neurodegeneration in the striatum injected with QUIN but not with saline. Flow cytometry detected no differences in the viability of electroporated and non-electroporated cells. Immunohistochemistry showed iron presence in cells electroporated with SPIO. Cells electroporated without SPIO showed little fluorescence. The brains of rats with intra-striatal injections of BMSCs showed intense staining for iron, indicating the presence of cells, and the brains of rats with endovenous injections showed no vestige of iron.

The QUIN model exhibits typical cellular death in the striatum. Electroporation does not affect significantly the viability of BMSCs. There is a tendency of cells electroporated with SPIO to incorporate the nanoparticles, and local transplantation is probably the best option in HD, but additional experiments using MR are required to confirm the potential of this technique.

Palavras-Chave: Huntington's disease, bone marrow stem cells, nanoparticles

Apoio Financeiro: CNPq

A.03.003

HOMOCYSTEINE AND FOLIC ACID AFFECT MURINE NEURAL CREST CELLS *in vitro*.

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The neural crest (NC) is a transient structure of the vertebrate embryo formed by the lateral borders of the neural primordium during neurulation. The NC originates the peripheral nervous system, pigment cell of body and bones and cartilage of the head. Folate (FA) deficiencies have been associated with many adverse congenital abnormalities, particularly neural tube closure defects and neurocristopathies. However, it is not clear, whether these defects are due to a FA deficiency or to an increase in homocysteine (HC) levels neither the involvement of the NC cells.

Primary cell cultures were performed by mechanical dissection of 8.5 days mouse embryos mesencephalon. Explants were applied on plastic dishes coated with fibronectin, being removed 48 hours later. The remaining migrated cells (NC cells) were then trypsinized, reapplied on the same culture condition and cultured for additional 10 days. Cells were cultured in the complex media containing HC (0, 75, 150 or 300 uM) or HC (300uM) and FA (22.5, 45 or 90uM). The medium was changed every 3 days. The cell phenotypes were identified by immunofluorescence using the lineage-specific markers to glial cells (*GFAP*), neurons (*β -III-Tubulin*) and myofibroblasts (*SMA*). It was also analyzed the NC cell migration area, the cell proliferation (by BrdU incorporation) and the proportion of apoptotic nuclei.

We could observe that HC reduces in a dose-dependent manner the differentiation of NC to myofibroblasts, an effect that is prevented by AF. The proportion of apoptotic nuclei was insignificant in all tested condition. In addition, we verified that HC progressively increased the proliferation of NC cells, effects prevented by FA.

These results suggest an effect of the HC in the differentiation and proliferation processes of the CN cells and may be involved in congenital anomalies.

Palavras-Chave: Neural crest, homocysteine, folic acid

Apoio Financeiro: PIBIC/UFSC, CNPq, CAPES, MCT/INFRA, CNPq/PRONEX, FAPESC.

A.03.004

EFFECTS OF BONE MARROW CELLS OVER VASCULARIZATION OF REDUCED ACETYLCHOLINE-RELEASE ELDERLY MICE

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Objectives: The present study aimed to evaluate whether the transplantation of bone marrow cells alters the brain vascularization and angiogenesis in elderly wild-type (WT) or *knockdown* mice for the vesicular acetylcholine transporter (VACHT). If the brain vascularization and the angiogenic capacity decrease with age and in the presence of cholinergic deficit, then the VACHT animals must present a reduction of the vascularization and reduction in the rate of angiogenesis.

Methods: Elderly VACHT and WT mice (20-24 months, n=5) were transplanted with 1×10^6 of bone marrow cells, obtained from young EGFP mice. Elderly control animals VACHT (n=5) and WT (n=5) were treated with saline. Forty days after transplantation, animals were perfused with paraformaldehyde 4% and brain slices were processed for immunohistochemistry and immunofluorescence for laminin and GFP. Vessels of at least 0,02 μ m diameter were quantified in the whole brain in 3 levels along the longitudinal axis.

Results: The brain areas with high expression of laminin in vessels were cortex, hippocampus, septal region and hypothalamic nucleus. Animals transplanted with bone marrow cells had a significant reduction in the number of vessels when compared with saline-treated animals ($P < 0.0001$). WT group did not differ from VACHT animals concerning the effect of transplanted cells. Microvessels were present in 100% of saline-treated against 30% of cell-treated animals. We found GFP⁺ cells throughout the brain across the rostro-caudal axis in both *knockdown* and wild-type mice. Double-stained GFP⁺/laminin cells could be found in the choroid plexus and ventricular walls.

Conclusions: Our data indicate that the infusion of bone marrow cells modified the vascularization in the brain of aged animals, diminishing the number of vessels throughout the neuronal tissue. Vessels formed from transplanted cells were present in brain regions not protected from the blood-brain barrier. *Knockdown* mice for the vesicular acetylcholine transporter did not differ from WT animals in terms of vessel quantification.

Palavras-Chave: VACHT, bone marrow cells, vascularization and elderly

Apoio Financeiro: CNPq, FAPESP

A.03.005

Análise do efeito de células produtoras de fatores neuroprotetores em modelo pré-clínico da doença de Parkinson

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Objetivos: A Doença de Parkinson é caracterizada pela perda progressiva de neurônios dopaminérgicos localizados no mesencéfalo ventral e conseqüente comprometimento das funções motoras. Terapias atuais proporcionam melhora parcial dos sintomas, além de efeitos colaterais indesejados. O GDNF (*glial derived neurotrophic factor*) é reconhecido por promover neuroproteção e regeneração dos neurônios dopaminérgicos. Células adultas da polpa dentária humana produzem GDNF e atuam como um bom substrato para maturação de neurônios. Neste trabalho avaliamos (1) a expressão de diversos fatores neuroprotetores por células-tronco imaturas da polpa de dente humano (CPDh) e desenvolvemos modelo experimental para avaliar o efeito de CPDh na recuperação motora de roedores hemi-parkinsonianos.

Métodos: Sob anestesia, camundongos suíços adultos receberam injeção estereotóxica de 6-hidroxidopamina (6-OHDA) (10µg em 2,0 µL) no corpo estriado esquerdo (AP+0,5; ML+2,0; DV-3,0). A avaliação comportamental foi realizada quinzenalmente, através de injeção subcutânea de apomorfina (0,5mg/Kg), que induz os animais hemi-parkinsonianos a girarem em sentido contra-lateral à lesão. Quatro semanas após a lesão, células foram injetadas no corpo estriado dos mesmos animais. A expressão de fatores neuroprotetores pelas CPDh *in vitro* foi analisada por RT-PCR.

Resultados: Verificamos por PCR a expressão dos seguintes fatores neuroprotetores: BDNF, CDNF, GDNF, IL-6, NGF-B, NT3 e NT4/5 nas CPDh. O modelo pré-clínico murino da Doença de Parkinson foi implementado e validado na UFRJ, na qual os animais apresentam o comportamento de rotação em torno do próprio eixo após indução com apomorfina (43,9 ± 7,86 rotações/5min.).

Conclusão: Os animais utilizados apresentaram comportamento característico após a lesão com 6-OHDA. As CPDh possuem características adequadas para serem usadas como fonte de fatores neuroprotetores, tornando factível a hipótese de uma recuperação do déficit motor dos animais parkinsonianos promovida por essas células. Camundongos hemi-parkinsonianos receberão transplantes de CPDh para avaliação de seu potencial terapêutico na Doença de Parkinson.

Palavras-Chave: Doença de Parkinson, célula imatura de polpa de dente, GDNF.

Apoio Financeiro: FAPERJ, Ministério da Saúde e CNPq

A.03.006

Potencialização da diferenciação neuronal de células-tronco embrionárias pelo co-cultivo com células derivadas da polpa de dente

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Objetivos: Células-tronco embrionárias murinas (mES) são capazes de proliferação ilimitada e de dar origem às três camadas germinativas do embrião, sendo por isso denominadas pluripotentes. A diferenciação de mES em neurônios é obtida pela formação de corpos embrióides (EB) na presença de ácido retinóico (RA), procedimento que, entretanto, induz considerável morte celular. O estabelecimento de protocolos de diferenciação que permitam maior rendimento na produção de neurônios é objeto de inúmeras pesquisas. Aqui avaliamos um possível efeito potencializador de diferenciação neuronal pelo co-cultivo da mES e EB com células-tronco imaturas da polpa de dente humano (CPDh), produtoras de fatores neurotróficos e tratamento com RA.

Métodos: mES foram cultivadas na presença de monocamada de fibroblastos (MEF) ou de CPDh por 1 semana. Para a formação de EB, mES foram transferidas para uma placa de cultura não aderente. EB provenientes do cultivo em MEF ou CPDh foram tratados por 4 dias com 2 μ M de RA e, posteriormente, plaqueados sobre CPDh ou uma matriz permissiva à migração por mais 4 dias. A incidência de migração e diferenciação celular foram quantificadas após imunocitoquímica.

Resultados: O co-cultivo das mES sobre a monocamada de CPDh promoveu aumento na migração de progenitores neuronais, em comparação com às cultivadas sobre MEF, de 852 $\pm$ 111,5 para 3008 $\pm$ 396,0. Da mesma forma, quando plaqueamos EB sobre uma monocamada de CPDh, verificamos ampla presença de células positivas para nestina (progenitores neurais) que migraram a partir dos EB.

Conclusão: Nossos resultados indicam que o co-cultivo de mES e CPDh é capaz de potencializar o processo de diferenciação neuronal e pode ser uma estratégia a ser utilizada para a geração de neurônios a partir de células-tronco embrionárias. **Palavras-Chave:** Células-tronco embrionárias, células imaturas de polpa de dente, Diferenciação

Apoio Financeiro: FAPERJ, Ministério da Saúde e CNPq.

EFEITO DO TRANSPLANTE DE CÉLULAS DE SANGUE DE CORDÃO UMBILICAL HUMANO SOBRE A MEMÓRIA DE RATOS ADOLESCENTES SUBMETIDOS À HIPÓXIA-ISQUEMIA NEONATAL

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Objetivos: Este estudo visa investigar o papel do transplante de células de sangue de cordão umbilical humano sobre a memória de ratos adolescentes submetidos à hipóxia-isquemia (HI) cerebral no período neonatal.

Métodos: Utilizaram-se ratos Wistar, machos e fêmeas, nos grupos: controle (CT; n=21), veículo 3 dias (CTV3; n=25), veículo 14 dias (CTV14; n=14), hipóxico-isquêmico (HI; n=19), célula-tronco 3 dias após HI (HISC3; n=15) e célula-tronco 14 dias após HI (HISC14; n=13). No 7º dia pós-natal, os ratos foram submetidos à isquemia, com oclusão permanente da artéria carótida comum direita e sofreram exposição à hipóxia (90 min - 8% O₂; 92% N₂). Três ou 14 dias após a cirurgia, os animais receberam transplante da fração mononuclear de sangue de cordão umbilical humano (10⁷ células em 300µl de PBS) via i.p.. Aos 30 dias de vida pós-natal, avaliou-se a memória dos animais através dos testes de reconhecimento de objetos e esQUIVA inibitória, e o comportamento motor através do teste do campo aberto. No teste de reconhecimento de objetos, a memória foi avaliada através do cálculo do índice de exploração do objeto novo (B) na segunda exposição (B-A/A+B).

Resultados: A análise dos dados indicou prejuízo de memória de reconhecimento nos animais HI (CT: 0,43±0,1; HI: -0,3±0,1), sem, no entanto, reversão deste pelo transplante de células da fração mononuclear. No teste da esQUIVA inibitória, os animais HI não demonstraram prejuízo da memória (CT: 180, 24/180s; HI: 180, 12/180s). O teste do campo aberto não demonstrou déficit motor em quaisquer dos grupos nas variáveis analisadas.

Conclusões: A hipóxia-isquemia cerebral neonatal resultou em déficit mnemônico no teste de reconhecimento de objetos, o qual não foi revertido pelo transplante das células de sangue de cordão umbilical. Tanto o teste de esQUIVA inibitória quanto o teste de campo aberto não revelaram quaisquer prejuízos nos animais, quando comparados aos controles.

Palavras-Chave: Hipóxia-isquemia; memória; células-tronco; cordão umbilical

Apoio Financeiro: CNPq

A.03.008

POTENCIALIZAÇÃO DA DIFERENCIAÇÃO NEURONAL PROMOVIDA POR BIFLAVONÓIDE EM CÉLULAS-TRONCO EMBRIONÁRIAS

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Objetivo: Células-tronco embrionárias murinas (mES) possuem capacidade de auto-renovação, bem como potencial de se diferenciar em todos os tipos celulares do animal adulto. A formação de corpos embrioides (EBs) é a estratégia básica para diferenciação aleatória das mES *in vitro*. No entanto, diferentes condições de cultivo podem induzir diferenciação orientada para um determinado fenótipo. Sabendo que alguns flavonóides potencializam a diferenciação desencadeada por estímulo indutor adicional, avaliamos se o biflavonóide Agathisflavona (FAB) potencializaria o efeito da diferenciação neuronal induzida por ácido retinóico (RA) em mES.

Métodos: mES foram cultivadas sob a forma de colônias sobre monocamada de fibroblastos (MEF). Para a formação de EBs, mES foram transferidas para uma placa de cultura não aderente. EBs foram tratados por 4 dias com 2µM de RA, 60µM de FAB ou com 2µM de RA+60µM de FAB. Os controles foram realizados com meio de cultura ou com a adição do veículo de diluição do FAB e do RA, DMSO. Tanto a diferenciação, quanto a proliferação celular foram analisadas através da técnica de imunohistoquímica. A morte celular foi identificada por incidência de fragmentação de DNA *in situ*.

Resultados: Nossos resultados revelam que o FAB é um excelente potencializador da diferenciação neuronal induzida pelo RA, uma vez que dobrou a eficiência do protocolo. Observamos também um aumento na área de 30% nos EBs tratados com FAB e 80% nos tratados com RA+FAB, sendo expressivo em relação aos demais grupos. Associado a isso, observamos que o FAB exerce efeito protetor, reduzindo significativamente (50%) a morte celular em EBs tratados com RA. Estatística realizada por ANOVA, sendo $P < 0,05$.

Conclusão: O desenvolvimento de protocolos de diferenciação eficientes é um avanço tanto para o entendimento de mecanismos envolvidos nesse processo, como prova de princípio para testes clínicos de células-tronco embrionárias. Com base na intensa taxa de morte celular associada à diferenciação neural com RA e os resultados obtidos nesse trabalho, concluímos que o FAB demonstra grande potencial para ser aplicado em protocolos de diferenciação neuronal com células-tronco embrionárias. **Palavras-Chave:** Células-tronco embrionárias; Diferenciação neuronal; Biflavonóide

Apoio Financeiro: CNPq e FAPERJ

A.03.009

Expressão e Atividade do Receptor B2 Durante a Diferenciação de Neuroesferas de Ratos

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Células progenitoras neurais (CPN) podem ser isoladas do telencéfalo de embriões de 14 dias (E14) de ratos Wistar. Essas células proliferam e formam agregados denominados neuroesferas, sendo cultivadas em suspensão na presença de FGF-2 (fibroblast growth factor 2) e EGF (epidermal growth factor). Após remoção desses fatores de crescimento e adesão à matriz, as neuroesferas se diferenciam em neurônios, astrócitos e oligodendrócitos. Foi demonstrada anteriormente a presença de um sistema autócrino entre a ativação do receptor B2 e a secreção de seu ligante, a bradicinina (BK), na linhagem de carcinoma embrionário P19. Esse sistema é um modelo para o estágio final da diferenciação neuronal (Martins et al., J Biol Chem 2005; 280: 19576–19586). O objetivo desse trabalho foi verificar a atividade do sistema caliceína-cinina (KKS) durante a diferenciação de CPN. Através de análise por imunofluorescência e citometria de fluxo, observou-se um aumento na população de astrócitos (GFAP+) e neurônios (β -tubulin III+) e uma diminuição no número de células não diferenciadas (Nestina+) no decorrer do processo de diferenciação, indicando uma transição de CPN para astrócitos e neurônios. A expressão e atividade do receptor B2, a secreção de BK para o meio de cultura e a presença de cininogênio de alto peso molecular, sugerem a participação do sistema caliceína-cinina na diferenciação das neuroesferas de ratos. A presença de receptores B2 funcionais e a secreção de BK indicam um loop autócrino durante a diferenciação das neuroesferas em neurônios, astrócitos e oligodendrócitos, que pode ser semelhante aos eventos que ocorrem durante o desenvolvimento cerebral inicial. **Palavras-Chave:** células progenitoras neurais, diferenciação, neuroesferas, sistema caliceína-cinina

Apoio Financeiro: FAPESP, CNPq

A.03.010

Neuroesferas como modelo *in vitro* para estudo da Esclerose Lateral Amiotrófica

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Neuroesferas derivadas do sistema nervoso em desenvolvimento podem originar os três principais tipos de células neurais que o compõe e são capazes de manter especificação regional em condições de cultivo. **OBJETIVO:** O presente trabalho visa a caracterização de neuroesferas obtidas a partir da medula espinal em desenvolvimento de ratos transgênicos que super-expressam superóxido dismutase 1 (SOD1), um modelo experimental de ELA. **MÉTODOS:** A medula espinal de fetos E15 de ratos selvagens (Sprague-Dawley, CTR) e SOD1 foram dissecadas e cultivadas como neuroesferas na presença de FGF-2 e EGF. As subpopulações celulares, tanto das neuroesferas como após diferenciação *in vitro*, foram quantificadas por citometria de fluxo. Essas células também foram analisadas com relação à sua morfologia e localização por imunocitoquímica. A variação da expressão de fatores neurotróficos foi verificada por PCR em tempo real. Como medida indireta do balanço redox das células, os conteúdos de cisteína (Cys) e homocisteína (Hcy) foram determinados por HPLC. **RESULTADOS:** As percentagens de progenitores neurais indiferenciados (Nestina⁺), astrócitos (GFAP⁺), oligodendrócitos (Gal-C⁺) e neurônios (b-tubulina III⁺), assim como suas morfologias, não apresentaram diferenças entre os grupos CTR e SOD1. A localização tridimensional dessas subpopulações neurais também era similar entre os grupos SOD1 e CTR. Com relação aos fatores neurotróficos, PDGFA foi significativamente mais expresso nas neuroesferas SOD1 (CTR 0,8±0,2/SOD 1,2±0,1) enquanto as expressões de EGF, CTNF e CT1 não diferiram entre os grupos. Por fim, o balanço do sistema redox parece estar alterado nas células derivadas de SOD1, de acordo com as dosagens de Hcy (CTR 6,3±1,2/SOD1 9,7±2,2), ainda que o conteúdo de Cys não tenha variado entre os grupos. **CONCLUSÃO:** A partir desses resultados podemos concluir que não há diferenças importantes na estrutura e plasticidade entre as neuroesferas SOD1 e CTR, sugerindo um desenvolvimento normal da medula espinal. No entanto, o desequilíbrio no sistema redox e a expressão diferencial de fatores neurotróficos específicos podem a longo prazo culminar na morte dos neurônios motores.

Palavras-Chave: neuroesferas, células-tronco neurais, esclerose lateral amiotrófica, superóxido dismutase,

Apoio Financeiro: FAPESP, CNPq

A.03.011

Limiar de Crises Convulsivas de Ratos com Epilepsia Tratados com Células-Tronco Mesenquimais

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Objetivo: Avaliar a influência do tratamento com Células-Tronco Mesenquimais (CTMSCs) sobre o limiar de crise de ratos epiléticos.

Materiais e Métodos: Ratos Wistar machos foram divididos em quatro grupos: salina, salina+CTMSCs, pilo+salina e pilo+CTMSCs. Receberam cloreto de lítio, e 14 horas após, foi administrado metilescopolamina e pilocarpina. O status epilepticus (SE) foi mantido por 90 minutos, e abortado com diazepam. Após 21 dias de SE, 05 animais do grupo salina e 08 do pilo foram transplantados com CTMSCs GFP+(10⁶/200µl), provenientes de camundongos C57BL6/EGFP, por meio de injeção intravenosa na veia caudal. Decorridos 60 dias de transplante, os animais foram testados no eletrochoque máximo, para avaliar a influência da administração de CTMSCs sobre o limiar de crise convulsiva. Este teste consistiu na aplicação de corrente alternada de 50Hz (entre 65 mA e 170 mA) através de eletrodos posicionados no canal auricular, com auxílio de um software gerador de onda e temporizador de estímulo. Para todos os animais a indução da crise foi iniciada com uma duração única e fixa de 200 ms e por pulso de corrente alternada, que progrediu com incrementos de 5 mA até a obtenção de convulsão generalizada com extensão tônica. A cada degrau de corrente no eletrochoque a corrente em que o animal atingia extensão tônica foi registrada. Em todos os casos os animais receberam estimulações repetidas, com intervalo de 48 horas entre elas para permitir sua recuperação neurológica. **Resultados:** Observou-se que os animais do grupo pilo+salina (80±3,78) convulsionaram ao receberem aplicações de corrente menor do que os animais salina (111±5,33), salina+CTMSCs (126±14,53), pilo+CTMSCs (106±8,59 $p<0,05$ na Anova de 1 Via), e que, portanto, seu limiar convulsivo é mais baixo. **Conclusão:** A administração intravenosa de CTMSCs é capaz de aumentar o limiar convulsivo no teste de eletrochoque máximo, apresentando um comportamento similar ao grupo controle. Este tipo de tratamento pode ser útil no tratamento da epilepsia crônica, dado o potencial que estas células têm de, independentemente do mecanismo, restaurar o micro ambiente neuronal, e re-estabelecer a excitabilidade neuronal fisiológica.

Palavras-Chave: eletrochoque máximo, epilepsia, células-tronco mesenquimais

Apoio Financeiro: PUC/RS, CEFET/SC, FUNPAR, Bauducco/PUCRS, Faculdade Gama e Souza/RJ.

A.03.012

BDNF producing mesenchymal stem cells increase neuronal survival and synaptic stability following ventral root avulsion

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Objective: Ventral root avulsion (VRA) is an experimental model used to induce motoneuron degeneration. Such a degenerative process can be delayed and minimized by an exogenous treatment with neurotrophic factors. The aim of this study is to investigate the neurotrophic action of the mesenchymal stem cells (MSC) into the motor nucleus after VRA.

Methods: Lewis rats were subjected to unilateral VRA, receiving 3×10^5 cells injected through the lateral funiculus. The neuronal survival, the astroglial reaction and the density of synaptic innervation within the motor nucleus was analyzed. The levels of mRNA for BDNF and GDNF were also studied *in vitro* and *in vivo* by quantitative PCR.

Results: The results indicated that the MSC treatment significantly rescued avulsed motoneurons (avulsion, $31.33\% \pm 5.13$; avulsion+PBS, $34.11\% \pm 4.28$; avulsion+MSC, $64.44\% \pm 4.24$; mean $\pm$ SD; $p < 0.001$). A preservation of 85% more synaptophysin positive inputs in the motor nucleus was observed after MSC injection (avulsion+PBS, 0.30 ± 0.02 ; avulsion+MSC, 0.85 ± 0.005 ; $p < 0.001$). Regarding the astrocytic response, MSC presence resulted in a decrease of 43% of GFAP labeling (avulsion+PBS, 2.22 ± 0.42 ; avulsion+MSC, 1.26 ± 0.17 ; $p < 0.001$). Additionally, the real-time PCR analysis showed that the MSC were able to produce about 16 times more BDNF mRNA than the control. Nevertheless, the GDNF mRNA level was not different from the control. Interesting, after avulsion and the MSC treatment, the levels of both neurotrophic factors in the MSC group were significantly greater than in the PBS and avulsion groups (BDNF mRNA: avulsion, 6.40 ± 2.43 ; avulsion+PBS, 4.80 ± 0.98 ; avulsion+MSC, 36.80 ± 4.90 ; $p < 0.001$. GDNF mRNA: avulsion, 2.92 ± 2.55 ; avulsion+PBS, 3.45 ± 1.02 ; avulsion+MSC, 16.62 ± 0.19 ; $p < 0.001$).

Conclusions: Grafting of MSC into avulsed spinal cord segments increased BDNF and GDNF expression, decreasing synaptic stripping, astroglial reaction and neuronal death.

Palavras-Chave: Motoneuron, avulsion, mesenchymal stem cells, BDNF, GDNF

Apoio Financeiro: CNPq

A.03.013

Stress inducible protein 1 modulates neural stem cells self-renewal when engaged to cellular prion

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The misfolding of the cellular prion (PrP^C) is an essential event in prion diseases however the physiological functions of PrP^C remain in debate. We have demonstrated that the interaction between PrP^C and STI1 (Stress inducible protein 1) is involved in relevant functions associated to central nervous developmental such as neural survival, neuritogenesis and memory formation. PrP^C has been related to neurogenesis but the role of PrP^C-STI1 in cell fate related-mechanisms is unknown. To address this issue neurospheres cultures, which contain a mix of both multipotent and restricted progenitor populations, derived from telencephalon of wild-type and PrP^C-null E14 mice were used. These cultures were maintained for several passages in the presence of EGF and FGF-b without apparent loss of either self-renewal or multipotenciality as assessed by their continuous capacity to generate astrocytes (GFAP positive cells), oligodendrocytes (O4 positive cells) and neurons (bIII-tubulin positive cells) upon induction of differentiation. The expression of STI1 as well as nestin, an undifferentiated cell marker, was uniform and presented similar levels in neurospheres suspensions derived from both wild-type and PrP^C-null embryos. The capacity of cells to form neurospheres was significantly affected in cultures derived from PrP^C-null embryos when compared to their wild-type counterparts, suggesting the participation of PrP^C in self-renewal. When STI1 was added to cultures of wild-type cells a significantly increase in the number of neurospheres was observed when compared to the untreated group. Conversely, in PrP^C-null neurospheres STI1 had no effect. These data suggest that STI1 could play a role in proliferation and/or survival through its binding to PrP^C. Indeed, PrP^C-STI1 interaction modulates the self-renewal of progenitor cells and may represent a novel therapeutic target for the treatment of neurodegenerative illness. Supported by FAPESP and HHMI.

Palavras-Chave: neural stem cell, cellular prion, STI1, neurosphere

Apoio Financeiro: FAPESP and HHMI

BLOQUEIO DA ATIVIDADE DE TELOMERASE INDUZ ANEUPLOIDIA E DIFERENCIAÇÃO NEURONAL EM CÉLULAS-TRONCO EMBRIONÁRIAS

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Objetivos: A aneuploidia (perda e ganho de cromossomos) é característica de progenitores neurais e neurônios derivados do cérebro de camundongos e humanos (Rehen *et al*, PNAS. 98: 13361, 2001; Rehen *et al*, J Neurosci. 25: 2176, 2005). Não é sabido, entretanto, se sua indução está associada aos processos de neurogênese. Células-tronco embrionárias murinas (mES) são capazes de gerar todos os tecidos de um indivíduo adulto e podem se dividir indefinidamente em virtude da alta expressão de telomerase. Na maioria das células diferenciadas esta enzima não é expressa, sugerindo uma correlação entre perda de atividade da telomerase e diferenciação celular. No presente trabalho investigamos se o bloqueio da função da telomerase pelo anti-retroviral zidovudina (AZT) é capaz de induzir aneuploidia associada à diferenciação neuronal em mES.

Métodos e resultados: mES foram cultivadas sob a forma de colônias na presença de monocamada de fibroblastos (MEF). Durante 4 dias as colônias foram tratadas com AZT nas concentrações de 30, 50, 100µM. Os cromossomos foram preparados e corados com DAPI para análise em microscópio óptico de fluorescência e pelo menos 40 metáfases foram analisadas por grupo experimental. Para avaliar a indução de diferenciação neural nas diferentes condições experimentais, corpos embrióides (EB) foram tratados por 4 dias com 2µM de ácido retinóico (RA), 30, 50 ou 100µM de AZT. mES controle apresentaram cariótipo estável com 28,8% de aneuploidia e mES tratadas com AZT (30, 50 e 100µM), apresentaram 70,5%; 71,9% e 67,4% de aneuploidia, respectivamente. Enquanto EB controle apresentaram 1% de células nestina positivas e EB tratados com RA (2µM) 19% ($\pm 5,37$), o tratamento com AZT (30, 50 e 100µM) induziu 22% ($\pm 7,6$); 22,3% ($\pm 5,9$) e 12,3% ($\pm 7,5$), respectivamente, de células nestina positivas.

Conclusão: Nossos resultados sugerem que instabilidade cromossômica causada pela inibição da atividade de telomerase induz neurogênese em células-tronco embrionárias. **Palavras-Chave:** Células-tronco embrionárias, Diferenciação Neural, Aneuploidia, AZT

Apoio Financeiro: Ministério da Saúde e CNPq

A.03.015

TERAPIA COM CÉLULAS MONONUCLEARES DE MEDULA ÓSSEA APÓS ACIDENTE VASCULAR CEREBRAL EM CAMUNDONGOS

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A finalidade deste projeto foi investigar o curso temporal da neurodegeneração na área de penumbra isquêmica após oclusão da artéria cerebral média (ACM) em camundongos e avaliar o efeito neuroprotetor das células mononucleares da medula óssea (CMMO) sobre a penumbra isquêmica, utilizando-se o marcador fluorescente de neurodegeneração FluoroJade-C (FJ-C).

Foram utilizados camundongos BALB-C (20-30g). Fêmeas foram doadoras das CMMO. Os animais foram esquiados pela oclusão da ACM. Aqueles destinados à avaliação do curso temporal foram perfundidos com paraformaldeído 4%, 3, 7, 10 e 14 pós-isquemia. Foram obtidas fatias coronais (20 microm) do cérebro destes animais. Estas lâminas foram submetidas à reação com FJ-C. Outros animais receberam 10 milhões de CMMO (n=4) ou salina fisiológica (n= 3) pela veia femoral 1 dia pós-cirurgia. Estes animais foram perfundidos 4 dias após a isquemia e os cérebros processados conforme descrito anteriormente. As fatias cerebrais foram analisadas ao microscópio de fluorescência.

Os resultados mostraram que o maior número de células FJ-C-positivas foi encontrado na região de penumbra 3 dias após a isquemia, indicando ser este o período de maior neurodegeneração, enquanto nenhuma foi observada após 14 dias. Animais que receberam CMMO 1 dia pós-cirurgia apresentaram menor número de células FJ-C-positivas (Média: $256,7 \pm 59,2$) do que animais que receberam apenas salina (Média: $663,0 \pm 116,5$; $P = 0,02$).

No modelo de isquemia focal pelo bloqueio permanente da ACM de camundongos, a análise do curso temporal de morte neuronal mostrou que o pico de degeneração ocorre no terceiro dia pós-isquemia, sendo assim, este se mostrou o melhor período para a administração das CMMO. A administração de CMMO diminuiu o número de células FJ-C positivas na região de penumbra, isto mostra que provavelmente, as CMMO poderiam produzir fatores que impediriam a morte celular nesta região.

Palavras-Chave: Células Mononucleares de Medula Óssea, Acidente Vascular Cerebral, FluoroJade - C, Neurodegeneração

Apoio Financeiro: Apoio financeiro: CNPq e FAPERJ.

DIFFERENTIATION OF HUMAN DENTAL PULP STEM CELL FROM PERMANENT TOOTH INTO NEURAL LINEAGES.

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Objectives: According to the current knowledge, different stem cell populations of ectomesenchymal origin can be isolated from dental pulp from adult and young patients. This work focuses on the isolation, culture, characterization of the human dental pulp stem cells (hDPSC), which can be isolated from permanent tooth, as well as on the development of approaches using these cells for differentiation into neural lineages.

Methods: The pulp tissue from a permanent second molar tooth of a 49-year-old healthy male patient was extracted and hDPSC were cultured according to the literature **(1)**. These cells were examined with stem cells markers, such as Oct 3/4, SH-2, SH-3, SH-4, CD 45, CD90, anti-DPSC, CD44, Nestin and others. Neural differentiation of hDPSC was achieved using a protocol modified by us which was previously developed for human BM-MSC **(2)**. Neurogenic potential was evidenced by the expression of neural and glial markers, such as Nestin, Beta III tubulin, Neurofilament M (NFM), GFAP and other markers.

Results: This cell population presents rapid proliferation and expansion *in vitro*. The cells can be maintained for a long period in culture, which is an indicative for their self-renewal potential. The hDPSC express markers of mesenchymal stem cells. Moreover, approximately half of hDPSC population reacted positively with markers of human embryonic stem cells. After the induction of neurogenic differentiation in monolayer, our morphological analysis revealed the presence of neuron-like as well as glial-like cells formation. These cells were also positive for several neural and glial markers. We observed neurosphere formation from hDPSC, which is similar to the one formed by primary neural stem and progenitor cells within central nervous system **(3)**. Functional characterization of neuron-like cells obtained from hDPSC is in progress.

Conclusions: Here, we demonstrated the feasibility of deriving hDPSC from a permanent tooth, and their differentiation potential into neural lineages.

References: 1- Cells Tissues and Organs 184:105-116, 2006; 2- Journal of Neuroscience Research 83:1502-1514, 2006; 3- Cell 60:585-595, 1990.

Palavras-Chave: human dental pulp stem cells, permanent tooth, differentiation, neural lineages

Apoio Financeiro: FAPESP

A.03.017

Propagação de células-tronco embrionárias em biorreatores visando aumento de escala para transplante em doenças neurodegenerativas.

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Células-tronco embrionárias de camundongos (mES) cultivadas em placas estáticas foram comparadas a mES aderidas a micropartículas esféricas (microcarregadores) e mantidas em meio condicionado por fibroblastos embrionários murinos no interior de frascos agitados (*spinner*). Com esse sistema foi possível realizar um cultivo em três dimensões, onde as limitações de oxigênio e de nutrientes são bem menores comparados aos cultivos tradicionais.

A quantidade total de células geradas por intervalo de tempo, morfologia das colônias, estabilidade cromossômica, expressão dos marcadores de pluripotencialidade Oct-4, SOX2 e SSEA-1 e o potencial de diferenciação em corpos embriões foram comparados entre mES propagadas no sistema estático e nos frascos agitados.

O cultivo de mES em placas permitiu a geração de $2,01 \pm 0,09 \times 10^5$ células/cm² após 5 dias, organizadas em colônias de $3,6 \pm 2,1 \times 10^3 \mu\text{m}^2$ que quando transferidas para placas não aderentes formaram corpos embriões de $147 \pm 56 \mu\text{m}^2$. Quando mES foram cultivadas em frascos *spinner* foi possível produzir $7,88 \pm 1,41 \times 10^6$ células/mL após 7 dias, preservando as características de crescimento, estabilidade cromossômica e pluripotencialidade. Quando replaqueadas se organizaram em colônias de $3,6 \pm 1,8 \times 10^3 \mu\text{m}^2$ e depois formaram corpos embriões de $129 \pm 49 \mu\text{m}^2$.

Nossos resultados indicam a possibilidade de cultivo de células-tronco embrionárias em biorreatores com rendimento pelo menos 10 vezes superior ao obtido em placas estáticas sem que fossem observadas alterações em suas características intrínsecas. Além disso, os microcarregadores utilizados são até 40 vezes mais baratos quando comparados às placas e frascos estacionários utilizados tradicionalmente. Esse procedimento de cultivo permitirá a produção de células-tronco embrionárias em quantidades compatíveis com sua eventual utilização em testes pré-clínicos e clínicos visando o tratamento de doenças neurodegenerativas

Palavras-Chave: células-tronco embrionárias, scale-up e spinner

Apoio Financeiro: Faperj e Ministério da Saúde

LECTIN from *Canavalia brasiliensis* (ConBr) may produce selective citotoxicity and induces mesenchymal stem cell differentiation.

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Carbohydrates may act as cellular signaling components in biological systems, regulating differentiation, proliferation and cell death in physiologic and pathological conditions. The information in the structure of oligosaccharides, normally conjugated to proteins or lipids in the cell surface, can be recognized by a group of proteins, the lectins. ConBr is a lectin isolated from *Canavalia brasiliensis* seeds which can reduce the viability of tumoral cells of glioma C6. Meantime, it is not known if this lectina can cause death in non tumoral cells such as mesenchymal stem cells. Besides, ConA, a lectin similar to ConBr, can induce protection against mechanical insult or action of proteases in mesenchymal stem cells from bone marrow. In the present work we carry out a comparative study of the action of the ConBr regarding production of cell death and modulation of ERK1/2 and p38^{MAPK} in mesenchymal stem cells originating from the human umbilical cord, and cells of glioma C6. The stem cells were treated with ConBr (10 µg/ml) for 12-72h and its viability was measured through immunocytochemistry and staining with Hoesht. The C6 cells were treated with ConBr (1-100 µg/mL; 24 h) and the viability evaluated through the incorporation of propidium iodide. The modulation of ERK1/2 and p38^{MAPK} were analysed by western blotting. The results showed that ConBr (10 µg/mL; 24h) induced C6 glioma cell death (approaching 30% ±5; p<0.05) and strongly activated the phosphorylation of p38^{MAPK} (50% ±7; p<0.05) without changing the entire enzyme. In mesenchymal stem cells from the human umbilical cord it was not observed induction of cell death or modulation of ERK1/2 or p38^{MAPK} by ConBr (10 µg/mL; 12-72h). However, it was observed by immunocytochemical labeling, using anti-β-tubulin III antibody, that ConBr treatment (10µg/mL; 12h) increased mesenchymal stem cell differentiation to a neuronal phenotype (35% ±4.5; p<0.05). The total number of mesenchymal cells was not changed by ConBr treatment. These results suggest a cellular selectivity regarding the cytotoxic actions of ConBr and suggest a potential application of this lectin as a tool to induce stem cell differentiation.

Palavras-Chave: Lectin, stem cell, citotoxicity, cell differentiation.

Apoio Financeiro: CNPq, FAPESC and FINEP (IBN-Net #01.06.0842-00)

A.03.019

A proteína príon celular (PrP^c) e seu ligante, a proteína 1 induzida por estresse (STI1), influenciam a diferenciação das células da crista neural truncal, *in vitro*.

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A crista neural (CN) compreende uma população de células altamente pluripotentes que possuem propriedades migratórias. A diferenciação destas células é fortemente influenciada por fatores do microambiente, dando origem a neurônios, células gliais, melanócitos e a diversos derivados mesenquimais. A proteína príon celular (PrP^c) é abundantemente expressa no sistema nervoso e tem sido associada a vários fenômenos celulares como neuritogênese e diferenciação neuronal. Muitas das funções de PrP^c dependem da sua ligação com outras proteínas, tais como a proteína 1 induzida por estresse (STI1). Neste trabalho, avaliamos o papel de PrP^c e de STI1 na diferenciação das células tronco da CN truncal (CNT) de aves. Para isso, as células da CNT (CCNTs) de embriões de codorna de 48 horas foram tratadas com PrP^c ou STI1. O tratamento foi feito na fase de migração das CCNTs ou na fase de diferenciação celular (5 dias em cultura). As células foram analisadas por imunofluorescência usando marcadores específicos para neurônios (tirosina hidroxilase e β -tubulina III), células da glia (proteína mielínica de célula de Schwann) e melanócitos (melanina). Nossos resultados mostraram que as CCNTs expressam tanto PrP^c quanto STI1. Além disso, ambos os tratamentos com PrP^c ou STI1 na fase de migração foram capazes de aumentar em cerca de quatro vezes a proporção de neurônios e em cerca de três vezes a proporção de melanócitos. A proporção de células gliais diminuiu ao mesmo tempo em que a proporção de células neuronais aumentou. Quando as CCNTs sofreram tratamento somente na fase de diferenciação a proporção dos fenótipos analisados não apresentou diferenças significantes em relação ao controle. Até o momento, não existem trabalhos relacionando PrP^c e STI1 com as CCNs e a exata função do PrP^c durante o desenvolvimento embrionário ainda não é conhecida. Nossos resultados são inéditos na literatura e sugerem que ambos os fatores, PrP^c e STI-1 têm importante efeito na diferenciação das CCNTs principalmente para os fenótipos neuronal e melanocítico. Além disso, sua ação parece estar na determinação e/ou especificação dos progenitores mais indiferenciados da CN. **Palavras-Chave:** crista neural, microambiente, proteína príon celular (PrP^c), proteína 1 induzida por estresse (STI1), diferenciação celular

Apoio Financeiro: PIBIC/UFSC, CNPq, CAPES/PROCAD, MCT/INFRA, CNPq/PRONEX, FAPESC, Industrias Macedo

A.03.020

DETERMINAÇÃO *IN VITRO* DOS NÍVEIS DE CD133 EM GLIOBLASTOMA MULTIFORME HUMANO GL-15

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Objetivos: Os gliomas são os tumores cerebrais mais freqüentes, representando mais de 50% de todas as neoplasias do SNC em adultos. Geralmente são difusos e agressivos, adquirindo rápida resistência à radioterapia. Atualmente, vem sendo sugerido que células-tronco tumorais estão relacionadas a radioresistência e re-população. Estudos recentes relatam um aumento da expressão do marcador de células-tronco neural e tumoral CD133 (Prominina-1), após radiação em gliomas. Neste estudo, foi avaliada a determinação dos níveis de CD133 em cultura de GBM humano GL-15.

Metodologia: Células GL-15 foram cultivadas em placas de 35mm, com meio DMEM suplementado com penicilina G, streptomicina, glicose, L-glutamina, piruvato e 10% de soro fetal bovino, em incubadora de CO₂ a 37°C. 10x10⁶ células foram incubadas, após 48h, com anticorpo monoclonal anti CD133 (1:50 e 1:100) e marcadas com anticorpo anti-IgG de camundongo conjugado com FITC (1:100). As células foram, então, analisadas por citometria de fluxo (FACScan BD /US-BD Biosciences Clontech).

Resultados: Foi verificado, através da citometria de fluxo, que 12% das células GL-15 expressaram marcador CD133, na diluição 1:100.

Conclusões: Em comparação a estudos efetuados até o momento, nossos resultados indicam uma alta porcentagem de células CD133⁺ na linhagem GL-15. Devido ao importante papel desse tipo celular na manutenção do tumor, acreditamos que serão necessários maiores estudos para elucidar um melhor entendimento acerca do comportamento da neoplasia. A identificação dos mecanismos envolvidos na resistência e repopulação tumoral, poderá contribuir para a produção de futuros alvos terapêuticos.

Bibliografia: 1- Nature 444:756, 2006; 2- J. Sem. Cancer 17:214, 2007.

Palavras-Chave: Glioblastoma, células-tronco, CD133

Apoio Financeiro: CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

A.03.021

A role of the purinergic system in the development of cancer stem-cells

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Objectives: The aim of this study was to analyze the role of the purinergic system in the formation of spheres in the human glioma cell line U87. The spheres observed in culture are similar to the neuro-spheres produced by neural stem-cells, and were already described in gliomas as containing a population of cancer stem-cells (1).

Methods: "Spheroenic assay": 250, 500, 750 and 1000 U87 cells were plated in a 96 well plate. DMEM with 5% fetal calf serum was used. In the treatment, ATP was added to obtain a final concentration of 100uM ATP. The plate was analyzed under optic microscope from days 3-7, and spheres were counted. We considered as spheres groups of cells not completely adhered to the plate, and presented certain volume observed in phase contrast. PCRs: U87 cells were grown in plates covered with agar to induce the formation of spheres, and in plates without agar. RNA was extracted from those two populations (spheres and adherent cells), and PCR for differentiation genes CD133, Oct4, CamKII, GLT, Nestina, GLAST, Enolase 2, AMPA, NMDA and purinergic genes A1, A2a, A2b, A3, P2X1-7, P2Y1, P2Y2, P2Y4, P2Y6, P2Y11-14 was performed.

Results: Preliminary results showed that spheres expressed undifferentiated marker genes such as Oct4 and P2Y1, which were not present in the cells from the monolayer. Cells treated with ATP 100uM formed less spheres than control. For example, when 750 cells were plated, there were a mean of $8,74 \pm 3,0$ spheres per well at the seventh day at the control, while there were only $4,08 \pm 2,3$ spheres at the ATP treatment ($P < 0,05$). The percentage of wells without spheres was used to calculate the number of cells that are necessary in order to form a sphere, and this number was higher when cells were treated with ATP.

Conclusion: Purinergic receptors are reported in stages of embryonic development and not only in the adult brain. ATP induces several cellular responses, such as proliferation and differentiation (2). Our work shows that the purinergic system is probably involved in the formation of cancer stem cells, and ATP is playing a role in the formation of spheres.

References: 1 – Nature 432:396, 2004; 2 – Neuroscience 146:1169, 2007. **Palavras-Chave:** cancer stem cells, glioma, purinergic system, spheres

Apoio Financeiro: CNPq, CAPES, Fapergs

A.03.022

Células-Tronco Embrionárias Humanas: expansão em larga escala visando aplicações industriais e terapêuticas

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Objetivo: Expansão de células-tronco embrionárias humanas (hES) em biorreatores para futura utilização em terapias celulares e biotecnologia.

Metodologia: hES cultivadas em placas aderentes sobre fibroblastos embrionários murinos inativados (MEF) em meio DMEM/F12 com 20% knockout serum replacement e FGF-2 (8ng/mL) foram comparadas a hES cultivadas em biorreatores, aderidas a microcarregadores revestidos de colágeno (0,3% m/v) em meio condicionado de MEF e FGF-2 (8ng/mL). Análises de pluripotencialidade e diferenciação espontânea (formação de corpos embrióides) foram realizadas por imunocitoquímica para Oct-4, SSEA-4, TRA 1-60 e alfa-feto proteína (AFP), actina de músculo liso (AML) e nestina, respectivamente. Análise da estabilidade cromossômica foi realizada em 100 metáfases por grupo (Giemsa).

Resultados: O cultivo em biorreatores produziu 17 vezes mais hES do que o cultivo tradicional após 380 h (90×10^6 céls x $5,2 \times 10^6$ céls) pela metade dos custos (R\$760,00 x R\$1300,00). Os marcadores de pluripotencialidade Oct-4, SSEA 4 e TRA 1-60 estiveram presentes durante todo o cultivo e se mantiveram após replaqueamento em MEF. O cultivo das hES em biorreatores não ocasionou alterações de cariótipo. A capacidade de diferenciação espontânea não foi alterada pelo cultivo das células em microcarregadores, confirmado pela imunocitoquímica para marcadores dos três folhetos embrionários.

Conclusão: O cultivo em biorreatores permite a produção de células-tronco embrionárias humanas com pluripotencialidade e potencial de diferenciação equivalentes ao observado no cultivo tradicional e tornará possível sua utilização em prospecção biotecnológica e terapia celular.

Palavras-Chave: Células-tronco embrionárias; Biorreator;

Apoio Financeiro: Faperj, Ministério da Saúde e CAPES

ANÁLISE DA EXPRESSÃO DE *NEPH* EM CÉLULAS TRONCO MESENQUIMAIS DIFERENCIADAS PARA O FENÓTIPO NEURAL *IN VITRO*

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Objetivos: Analisar a expressão de *NEPH* no processo de diferenciação das células tronco mesenquimais (CTM) obtidas de placenta humana, para o fenótipo neural através da indução química pelo ácido trans-retinóico (ATRA).

Métodos: Uma pequena porção da placenta, próxima a região do cordão umbilical, foi dissociada mecanicamente e enzimaticamente com pancreatina, por aproximadamente 20 minutos, em temperatura ambiente. Essas células foram plaqueadas em garrafas de cultura e mantidas em estufa úmida. A troca do meio ocorreu periodicamente (3-4 dias), selecionando assim, as CTMs por sua adesão ao plástico. Após as culturas atingirem a monocamada confluyente (80 a 90%), as células aderentes foram retiradas usando solução de tripsina e EDTA, e re-plaqueadas na concentração de 1×10^5 células/mL (passagem 1-P1). Afim de induzir a diferenciação neural das CTMs, foi adicionado ATRA, na concentração de 0,5 μ M, em células com passagem superior a cinco (P5), durante 48 horas. Após tratamento, analisamos a expressão de diferentes genes por Reação de polimerase em cadeia via transcriptase reversa (RT-PCR). Utilizamos “primers” específicos para estas reações: β -tubulina III; proteína fibrilar ácida glial (GFAP); Nestina; NEPH1; NEPH2; NEPH3. E ainda, a morfologia foi analisada pela técnica de imunocitoquímica, utilizando os seguintes anticorpos: anti-GFAP; anti- β -tubulina III; anti-neurofilamento e anti-nestina.

Resultados: Através da análise de imunocitoquímica e RT-PCR, constatamos que as CTMs obtidas de placenta humana, expressam marcadores neurais, tais como β -tubulina III; GFAP e Nestina, quando estas são mantidas somente em IMDM, ou seja, na ausência de fatores indutivos. Quando as CTMs são submetidas a indução neural com ATRA, estas mantêm a expressão dos marcadores neurais, porém não visualizamos mudanças morfológicas. Quanto a expressão de *NEPH*, houve expressão apenas do gene *NEPH 1* nas células tratadas com ATRA, sendo que a expressão dos tipos 2 e 3, não foram observadas. Enquanto que nas células mantidas com IMDM não houve expressão de *NEPH*.

Conclusão: Com base nessas observações, há forte evidência que a expressão deste gene *NEPH*, está envolvido na diferenciação neural.

Palavras-Chave: Ácido trans-retinóico; Células tronco mesenquimais; Placenta; NEPH

Apoio Financeiro: CNPq

A Synthetic Inhibitor of the Cysteine Protease (E64) Promotes Differentiation of Murine Embryonic Stem Cells into Neural Cells

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Mouse embryonic stem cells (mESC) are capable of unlimited proliferation and pluripotent differentiation. However differentiation into neural cells is difficult to be achieved. Cystatin C, a cysteine protease inhibitor, is a small molecule secreted from neurospheres that has a powerful effect on differentiation mESC into neural cells (Kato *et al.*, 2006). In this work, we used a synthetic inhibitor of the cysteine protease, E-64, to induce a mESC lineage (USP-1) to differentiate into a neural phenotype. The USP-1 cell line was initially cultivated onto fibroblastic embryonic mouse feeder layer in DMEM-F12 medium supplemented with fetal bovine serum (FBS) and leukemia inhibitor factor (LIF). After enzymatic passage embryoid bodies (EBs) were formed in bacteriologic plates in DMEM-F12 medium, without LIF and supplemented with retinoic acid (10 μ M) for two days (Fraichard *et al.*, 1995]. Approximately 5 EBs were seeded over glass coverslips treated with Poly-L-lysine and laminin (20 μ g/mL) and kept in Neurobasal medium and B27 supplement for three or fourteen days. The EBs and the mESC colonies were fixed for 10 minutes at 37°C in 4% paraformaldehyde and then processed for immunocytochemistry to assess the presence of nestin, GFAP, Msi-1, Pax-6, NeuN and/or β III-tubulin positive cells (neural markers) or SSEA-1 and oct-4 (undifferentiated cell markers). The analyses of mESC colonies showed the expression of SSEA-1 and oct-4 markers. Markers from neural progenitors stem cells were founded (nestin, PAX-6, Msi-1 and GFAP) three days after that EBs were seeded. After fourteen days in culture the cells showed many processes characteristic of neural differentiation observed by phase-contrast microscopy. These cells were positive for β III-tubulin and NeuN. GFAP positive cells were found after fourteen days, however only a small number. We conclude that the protocol using E-64 during the formation of the EBs for two days is adequate to differentiate mESC into neural cells. **Palavras-Chave:** Stem Cell; Embryonic Stem Cells; Differentiation; Neuron; cysteine protease inhibitor

Apoio Financeiro: Edital CT-Biotecnologia/MCT/CNPq/MS/SCTIE/DECIT nº 24/2005, CAPES e FAPERJ

Isolation and Culture of Neural Stem Cells from Adult Human Brain

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Recent studies demonstrated that neurogenesis occurs in two regions in the adult mammalian brain: the subventricular zone (SVZ) around the lateral ventricles and the subgranular layer in the hippocampus. It has also been shown that neuronal stem cells (NSC) can be isolated from the same regions. This discovery may lead to new therapeutical strategies to treat central nervous system disorders through the propagation of NSC *in vitro*. However, only a few studies investigate the presence of these cells in human adult brains. In this work our goal was to isolate and characterize NSC from neuronal tissue obtained from the SVZ of adult patients undergoing surgery. Biopsies from the SVZ were harvested during routine neurosurgical procedures to treat epilepsy surgery in patients. The tissue was mechanically separated by scalpel and dissociated with papain for 60 minutes at 37°C. The cells were washed with PBS and the pellet was transferred to bacteriologic plates in DMEM/F12 with B27 supplement, 10ng/mL basic fibroblast growth factor (β FGF) and 20ng/mL epidermal growth factor (EGF). We observed formation of neurospheres after 13 days in culture. Neurospheres were then transferred to laminin coated glass slides in Neurobasal medium with B27 supplement without β FGF and EGF and the cells were allowed to differentiate. We isolated 26 neurospheres from the tissue of one patient. Two neurospheres were positive to β III-tubulin and GFAP after seventeen days *in vitro*. In two of these cells, the patch-clamp technique under whole-cell configuration, the outward current was observed in response to depolarizing stimulus. We demonstrate that NSC can be harvested from human adult brain SVZ and they give origin to neurons and glial cells with electrophysiological activity. These results opens the possibility of expansion of human adult stem cells *in vitro* obtained from small biopsies or from surgical procedures to treat the disease.

Palavras-Chave: Stem Cell; Neural Stem Cells; Epilepsy; Neuron; Neurosphere; Electrophysiological Activity

Apoio Financeiro: Brazilian Ministry of Health, CNPq, CAPES and FAPERJ

A.04.001

PRESENÇA DE MARCAÇÕES NO NÚCLEO GIGANTOCELULAR APÓS APLICAÇÃO DE HRP NA ÁREA PRESSORA CAUDAL BULBAR.

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Objetivos: Identificar diferentes projeções de neurônios da região bulbar, com a área pressora caudal (CPA), descrita como importante núcleo no controle cardiovascular.

Métodos: Quatro ratos wistar machos (270 – 300 g.), anestesiados com cloral (0,4g/kg), posicionados no aparelho estereotáxico com barra incisiva a -11mm. Cânula de microinjeção posicionada na APC (1,0mm caudal e 1,7mm lateral ao obex; 2,0mm da superfície encefálica) e o traçador axonal retrogrado horseradish peroxidase (HRP; 20%; 50nl), injetado unilateralmente. A incisão foi suturada e os animais mantidos vivos durante três dias, tempo necessário para o deslocamento do traçador. Os ratos foram profundamente anestesiados e perfundidos transcardiacamente com 500ml de solução salina; 500ml de paraformaldeído a 1% e 1,25% de glutaraldeído em buffer fosfato 0,1M (pH 7,6). O tronco cerebral foi seccionado com uma espessura de 40 µm e tratado com Diaminobenzidina a 0,05%. Esta reação promoveu uma coloração escura nos neurônios marcados. As secções foram coradas com vermelho neutro e os neurônios marcados visualizados com o auxílio do microscópio óptico, sendo suas coordenadas analisadas com o auxílio de um atlas histológico.

Resultados: Foram encontrados neurônios marcados no núcleo reticular gigantocelular (Gi), núcleo reticular gigantocelular alfa (GiA), núcleo reticular gigantocelular ventral (GiV). Quatro neurônios com HRP intracelular foram encontrados no núcleo GiA, três estavam -11,30 mm e um -11,60 mm caudais ao Bregma. A profundidade em relação à superfície do crânio era de 10,70 mm aproximadamente, ou seja, na superfície ventral bulbar. Outros quatro neurônios foram observados no núcleo GiV entre -11,80 mm e -11,96 mm do Bregma estando com aproximadamente 10,50 mm de profundidade da superfície craniana. Um único neurônio foi encontrado no núcleo Gi a -11,96 mm caudal ao Bregma, com profundidade de 10,30 mm da superfície craniana.

Conclusão: O estudo demonstra a presença de projeções eferentes monossinápticas dos núcleos Gi, GiA e GiV para a APC. Com base nestas evidências é possível sugerir que estas projeções estejam relacionadas, pelo menos em parte, com o controle das atividades cardiovasculares em ratos.

Palavras-Chave: HRP, APC, GiA, GiV

Apoio Financeiro: CAPES

A.04.002

AXONAL BIFURCATION AS TARGET-FINDING STRATEGY DURING DEVELOPMENT

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The main efferent routes from the cerebral cortex are the internal capsule (IC) and the corpus callosum (CC). In adults, cortical neurons project through one of these routes, but never to both, a feature that is considered to exist since early in development. We aimed to investigate (1) if axonal bifurcation is a possible strategy used by cortical neurons to find the midline (ML), (2) how neuronal position in the cortex influences bifurcating behavior, and (3) if bifurcation is cell-dependent or driven by environmental signals. We performed three different experiments in mice from E17 to P9. (1) DiI crystals were inserted into the CC of one hemisphere or into the IC, looking for labeled somata in different mediolateral cortical sectors. Moreover, (2) E17.5 EGFP+ dissociated cortical cells from medial or dorsolateral sectors were decanted onto wild-type slices (Slice Overlay) with preserved ML and IC or without ML and/or IC. Finally, (3) slice overlay assays were performed with blockage of netrin-1 or Src-family kinases (SKF). All three experiments were analyzed to quantify the number of bifurcated and non-bifurcated callosal axons in the white matter, in correlation with the mediolateral positions of the cell body. Our results indicated that in E18 and P1, bifurcated axons are 40% more frequent than in older animals (P9). Also, the majority of bifurcated axons are originated from neurons dorsolaterally placed in the cerebral cortex. In addition, callosally projecting neurons bifurcate more frequently than subcortically projecting cells. *In vitro*, cells originated from any mediolateral position bifurcate when plated on dorsolateral but not on medial cortex, indicating that environmental cues are important to regulate this behavior. The intermediate targets are important to promote bifurcation in cerebral cortex, since the absence of ML or IC hinders bifurcating behavior of axons. Netrin-1 blockage also induces lack of bifurcation and lead to misrouting of axons to the ipsilateral cerebral cortex. Inhibition SKF converts the axonal behavior: medial cells bifurcate and the dorsolateral do not. These results suggest that transient bifurcation could be a strategy used by callosal neurons searching for their target. The existence of a transient, spatially regulated bifurcating phenotype supports this hypothesis. Our results also suggest that extrinsic factors from both intermediate targets regulate bifurcation. The guidance molecule netrin-1, involved in the assembly of CC, and SKF could be responsible for this regulation of axonal bifurcations.

Palavras-Chave: corpus callosum, netrin, Src kinase, axonal guidance,

Apoio Financeiro: FAPERJ, CNPq, Ministério da Saúde.

A.04.003

Evidence for the involvement of SARA in endosomal trafficking and neuronal polarization

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SARA is a protein, which has a binding domain to Smad2 and Smad3 proteins and a double zinc-finger domain FYVE (Fab1p, YOTB, Vac1p y EEA1) that enables it to attach to PI3P with high specificity and to localize to early endosomes. It has been demonstrated in cell lines that SARA plays a crucial role in the Rab5-regulated endosomal trafficking. Since membrane recycling is an essential event in the establishment of neuronal polarity, in this study we have analyzed the function of SARA in the regulation of process formation and membrane delivery in cultured hippocampal pyramidal neurons.

Confocal microscopy revealed that SARA is endogenously expressed in hippocampal neurons and partially colocalize with Rab5. The overexpression of SARA causes an enlargement of the early endosomal compartment labelled with Rab5-GFP. The cells coexpressing SARA and Rab11-GFP were unable to form a typical recycling endosome as evidenced by the lack of the yuxtanuclear Rab11 positive structure. The suppression of SARA using siRNA and cotransfected with Rab11GFP display a large recycling endosome.

Morphometric analysis of neurons ectopically expressing SARA revealed a significantly reduction in the total neuritic length; by contrast, SARA suppression induced neurons with multiple and large axons compared with GFP control cells. These observations prompted us to analyse with more detail the participation of SARA in membrane addition.

L1 is a neuronal adhesion molecule that is important for axon outgrowth and pathfinding, being constantly endocytosed and recycled back to the plasma membrane in the axonal growth cone. Neurons overexpressing SARA shown less L1 at the axonal membrane compared with control ones, while those transfected with SARA siRNA showed an enhanced membrane delivery. In support of this, TIRF microscopy revealed that neurons co-expressing SARA and the transferrin receptor fused to GFP (TfR-GFP) showed a significant reduction of fusion events at the somatodendritic plasma membrane. Taken together, our results suggest that SARA regulates neuronal polarity by regulating the trafficking of membrane from the early endosome to the recycling endosome, both in axons and dendrites.

Palavras-Chave: SARA, endosome, trafficking, neuronal polarization

Apoio Financeiro: CONICET and FONCyT

Differential Expression of Connexins During Histogenesis of the Chick Retina

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Objectives: Gap junction (GJ) channels couple adjacent cells, allowing transfer of second messengers, ions, and molecules. These channels are composed by a multigene family of integral membrane proteins called connexins (Cx). In the retina, besides being essential circuit element in the visual processing, GJ channels also play important roles during its development. Herein, we analyzed Cx43, Cx45, Cx50, and Cx56 expression during chick retinal histogenesis.

Methods: Experiments were carried out with *Gallus gallus*, ranging from embryonic day 4.5 (E4.5) to 15 days after hatching (E4.5, E7, E12, E15, E18, and P15). The retinas were collected and prepared for Immunohistochemistry, Real-Time PCR, and Western Blotting.

Results: Cx43 immunolabeling was observed at early development, in the transition of ventricular zone and pigmented epithelium. Later, Cx43 was seen in the outer plexiform and ganglion cell layers and in the inner plexiform layer. Cx45 showed stable gene expression levels throughout development and ubiquitous immunoreactivity in progenitor cells. From later embryonic development, Cx45 was mainly observed in the inner retina, by glial cells and neurons. Cx50 was virtually absent at initial embryonic phases but expressed by neurons afterwards. In turn, Cx56 was not detected in the retina. PCR results indicated that Cx43 gene is highly expressed in E4.5 (67-fold, $p<0.001$) and E7 (64-fold, $p<0.01$) when compared with P15. Cx45 transcripts showed constant steady-state levels during the development. Cx50 gene is poorly expressed in retinas from E4.5 (23%, $p<0.001$) and E7 (35%, $p<0.01$) when compared to P15. In the western blotting, Cx43 is highly expressed at E4.5 (255%, $p<0.001$) when compared to P15. Cx45 optical density did not vary through studied ages. Cx50 was shown to be virtually absent in retinas from E4.5 (4%, $p<0.01$) and E7 (9%, $p<0.01$), and poorly expressed in E15 (29%, $p<0.01$) when compared to the mature retina.

Conclusions: Characterization of Cx expression in the developing chick retina indicated particular roles for these proteins and revealed similarities and differences when compared to other species.

Palavras-Chave: development, gap junction, connexin, retina, gene expression, histogenesis

Apoio Financeiro: CNPq, FAPESP

A.04.005

Efeito da degradação do condroitim sulfato na plasticidade cortical

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Os mamíferos possuem a representação completa da superfície do corpo no córtex somestésico primário (S1). Em alguns roedores, a região cortical de representação do focinho apresenta uma distribuição tanto de axônios tálamo-corticais quanto de células da camada IV que formam módulos chamados de barris. O término do período de plasticidade no campo dos barris corresponde à maturação da matriz extracelular e suas especializações, como as redes perineuronais (RPNs), compostas por macromoléculas como os proteoglicanos de condroitim sulfato (PGCS), que inibem o crescimento axonal e a plasticidade. Para verificarmos se a degradação do PGCS pela enzima condroitinase ABC prolonga a plasticidade em S1, ratos neonatos tiveram todas as vibrissas de uma das faces retiradas durante o período de P0 a P10, para causar uma desorganização no padrão dos barris. Nas idades de P20, P40 ou P70 esses animais foram submetidos ao implante de fatia do polímero ELVAX saturado ou com a enzima condroitinase ABC (50 U/ml) ou enriquecido com tampão fosfato salina (controle). Três ou dez dias após o implante, os córtices foram aplanados, cortados em criostato e submetidos a diferentes reações imuno e histoquímicas para determinar a eficácia da enzima e a distribuição do PGCS. Foram feitas imunofluorescências para o anticorpo que reconhece o glicosaminoglicano (GAG) de condroitim sulfato e para o anticorpo que reconhece um epítipo exposto no núcleo protéico após a digestão do GAG do PGCS, além da histoquímica para a atividade da enzima citocromo oxidase, que revela o padrão de barris após o tratamento, e para a lecitina *Vicia villosa*, que marca o PGCS presente na RPN. O uso do ELVAX como veículo para a difusão lenta da droga mostrou-se eficaz, com uma liberação crescente até 96 horas, com uma grande variação entre as amostras. Todos os animais sacrificados após dez dias apresentaram a recuperação morfológica dos barris. Após três dias de implante somente os animais que sofreram implante em P20 tiveram uma resposta imediata significativa. A alteração da RPN promoveu a plasticidade em S1, já que foi possível recuperar o padrão normal de barris que havia sido alterado pela retirada das vibrissas.

Palavras-Chave: barril, plasticidade, período crítico, córtex somestésico primário

Apoio Financeiro: FAPERJ, PRONEX

A.04.006

Developmentally regulated expression, localization and functional regulation of CALEB, an EGF-like protein

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The establishment of precise and selective synaptic connections between neurons during embryonic and early postnatal development depends on the coordinated interplay of an orchestra of molecular components. One these components is CALEB (chicken acidic leucine-rich EGF-like domain containing brain protein), a transmembrane protein. Due to its structural features, it belongs to the EGF-family. It was shown that CALEB is converted by neuronal activity and involved in regulating neuronal connectivity early in development.

Analysis of the expression of CALEB by immunohistochemistry, immunoblot and RT-PCR showed that it is restricted to the central nervous system. It appeared in embryonic and early neonatal stages in development and expression peaks between postnatal day 10 and 20, a period of synaptogenesis and synapse refinement. Subcellular localization of CALEB studied by bio- and immunocytochemical methods in dissociated neuronal cell cultures revealed that CALEB is predominantly located in plasma membranes of dendrites and cell bodies, but not in axons. Double immunostaining of CALEB together with pre- or postsynaptic proteins provided evidence for a partial localization on synapses.

Protein phosphorylation and dephosphorylation are pivotal steps in signal transduction and play a key role in the regulation of many cellular processes. Therefore, potential Phosphorylation sites of CALEB were analyzed by mass spectroscopy and phospho-motif antibodies.

In summary, the developmentally regulated expression pattern and localization of CALEB supported our physiological studies and suggests that CALEB is implicated in the formation of neuronal circuits.

Palavras-Chave: synapse, subcellular localization, development

Cellular and subcellular localization of Marlin-1

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Objectives

Marlin-1 (Jamip1, Jakmip1) belongs to a family of three structurally related proteins which are highly conserved in vertebrates. Marlin-1 is a GABA_B receptor-binding protein that associates specifically to the GABA_BR1 subunit in neurons. It also binds microtubules and kinesin-1. This work describes the cellular and subcellular localization of Marlin-1 in the rodent brain and in hippocampal neurons, with emphasis in its relationship to the cytoskeleton.

Methods

Immunohistochemistry: Immunostaining was performed with Marlin-1 antibody using the Universal ICQ LSAB plus kit in sagittal sections of mouse brains.

Immunofluorescence: Staining was carried out in fixed hippocampal neurons attached to glass coverslips.

Pre-embedding electron microscopy: Coronal sections of rat brains were stained with Marlin-1 and secondary antibodies coupled to 1.4 nm gold particles. Gold particles were enhanced with the HQ Silver kit. The CA1 region of the hippocampus was selected for analysis.

Microtubule association assay: Rat brains were homogenized, fractionated and the microsomal fraction containing Marlin-1 was left untreated or treated with 20 μ M Taxol or 10 μ M nocodazole for 40 min.

Results

Marlin-1 is abundant in restricted regions of the brain such as hippocampus, olfactory bulb, cerebral cortex, medulla and cerebellum. Marlin-1 is present in dendrites and axons of gabaergic and non-gabaergic hippocampal neurons. In the axon it shows a preferential colocalization with acetylated tubulin. At the ultrastructural level Marlin-1 is present in the cytoplasm and the nucleus of CA1 neurons. In the cytoplasm it associates to microtubules in the dendritic shaft. Marlin-1 is excluded from synapses, the Golgi apparatus and the endoplasmic reticulum. In the nucleus, clusters of Marlin-1 associate to euchromatin. A biochemical assay confirms the association of Marlin-1 to microtubules in the brain.

Conclusions

The subcellular distribution of Marlin-1 and its association to microtubules is consistent with a role in the organization of the neuronal cytoskeleton. **Palavras-Chave:** Marlin-1, microtubules, electron microscopy.

Apoio Financeiro: FONDECYT 1071001, ICM-P04-068-F

TREINAMENTO FÍSICO NÃO ALTERA ESTRUTURA DO NERVO CIÁTICO DE RATOS ADULTOS DESNUTRIDOS NO PERÍODO CRÍTICO DE DESENVOLVIMENTO.

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Objetivo: Avaliar o efeito do treinamento físico sobre a estrutura do nervo ciático de ratos adultos desnutridos na gestação e na lactação. **Método:** Ratos machos *Wistar* (n=24) foram mantidos em condições padronizadas de biotério. Os animais foram obtidos acasalando-se ratas primíparas (*Wistar*, n=17, peso corporal = 241,2±23,1g) com um macho da mesma linhagem segundo o ciclo estral. Detectada a prenhez através da presença de espermatozóides na secreção vaginal, as ratas foram distribuídas em grupo Nutrido (N, n= 9, caseína 17%) e grupo Desnutrido (D, n = 8, caseína 8%) e receberam dieta conforme seu grupo nutricional na gestação e lactação. A recuperação nutricional dos filhotes iniciou no 21º dia de vida (Nuvilab CR1-NuvitalO). Aos 60 dias de idade, os animais foram subdivididos em grupos: nutrido não-treinado (NNT, n=6), nutrido treinado (NT, n=6), recuperado não-treinado (RNT, n=6) e recuperado treinado (RT=6). O programa de treino físico moderado utilizou esteira ergométrica (Insight[®]) durante 8 semanas, 5 dias/semana, 60 minutos/dia e após o seu término os animais foram anestesiados para a coleta do nervo ciático. O material foi fixado em solução de glutaraldeído a 2,5% e processado para obtenção de cortes semi finos (0,38 mm) corados com azul de toluidina. Foi realizada a mensuração da área do nervo (mm²), da contagem do número de fibras mielinizadas e da densidade de fibras. Foi aplicada a análise de Variância (ANOVA) seguida do teste de Tukey e o teste Mann-Whitney considerando significância de 5%. **Resultados:** A área do nervo ciático (NNT: 0,58±0,4; NT: 0,53±0,5; RNT: 0,52±0,5; RT: 0,57±0,6, M±SEM), a densidade nervosa (NNT: 6233,93±521,91; NT: 5900,56±1029,41; RNT: 6251,69±541,14; RT: 5594,95±530,89, M±SEM) e o número de fibras nervosas mielinizadas (NNT: 3609,50±391,52; NT: 3019±185,38; RNT: 3260±375,18; RT: 2929±364,18, MED±SEM) não revelaram diferença na comparação entre os grupos (p>0,05). **Conclusão:** O modelo de desnutrição protéica utilizado não causou danos ao nervo ciático dos animais e o treino físico aplicado nos ratos adultos não modificou a estrutura do nervo nos grupos avaliados.

Palavras-Chave: nervo ciático; desnutrição protéica; treinamento físico.

Apoio Financeiro: CNPq

A.04.009

Urokinase-type plasminogen activator and the plasminogen activator inhibitor-1 (uPA-PAI) complex promote cell migration and axonal outgrowth in the developing optic tectum.

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Objectives: The aim of this work is to investigate the role of the urokinase-type plasminogen activator - plasminogen activator inhibitor-1 (uPA-PAI) complex and the uPA receptor in cell migration and neuritogenesis using the avian optic tectum (OT).

Methods: OT obtained from embryos of 6 days were dissected. Each portion was grown in DMEM with glutamine and N2, incubated at 37 °C for 20 hours and then treated with uPA, PAI1 or both at a final equimolar concentration of 0.74 nM. After 4 hours, the explants were observed by phase-contrast microscopy and photographed. The images were assembled and two different variables were measured: cell migration pattern and axonal length.

Immunohistochemistry: After the treatment explants were fixed in 4% paraformaldehyde in 0.1M phosphate buffer, blocked with 5% normal goat serum and incubated with anti-PAI-1 (Calbiochem[®], final concentration 25 µg/ml) or with the primary antibody anti- urokinase-type plasminogen activator receptor (uPAR, Santa Cruz[®], final dilution 0.4 mg/ml) for 48 hours at 4°C. After incubation, the primary antibodies were removed and explants were incubated with the appropriate secondary antibodies Alexa Fluor (Molecular Probes[®]) in order to detect uPAR and PAI1 or with phalloidin-rhodamine (Sigma[®], 1/1000) to detect actin cytoskeleton.

Results: The results show a significant increase in the number of cells (48.16% +/-16%) and the covered distance (19% +/-1.8%) of migrating cells as well as an increase in the number of axons (13.3 % +/-1.9%) in the explants treated with uPA-PAI1 0.74 nM compared with the control explants. The immunohistochemistry shows an increase in the number of stress fibres in treated explants compared with the controls and the same expression pattern of PAI1 and uPAR

Conclusion: These results indicate that uPA-PAI1 complex participates actively in the process of cell migration and neuritogenesis. The appearance of stress fibres in treated explants suggests that the reorganization of the actin cytoskeleton is the result of the activation of an intracellular signaling pathway mediated by the complex uPA-PAI- uPAR.

Palavras-Chave: development- plasminogen activator system- neuritogenesis- neuronal migration

Apoio Financeiro: This work was supported by grants of UBACyT and CONICET. Argentina.

A.04.010

Post-endoplasmic reticulum assembly of GABA_BR subunits in dendrites of hippocampal neurons

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OBJECTIVES

Neuronal GABA_B receptors are heterodimers composed of GABA_BR1 and GABA_BR2, and their availability at the plasma membrane is required to maintain the efficacy of synaptic inhibition. However, the mechanisms that regulate their transport, assembly and insertion remain poorly understood. For example, it is not known if synthesis and assembly of GABA_B receptors occur in the somatic endoplasmic reticulum or whether somatic synthesis is followed by independent transport of the subunits for assembly in distal projections. To discriminate between these possibilities we studied the association of GABA_B receptor subunits relative to the topology of the neuron.

METHODS

We combined fluorescence microscopy, an improved quantitative colocalization analysis and biochemistry.

RESULTS

GABA_B receptor subunits are segregated in intracellular compartments of dendrites and axons. The subunits are independently mobile in dendrites and the proportion of non-associated intracellular subunits is high in the brain. Both subunits localize preferentially to the endoplasmic reticulum in dendrites, but the colocalization between GABA_BR1 and GABA_BR2 is higher in post-ER compartments including Golgi outposts.

CONCLUSIONS

Our findings demonstrate that GABA_B receptors are synthesized in the cell body and remain segregated in the endoplasmic reticulum of neuronal projections consistent with a mechanism of independent transport and distal assembly in a post-ER compartment.

Palavras-Chave: GABAB, axons, dendrites, colocalization, Manders coefficient, endoplasmic reticulum, Golgi apparatus.

Apoio Financeiro: FONDECYT 1071001, MECESUP, CONICYT

A.04.011

Regulation of Phospholipid Synthesis by the Protein c-Fos

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OBJECTIVES: Our group described that the oncoprotein c-Fos associates with the Endoplasmic Reticulum (ER) and activates phospholipid synthesis (*FASEB J.* 15:556, 2001). This activity of c-Fos is required for PC12 cells to develop neurites; the 22 amino acids (aa) of the Basic Domain of c-Fos (aa 139-159) are capable of promoting comparable levels of phospholipid synthesis activation as that obtained with full length c-Fos (*Mol Biol Cell.* 15:1881, 2004).

*As of the 22 aa of BD, 12 are basic ones, we examined which of these are relevant for c-Fos/ER association and for phospholipid synthesis activation.

*As c-Fos activates the synthesis of phospholipids, to obtain information respect to the molecular mechanism involved, we first determined that c-Fos activates CCT β 2, the rate-limiting enzyme of Kennedy Pathway of synthesis of phosphatidylcholine and important in neurite outgrowth and branching (*J. Biol. Chem.* 283:202, 2008), and then examined if there is a direct interaction between c-Fos and CCT β 2.

METHODS: The following methods were used in cultures of T98G cells that derive from a human brain tumor and of NIH3T3 fibroblasts:

*Site directed mutation for Overlap Extension-PCR for heterologous expression and purification of proteins;

**In vitro* phospholipids labeling;

* Immunofluorescence and colocalization analysis of c-Fos and CCT β 2 proteins;

* Immunoprecipitation assays between endogenous c-Fos and transfected CCT β 2 tagged enzyme.

RESULTS: We prepared 3 point-mutated c-Fos proteins by substituting one basic aa with a neutral one. The substitution of aa 146 is enough to promote the loss of the lipid synthesis activating capacity of c-Fos whereas the same substitutions in positions 139 and 144 have no effect.

By immunofluorescence and immunoprecipitation assays, c-Fos was found associated to the ER and co-localizing with the enzyme CCT β 2.

CONCLUSIONS: The results presented herein indicate that the aa in position 146, a central position of the BD that shows an α -helix structure, is important for the c-Fos/ER association required for phospholipid synthesis activation. Having found that c-Fos interacts with CCT β 2 further supports our hypothesis that a direct interaction between c-Fos and the enzymes it activates is required.

Palavras-Chave: c-Fos, Phospholipid, CCT β 2

Apoio Financeiro: CONICET and SECyT-Universidad Nacional de Cordoba, JS McDonnell Foundation

A.04.012

Estradiol induces the expression of an estrogen receptor α variant at the cell- surface of hypothalamic neurons.

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Previous studies from our laboratory have demonstrated that the axogenic effect of 17- β -estradiol (E2) on male hypothalamic neurons is not exerted through the classical intracellular estrogen receptor (ER) but depends on a membrane mechanism involving Ca²⁺, PKC and ERK signaling. Recently, we found that a truncated ER α was localized in membrane fractions of hypothalamic tissue from E16 embryos. Moreover, our experiments extend these results to hypothalamic neurons *in vitro* showing that ER α can be detected from the cell exterior as a biotinylated cell-surface protein.

In the present study we investigate the effect of E2 treatment on ER α localization. To this end, we used the membrane impermeant sulfo-NHS-biotin to label cell-surface proteins of hypothalamic neurons grown for 48h with or without 10 nM E2. The labeled proteins were collected with avidin-agarose beads to obtain 2 fractions: the pellet fraction, with the cell-surface biotinylated proteins; and the supernatant fraction, with the intracellular un-biotinylated proteins.

In E2 treated cultures, Western blot analysis revealed the presence of ER α in both fractions; in the supernatant there appeared the full-length (66 kDa) and the truncated (~ 55 kDa) ER α , whereas in the pellet there appeared only the truncated isoform of ER α . In control cultures, ER α was not evident in the pellet fraction. Using different antibodies against ER β (ZYMED and SCBT) and GPR30 (MBL), no immunoreactive bands were detected in the pellet fraction. Immunocytochemical analysis showed the expression of ER α in the cell- surface of non-permeabilized neurons. A strong staining at the cytoplasmic and nuclear levels was observed in permeabilized neurons.

In summary, these results confirm the presence of an ER α on the plasma membrane of hypothalamic neurons *in vitro*. We have also shown that E2 treatment induces the expression of the truncated isoform of ER α at neuronal cell-surface. Although the mechanisms of E2 translocation to the neuronal cell-surface will have to be addressed, the evidence presented in this report indicates the need to re-evaluate the current estrogen signaling models.

Palavras-Chave: Estradiol, estrogen receptor, cell-surface, hypothalamic neurons

Apoio Financeiro: CONICET and FONCyT

A.04.013

Avaliação dos efeitos da estimulação elétrica ao músculo desnervado do rato por meio de análise funcional da marcha e eletrodiagnóstico clássico

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Introdução: A reconhecida reciprocidade entre o nervo e seu tecido alvo, fomenta a busca por modelos experimentais na tentativa de conjugar conhecimentos sobre regeneração nervosa e o desenvolvimento de estratégias que promovam a recuperação sensório-motora do segmento corporal afetado. **Objetivos:** Avaliar os efeitos funcionais e sensitivos da estimulação elétrica muscular aplicada em diferentes períodos após lesão nervosa periférica em ratos. **Métodos:** 30 ratos adultos machos foram submetidos a axonotmese do nervo ciático direito e divididos em três grupos de dez animais – cinco experimentais (GE) e cinco controle (GC), tratados pelo mesmo protocolo de estimulação (GC, aparelho desligado) e avaliados funcionalmente (IFC). Os três grupos diferiram entre si quanto ao período de tratamento após a lesão: o grupo 1 (G1), do 1º ao 7º dia; G2, do 8º ao 14º e o G3, do 15º ao 21º. Em dois grupos adicionais, o tempo de tratamento foi prolongado. Estes animais foram estimulados durante 21 dias, G4, do 1º ao 21º, e G5, do 8º ao 28º dia e comparados aos seus GC análogos por meio de cronaxia e reobase (valores elevados denotam processo de desnervação muscular) e do IFC. **Resultados:** A EE prejudicou a regeneração do nervo ciático dos animais tratados durante a segunda e terceira semana após a lesão, enquanto nos animais que receberam a EE na primeira semana a evolução funcional apresentada pelo GE foi levemente melhor comparada a do GC. O prolongamento do tempo de tratamento mostrou-se negativo quanto às variáveis analisadas (IFC, cronaxia e reobase) quando se compara GE e GC. **Conclusão:** Os efeitos funcionais e sensitivos da EE sobre o músculo desnervado do rato parecem depender tanto do momento em que se inicia o tratamento, quanto do tempo que ele dura. Se prolongado, pode ser prejudicial e se tardar muito a começar, também. Nossos dados demonstraram a existência de um momento mais propício ao tratamento elétrico da musculatura desnervada. Isto reforça a necessidade pela busca de parâmetros físicos de corrente mais adequados à terapia elétrica muscular no que se refere ao modelo de estudo da regeneração nervosa periférica em ratos.

Palavras-Chave: Lesão nervosa periférica; estimulação elétrica muscular; regeneração nervosa; análise funcional; eletrodiagnóstico

Apoio Financeiro: CNPq; FUNAPES; Depto Fisioterapia - FURB

A.04.014

Inhibition of EphA4 increases axon growth of retinal ganglion cells

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The retinotectal system is useful to study topographic map formation because nasal retinal ganglion cells (RGC) connect to the caudal tectum and temporal ones connect to the rostral tectum. Eph receptor tyrosine kinases and their ligands, the ephrins, are expressed in complementary gradients in both the retina and the tectum and contribute to guide retinotectal projections. Both Ephs and ephrins function as receptors and ligands producing bidirectional signaling. Ephrin-As located in the caudal tectum repel temporal axons by activating the axonal EphA3. Ephrin-As expressed in nasal RGC activate EphA4 converting nasal RGC less sensitive to tectal ephrin-As. We demonstrated that tectal EphA3 promotes axon growth of RGC toward the caudal tectum.

Our **objective** was to investigate whether the level of activation of axonal EphA4, produced independently of tectal ephrin-As, regulates the rate of axon growth of RGC.

Methods: We used retinal explants from 7 days-old chicken embryos to investigate: 1) the expression pattern of ephrin-As and EphA4 in growth cones in vitro by immunocytochemistry; and 2) the effect of inhibiting the axonal EphA4 activity by using an inhibitory peptide (KYL) (Molecular and Cel Neurosci. 24:1000, 2003) in vitro.

Results: EphA4 is expressed homogeneously in nasal and temporal growth cones. Ephrin-As are highly expressed in nasal and moderately in temporal growth cones. It was previously demonstrated that axonal ephrin-As activate axonal EphA4. Control temporal RGCs grow statistically significant longer axons than nasal ones.

Inhibition of axonal EphA4 produces a significant increase in axon growth of RGC. .

Conclusions: The fact that inhibition of axonal EphA4 increases axon growth independently of tectal ephrin-As suggests that activation of axonal EphA4 by axonal ephrin-As decreases axon growth in vitro. This supports the idea that topographic mapping is influenced by Eph-ephrin interactions not only between RGC axons and tectal cells but also among neighboring axons.

References:

Molecular and Cel Neurosci. 24:1000, 2003

Palavras-Chave: Eph-ephrin, retinotectal system, development, axon guidance

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A.04.015

Inducción de SVCT2 en la corteza cerebral postnatal. (SVCT2 induction in the postnatal cerebral cortex)

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Introducción. Debido a que el ácido ascórbico (AA) no puede ser sintetizado en el cerebro, los altos niveles registrados en este órgano se logran mediante mecanismos de captación específicos. SVCT2wt media el transporte dependiente de sodio con alta afinidad y es necesario para la captación de vitamina C en las células del SNC. La función de SVCT2wt podría estar regulada por una isoforma corta del transportador (SVCT2sh), recientemente clonada de cerebro fetal. Actualmente, no existe evidencia sobre la expresión y función de SVCT2 en el cerebro post-natal, aún cuando, AA ha sido relacionado con diferenciación y maduración neuronal.

Metodología. Analizamos la expresión de SVCT2 en el cerebro de ratones en desarrollo post-natal mediante análisis de RT-PCR, Western blot, hibridación *in situ* e inmunohistoquímica. Además, se utilizaron las construcciones SVCT2wt-YFP y SVCT2sh-YFP (dominante negativo) para sobre-expresar el transportador en células N2a y obtener datos funcionales.

Resultados. Nuestros datos demuestran que neuronas de la corteza cerebral, células troncales ventriculares y líneas celulares de neuroblastoma expresan el transportador de ácido L-ascórbico dependiente de sodio SVCT2. Esta proteína es inicialmente inducida en las neuronas más profundas de la corteza cerebral durante la vida post-natal. Mediante la utilización de células N2a y análisis de FRET demostramos una interacción intracelular entre SVCT2wt and SVCT2sh. Los parámetros cinéticos muestran que SVCT2sh inhibe la captación de AA en células N2a.

Conclusiones. La diferenciación y maduración postnatal de neuronas corticales esta asociada a un incremento en la expresión de SVCT2 y su actividad podría ser regulada a través de la hetero-oligomerización de SVCT2wt y su dominante negativo.

Palavras-Chave: SVCT2, Vitamina C y corteza cerebral postnatal

Apoio Financeiro: Proyecto Anillo ACT-02, Becaria CONICYT (KS).

A.04.016

The role for adenosine A2a receptor in the stabilization of retinotectal topography.

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The uncrossed retinotectal projections of rats undergo an extensive reorganization within the first ten post-natal days when the terminal fields acquire an adult-like appearance. The fine-tuning of these connections is a result from multiple factors. Previous studies had shown that adenosine, a purine molecule, acts as a neuromodulator involved in nervous system development and plasticity through activation of metabotropic receptors classified in A₁, A_{2a}, A_{2b} e A₃. Recently we had demonstrated the expression of both A₁ and A_{2a} receptors in the superior colliculus. In the present study, we investigated the role of A_{2a} receptor in the distribution of uncrossed retinotectal axons.

Lister Hooded rats at PND7 were submitted to a subpial ELVAX implant containing DMSO (vehicle) or SCH 58261 (1 μ M). Animals were maintained with treatment until PND28. Horseradish peroxidase intraocular injection was used as an anterograde tracer to stain retinal ganglion cells terminal axons.

Our data show that A_{2a} receptor blockade induces a fusion of terminal clusters compared to control, indicating a plastic phenomenon. Moreover, this pharmacological treatment also leads to an up-regulation in both A₁ and A_{2a} receptors.

Taken together the results suggest that adenosine A_{2a} receptors are involved in the formation of uncrossed retinotectal connections specificity. Furthermore, our data confirm that A_{2a} receptor control the expression of A₁ adenosine receptor, indicating that a balance of purinergic A₁ and A_{2a} signaling is crucial to retinotectal development.

Palavras-Chave: adenosine receptors; antagonist A_{2a} receptor; retinotectal development

Apoio Financeiro: CNPq, CAPES, FAPERJ, PRONEX-MCT

A.04.017

A local blockade of A1 adenosine receptor disrupts retinotectal topography within the critical period.

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The retinotectal projection of rats develops within a critical period. The plasticity of this pathway can be induced by lesions or experimental manipulations that interfere with the pattern of electrical activity, release of retrograde messengers or neurotransmitters. Adenosine is a neuromodulator that has been implicated in nervous system development and plasticity. The adenosine actions are mediated by metabotropic receptors which are classified as A1, A2a, A2b e A3. A1 receptor can exert pre-synaptic modulation by controlling neurotransmitter release. Previously we had shown that A1 receptors are expressed predominantly in the early stages of development. Herein, we investigated the role of A1 receptor in the formation and maintenance of retinotectal topography.

Lister Hooded rats were treated with A1 receptor antagonists (DPCPX – 8-cyclopentyl-1,3-dipropylxanthine, 1 μ M). Subpial ELVAX implants to deliver DPCPX or vehicle to the superior colliculus were done at postnatal day 7 (PND7) and the animals were maintained with drug until PND14 or PND21. To analyze the distribution of retinotectal axons under pharmacological or vehicle treatment, animals received intraocular injections of horseradish peroxidase (HRP) as an anterograde tracer. A group of treated-animals were used for western blotting to analyze the expression of adenosine receptors.

Our data showed that blockade of A1 adenosine receptor induced by DPCPX during the critical period leads to an exuberant sprouting of ipsilateral projections compared to controls, vehicle treated-rats. Both periods evaluated demonstrated the plastic potential of inhibition of A1 receptor. This treatment also induced an up-regulation of A1 expression.

In conclusion, the present study suggests that A1 adenosine receptor plays a crucial role in retinotectal topographical development.

Palavras-Chave: A1 adenosine receptor; DPCPX; retinotectal development

Apoio Financeiro: FAPERJ; CAPES; CNPq; PRONEX-MCT

A.04.018

Peptides analogous to the chemokine SDF-1 stimulates migration and axonal branching in cultured cerebellar neuronal precursors

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The axis stromal cell-derived factor 1(SDF-1/CXCL12)-CXCR4 plays an important role in CNS development by mediating cell migration, enhancing precursor cell proliferation and assisting in neuronal circuit formation. In the cerebellum, absence of SDF-1/CXCR4 signaling results in premature invasion of many proliferating granule cells into the cerebellar anlage, SDF-1 also acts as a chemoattractant for cerebellar granule cells in culture. Interactions with its receptor CXCR4 occur at two binding sites in amino acids 1–17 of SDF-1. We aimed to evaluate the activity of synthetic peptides analogous to the N-terminus of SDF-1 containing 8-19 aminoacids on cerebellar neuronal progenitors migration, survival and proliferation *in vitro*. We first observed that the synthetic peptides promotes chemotaxis of lymphocytes, cells that express CXCR4, in the Boyden chamber assay. To investigate the role of the peptides as a chemotactic factor for neuronal precursors we established an *in vitro* real-time migration assay. The peptides stimulated the percentage of migrating cells without affecting distance, duration or speed of migration. Cell viability was determined by MTT assay and the peptides were able to improve neuronal precursors survival in the absence of KCl and growth factors. To determine induced cell proliferation we used [³H] thymidine incorporation. The results showed that the peptides inhibited proliferation in the presence of growth factors. We have also developed a model of injury *in vitro* based on the classical wound healing *in vitro* assay. In the absence of the SDF-1 analogous peptides, glial cells are responsible for closing of the lesion, whereas in the presence of the synthetic peptides we observed increased amount of axons crossing over the lesion. Our study shows that peptides analogous to the N-terminus of SDF-1 are able to induce chemotaxis and migration of cells that express CXCR4, help neuronal progenitors survival in an adverse environment, inhibit proliferation of neuronal precursors while stimulates axonal outgrowth.

Palavras-Chave: SDF-1/CXCL12, cerebellum, axonal outgrowth, migration.

Apoio Financeiro: Support: FAPESP, CNPq, FADA/UNIFESP, MCT/CNPq, DECIT/MS, Fundo Setorial de Biotecnologia (CT-BIOTECNOLOGIA).

INFLUENCE OF BOVINE MILK GANGLIOSIDES ON NEURONAL MIGRATION AND NEURITE OUTGROWTH

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INTRODUCTION: Gangliosides are a group of glycosphingolipids which contain sialic acid as a component of their carbohydrate chain. They are expressed at higher concentration in nervous tissue. Moreover, milk is also an important source of these molecules. The ganglioside 9-O-acetyl GD3 (9acGD3) has been associated with neuronal migration in developing nervous system. The Jones monoclonal antibody (Jones mAb) that recognizes 9acGD3 inhibits *in vivo* and *in vitro* neuronal migration. The main goal of this study was to investigate whether the 9acGD3 ganglioside was present in a bovine milk fraction enriched in gangliosides – *Ganglioside 600 (G600)* – and investigate its involvement in mechanisms of neuronal migration and neurite outgrowth *in vitro*.

METHODS: To identify whether the 9acGD3 ganglioside was present in *G600* a lipid solution was obtained using Folch Partition. The ganglioside fraction was separated using Lipophilic Sephadex LH-20 column. The gangliosides in the samples were separated by HPTLC using solvents system. The 9acGD3 ganglioside band was identified by its specific mobility in the plate (between GM1 and GM2 gangliosides), by its immunoreactivity with the Jones mAb and by its sensitivity to mild-base treatment. To test a possible effect on neuronal migration and neurite outgrowth, explant cultures of rat cerebellum were maintained in the presence of two concentrations of ganglioside solution purified from *G600* (1,7% and 3,4%). As a control we have used explants grown only in DMEM/F12 + 10% fetal calf serum. The Jones mAb was used to block the neuronal migration.

RESULTS: The 9acGD3 ganglioside was identified as one of gangliosides presentes on *G600*. The addition of 1,7% of ganglioside solution in the medium did not have any effect. Whereas 3,4% of the ganglioside solution increased in 47% the neuronal migration in our model. Furthermore, this concentration reverted the blockade caused by the Jones mAb. Moreover, the addition of gangliosides with and without mild-base treatment increased in 100% the neurite outgrowth.

CONCLUSIONS: The increase on neuronal migration and neurite outgrowth is due the presence of 9acGD3 in *G600* bovine milk fraction.

Palavras-Chave: Gangliosides; 9-O acetyl GD3; Cerebellum; Migration; Neurite outgrowth

Apoio Financeiro: FAPERJ, CNPq, Fonterra

A.05.001

Comparative analysis of the retinal GABAergic system in neonate and adult *Octodon degus*

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Objectives. In the vertebrate retina, γ -aminobutyric acid (GABA) participates in inhibitory processes that modulate the visual response to permit adaptation and contrast enhancement. In addition, GABA is assumed to play a neurotrophic role during retinal development. Here, we compared the expression and function of the GABAergic system in the retina of neonate and adult *Octodon degus*, a diurnal rodent with diurnal-crepuscular activity and a gestational period of 90 days.

Methods. The retinal expression pattern of GABA, GAD65 and 67 and the three GABA receptor types GABA_A, GABA_B and GABA_C was compared by immunohistochemistry in neonate (P0) and adult *O. degus*. Electroretinography (ERG) was used to evaluate the overall function of the retina and to investigate the contribution of fast GABAergic transmission to the light-evoked responses at both developmental stages.

Results. GABA, its synthesizing enzymes and receptors displayed a similar cellular distribution in neonates and adults, but labeling of the outer plexiform layer, of Müller cells and of certain cell bodies in the inner retina was more intense in neonates, supporting a developmental role of GABA signaling. On the other hand, light sensitivity was ten times lower in neonates, light-evoked oscillatory potentials were undetectable and blockade of GABA_A receptors had no significant effect, while it increased the ERG b-wave by 22% in adults.

Conclusions. Our results show that full visual functionality is not yet accomplished in neonate *O. degus*, while the retinal GABAergic system presents a comparatively intense expression, suggesting a developmental function of GABA in the neonate retina.

Palavras-Chave: GABA, retina, *Octodon degus*, Müller cell, vision, development, electroretinogram

Apoio Financeiro: PBCT-Conicyt project ACT-45, "Research Ring in Sensory Neuroscience"

A.05.002

Transient exuberance of thalamocortical connections in the rat visual system.

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As a rule, cortico-cortical circuits develop through a stage of initial exuberance followed by the selective regression of many elements. But whether such events also occur for thalamocortical connections is unclear. By employing a new application of the lipophilic tracer DiI, we reveal massive transient projections in the visual thalamocortical pathway of postnatal rats.

Thalamocortical cells were retrogradely labeled by in vivo insertion of DiI crystals in the primary visual cortex of P1 rats. After different survival periods (2-14 days), the brains were fixed and (1) either processed and analyzed immediately, to visualize somatic fluorescent vesicles resulting from in vivo internalization and transport of tracer (vesicular labeling); (2) or stored in fixative for several months to allow for post-mortem diffusion of tracer along the cell membrane (membrane labeling). In control animals aged between P2 and P14, DiI crystals were inserted post-mortem.

In both groups 1 and 2, fluorescent cells were found throughout dorsolateral and medial thalamic regions, whereas they were restricted to dorsolateral nuclei in aged-matched controls. In group 2 brains, many cells were membrane labeled, indicating that they were connected with the site of crystal insertion at the age of sacrifice. Between P2 and P14, the proportion of such cells decreased markedly in medial nuclei and, to a lesser extent, in dorsal nuclei. In this latter region, we also encountered numerous cells with only vesicular labeling, suggesting that they had lost their connections before the day of sacrifice. Additional experiments using secondary insertion of DiO crystals, or in vivo injections of classical retrograde tracers (Diamidino Yellow and Fluorogold) confirm the results.

We conclude that, as it is the case for cortico-cortical pathways, ascending thalamocortical afferents undergo a marked regression, preceded by a stage of initial exuberance. In the rat visual system, this early postnatal stage involves several thalamic nuclei, including some that do not project to the adult visual cortex.

Palavras-Chave: Visual cortex, thalamus, postnatal development, transient connections, carbocyanines

Apoio Financeiro: CNPq, CAPES, FAPERJ, PRONEX.

A.05.003

Delayed development in rats prenatally exposed to nicotine

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Cigarette smoking during pregnancy is associated with intrauterine growth retardation and neuropathological abnormalities, leading to neonatal death, low birth weight, and long-term cognitive deficits in children. The aim of our study was to analyze the role of *in utero* nicotine on rats' maturation and development. Female Lister Hooded rats (n=19) received nicotine in drinking water at increasing concentrations up to 0,06 mg/ml during 3 weeks before mating and then continuously during pregnancy until the day of birth, leading to daily nicotine consumption in the range of 4.2±0.5 mg/kg body weight. Offspring from control and nicotine- exposed females were cross-fostered within 24 h after delivery to lactating dams not exposed to nicotine. Each litter was randomly reduced to 8 pups (4 males, 4 females), whenever possible. Maturation and developmental tests were conducted before weaning both on male and female offspring. There was no difference between groups and genders in maturation measured by appearance of fur, eye opening, and eruption of incisors, except a lower body weight in nicotine exposed animals. On the contrary, development of male and female rats exposed to nicotine was delayed in all assessed parameters: righting reflex, negative geotaxis, and grip strength. Our results suggest that nicotine might be responsible for developmental deficits described in children prenatally exposed to nicotine due to mothers' cigarette smoking.

Palavras-Chave: prenatal exposure, nicotine, maturation and development

Apoio Financeiro: Wellcome Trust

A.05.004

Influência da semente de linhaça (*Linum usitatissimum*) sobre o desenvolvimento cerebral de ratos filhotes

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Objetivo: Verificar o efeito da semente de linhaça no desenvolvimento cerebral de ratos filhotes.

Métodos: Foi efetuada cromatografia gasosa para determinar o perfil lipídico do leite materno e do cérebro de filhotes, bem como avaliação dos pesos cerebral, corporal e cerebral relativo. Utilizou-se filhotes da geração F1 obtidos de 18 ratas Wistar, divididas em 3 grupos (n=6): Linhaça (GL), recebendo ração à base de linhaça adicionada de caseína; Controle (GC), recebendo ração à base de caseína e Controle Modificado (GCM), recebendo ração à base de caseína suplementada com fibras e óleo. Aos dados de peso aplicou-se os testes de Scheffé e Bonferroni. Aos demais dados aplicou-se o teste de Mann-Whitney. A significância foi estabelecida ao nível de $p < 0,05$. **Resultados:** O leite das mães alimentadas com ração à base de linhaça apresentou maior valor energético (Kcal/100ml) (GL $228,85 \pm 5,93^b$; GC $186,57 \pm 5,57^a$; GCM $226,90 \pm 6,07^b$) e teor de gordura (%) mais elevado ao crematócrito (GL $31,79 \pm 0,20^c$; GC $23,10 \pm 0,65^a$; GCM $28,65 \pm 0,38^b$). Os ácidos da família n-3 (%) presentes na linhaça, foram achados em abundância na ração confeccionada com ela (GL $46,33 \pm 0,68^a$; GC $5,83 \pm 0,19^b$; GCM $5,84 \pm 0,04^c$) e foram transferidos para o leite materno (GL $23,01 \pm 4,33^b$; GC $2,15 \pm 0,62^a$; GCM $2,12 \pm 0,34^a$) de ratas que se alimentaram com esta. O DHA (%) (GL $14,33 \pm 0,25^c$; GC $10,41 \pm 0,06^a$; GCM $10,85 \pm 0,53^b$), assim como o somatório dos ácidos n-3 (%) (GL $17,32 \pm 0,03^c$; GC $10,67 \pm 0,01^a$; GCM $11,40 \pm 0,61^b$) estava bastante aumentado no cérebro de filhotes recém-natos de mães alimentadas com ração à base de linhaça. Esses filhotes também apresentaram maior peso cerebral (g) (GL $0,25 \pm 0,009^b$; GC $0,18 \pm 0,006^a$; GCM $0,20 \pm 0,008^a$) e peso cerebral relativo (%) (GL $4,68 \pm 0,21^b$; GC $3,42 \pm 0,14^a$; GCM $3,56 \pm 0,18^a$) no pós-natal imediato e, aos 30 dias, apenas maior peso cerebral relativo (GL $2,89 \pm 0,16^b$; GC $2,15 \pm 0,12^a$; GCM $3,16 \pm 0,12^b$). Todos os resultados descritos para GL foram estatisticamente significantes ($p < 0,05$).

Conclusão: A semente de linhaça influenciou positivamente a incorporação de ácidos graxos ômega-3 na composição do leite materno e no tecido cerebral, assegurando um bom desenvolvimento cerebral em ratos filhotes.

Palavras-Chave: Linhaça, Ômega-3, cérebro, ratos

Apoio Financeiro: Capes e FAPERJ

A INFLUÊNCIA DE UMA DIETA A BASE DE LINHAÇA (*Linum usitatissimum*) SOMADA AO ENRIQUECIMENTO AMBIENTAL, NO PESO CEREBRAL DE RATOS *WISTAR*

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Objetivos: Avaliar a influência de ácidos graxos *ômega-3* provindos da linhaça somados ao enriquecimento ambiental, no peso cerebral de ratos machos *Wistar*. **Métodos:** Foram utilizados 36 ratos *Wistar*, recém desmamados, divididos em seis grupos (n=6): Controle (GC), recebendo ração à base de caseína, Linhaça (GL), recebendo ração a base de linhaça, Controle Modificado (GCM), recebendo ração a base de caseína adicionada de fibras e óleo, e mais três grupos com a mesma dieta dos anteriores somadas a exposição ao Enriquecimento Ambiental, (GCEA), (GLEA) e (GCMEA). Durante 56 dias os grupos com a dieta de caseína e linhaça receberam ração e água *ad libitum*, e os grupos da dieta de caseína modificada se mantiveram em *pair feed* com o GL. Foram mantidos 3 animais/gaiola com temperatura constante de 22° C e ciclo claro-escuro 12/12h. Neste período foram controlados peso e consumo dos animais. Os animais foram sacrificados, tendo os cérebros retirados e pesados em balança analítica Bosh S2000 com sensibilidade de 0,0001g. O peso cerebral relativo foi calculado dividindo o peso cerebral, pelo peso corporal e multiplicando o resultado por 100. Utilizou-se *Kruskal-Wallis rank Sum test*, para detectar diferenças entre grupos e entre subgrupos, ao nível de $p < 0,05$. **Resultados:** Quanto à dieta, o peso cerebral relativo apresentou maiores valores no GL ($0,61 \pm 0,07$) comparado aos outros grupos, GC ($0,50 \pm 0,02$) e GCM ($0,54 \pm 0,06$), com significância estatística de $p < 0,05$. Já os grupos enriquecidos não apresentaram diferença estatística, mas numérica, com GLEA ($0,65 \pm 0,03$), GCEA ($0,50 \pm 0,03$) e GCMEA ($0,58 \pm 0,03$). Porém a média total dos grupos da Linhaça ($0,63 \pm 0,05$) foi superior aos demais grupos, Caseína ($0,50 \pm 0,03$) e Caseína Modificada ($0,56 \pm 0,05$). **Conclusão:** Uma dieta a base de linhaça somada a um ambiente enriquecido, aumenta o peso cerebral relativo favorecendo seu desenvolvimento.

Palavras-Chave: Linhaça, Enriquecimento Ambiental, Cérebro, Ratos

Apoio Financeiro: FAPERJ, CAPES e ARMA ZEN Ltda

HEMISFERECTOMIA UNILATERAL NA VIDA ADULTA SUGERE ASSIMETRIA CEREBRAL NO CONTROLE DA FUNÇÃO MOTORA DE CAMUNDONGOS

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Estudos em humanos sugerem que o hemisfério esquerdo é particularmente importante no controle e execução de movimentos. Em geral, lesões no hemisfério esquerdo promovem déficits motores mais pronunciados que lesões semelhantes no hemisfério direito. Neste trabalho utilizamos a hemisferectomia unilateral para avaliar a contribuição de cada hemisfério na função motora em camundongos.

Camundongos Suíços adultos machos foram submetidos a hemisferectomia unilateral direita (HD; n = 11) ou esquerda (HE; n = 11). O grupo controle (CONT) foi constituído por 14 machos. Quinze dias após a cirurgia, os animais foram submetidos ao teste da locomoção forçada em cilindro giratório (raio do cilindro = 3,2 cm; altura = 20 cm). Para tanto, os animais foram colocados individualmente sobre o cilindro e a velocidade de rotação foi aumentada de 4 a 40 RPM num intervalo de 120 segundos. Foram registradas a latência para a queda (LAT) e a velocidade de rotação do cilindro no momento da queda (VEL). ANOVAs classificando os animais pelo Tratamento (HD, HE ou CONT) foram realizadas para LAT e VEL. Diferenças entre grupos foram submetidas à análise post hoc pelo teste t de Student. A análise histológica confirmou que houve eliminação de praticamente todo o prosencéfalo. O tronco, a nível posterior, não foi afetado e a lesão ficou restrita a um dos hemisférios. Houve efeito do tratamento para LAT (F = 4,2, gl = 2, P = 0,02) e para VEL (F = 4,0, gl = 2, P = 0,03). Para LAT, o efeito do tratamento pode ser explicado pelo fato de que os tempos do Grupo HD ($8,0 \pm 1,9$) foram significativamente menores que a do grupo CONT ($15,4 \pm 1,7$; t = 3,2, gl = 23, P < 0,01). Para VEL, o efeito do tratamento pode ser explicado pelo fato de que as medidas do Grupo HD ($4,9 \pm 1,2$) foram significativamente menores que a do grupo CONT ($6,7 \pm 1,6$; t = 3,1, gl = 23, P < 0,01). Para LAT e VEL, o grupo HE não diferiu dos grupos HD e CONT.

De modo análogo ao observado em humanos, o pior desempenho em machos sem o hemisfério direito sugere uma ação assimétrica dos hemisférios cerebrais no controle da função motora em camundongos.

Palavras-Chave: HEMISFERECTOMIA; CONTROLE MOTOR; ROTAROD

Apoio Financeiro: FAPERJ/ PIBIC UERJ

LOCALIZATION OF ANGIOTENSIN II AT₂ RECEPTORS IN THE INFERIOR COLLICULUS.
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LUIS, ARGENTINA - BIOQUIMICA AVANZADA

Angiotensin II (Ang II) is not solely associated with fluid homeostasis and blood pressure control but it can also modulate neuronal activity in regions of the brain involved in cognition, motor control and sensory integration. Ang II recognizes two receptor subtypes, named as AT₁ and AT₂, based on pharmacological basis. AT₂ receptors are highly expressed in neonatal tissues, with a rapid decrease after birth and persist in adulthood in limited brain areas. The Inferior Colliculus (IC) is a critical midbrain station for the auditory processing, involved with motor activities related to audition. The aim of this work was to study the localization of Ang II type 2 receptor in the IC during rat development. We studied the localization of Ang II receptors by binding autoradiography in pups at different stages after birth. Newborn animals exhibited very low binding at the IC, which increase in postnatal days (P) 8 and P15 pups. Specific binding was stronger in P15 animals with prevalence of AT₂ receptors.

Immunohistochemistry and immunofluorescence staining with AT₂ receptor primary antibody evidence the presence of immunoreactive cells, confirming the observation by binding autoradiography. The expression of AT₂ receptors was detected and semiquantified by multiplex RT-PCR. mRNA of AT₂ receptors was co-amplified with GAPDH, allowing us to quantify messenger expression, which was higher in P15 animals.

The IC seems to be involved in the behavior related to sound input, such as patterns of hunting, escape or vocal communication. The pattern of Ang II AT₂ receptors expression in the IC encompasses the pattern of the brainstem maturation and connection to the cerebral system. We assume that AT₂ receptors might play a potential role in organogenesis and the establishment of neuronal circuits.

Palavras-Chave: angiotensin II receptors,inferior colliculus, development.

Apoio Financeiro: IMIBIO-CONICET, FONCYT, UNIVERSIDAD NACIONAL DE SAN LUIS. SAN LUIS, ARGENTINA.

Expressão da Dopa-descarboxilase em retina de vertebrados

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Na via de síntese da dopamina (DA), a tirosina é hidroxilada pela tirosina hidroxilase (TH) formando L-dopa, que é descarboxilada em DA pela ação da dopa-descarboxilase (DDC). Na retina de galinha, a TH surge aos 12 dias embrionários, no entanto, já existe atividade dopaminérgica através do receptor D1. Recentemente, foi mostrado que pode ocorrer formação de L-dopa na retina de aves em fases precoces do desenvolvimento, através da enzima tirosinase presente no epitélio pigmentado (EP). Propôs-se que L-dopa se difundiria para a retina e formaria DA, quando TH ainda não está presente, desde de que a enzima DDC esteja presente no tecido neural. Nosso objetivo foi verificar a expressão da DDC e caracterizar os tipos celulares que a contém, na retina de galinha, ao longo do desenvolvimento e no tecido maduro, de diferentes vertebrados. Utilizando imunohistoquímica em cortes de retinas caracterizamos pela primeira vez como dois tipos de células imunorreativas a DDC (DDC+ tipo 1 e 2) com base na localização do corpo celular e no nível de estratificação dos prolongamentos. Utilizando imunofluorescência foi possível caracterizar a subpopulação do tipo 1 como as células que contém DDC e que co-localizam com TH. As células DDC+ do tipo 2 possuem somente a DDC. As células DDC+ possuem uma distribuição topográfica homogênea pelo tecido durante todo o desenvolvimento e na retina madura, enquanto que células TH+ apresentam uma distribuição homogênea com o amadurecimento do tecido. A densidade das células DDC+ é muito maior quando comparada a TH+ em todas as idades estudadas. Retinas de macaco, gambá e ratos pigmentados adultos também apresentaram células DDC+. Em retina de gambá e ratos, as células DDC+ possuem uma densidade maior quando comparadas as TH. Os resultados apresentados neste trabalho contribuem para substanciar a proposta inicial de que na retina de galinha possam existir duas possíveis fontes de DA, uma baseada numa fonte alternativa de L-dopa oriunda do EP em fases onde a dopamina ainda não está presente, e outra baseada na via clássica de DA que ocorre no tecido mais tardiamente.

Palavras-Chave: Dopamina, Desenvolvimento, Tirosina hidroxilase, L-dopa

Apoio Financeiro: CNPq, FAPERJ, PRONEX

MEDIDAS DE SENSIBILIDADE AO CONTRASTE VISUAL EM CRIANÇAS E ADULTOS JOVENS

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Objetivo: A Função de Sensibilidade ao Contraste (FSC) tem se apresentado como um bom indicador teórico e clínico, sendo utilizada para caracterizar a percepção visual durante o desenvolvimento humano. Na literatura, não existe consenso a respeito do período em que a criança atinge o nível de sensibilidade ao contraste dos adultos. A presente pesquisa objetivou mensurar e comparar a FSC de frequências espaciais grades-senoidais em crianças e adultos jovens.

Método: Participaram do estudo 15 crianças, que foram devidas em três grupos etários: 6 a 7 anos (N=3), 8 a 9 anos (N=5) e 10 a 11 anos (N=7); e sete adultos com idade entre 20 e 30 anos. A FSC foi mensurada binocularmente, com o método psicofísico da escolha forçada, utilizando estímulos de grades senoidais (0,25; 1,0; 2,0 e 8,0 cpg) e um estímulo neutro, com luminância média de 42 cd/m², ajustada por um Fotômetro OPTICAL produzido pela Cambridge Research Systems. Os estímulos circulares, com diâmetro de 7,2 graus de ângulo visual, eram gerados em um monitor LG colorido, com resolução de 1280 x 1024 pixels. As medidas foram obtidas a 150 cm do monitor. O procedimento consistiu na apresentação de pares de estímulos e a tarefa do participante foi escolher, entre os estímulos, aquele que continha a frequência espacial.

Resultados: A análise dos resultados foi realizada através da ANOVA para medidas repetidas, que revelou diferenças significantes entre os grupos [$F_{(3,260)} = 41,50$; $p < 0,001$]. Já o teste *post-hoc* Tukey HSD mostrou diferenças significantes entre os grupos apenas na frequência grade senoidal de 8 cpg.

Conclusão: Esses resultados indicam que a maturação ocorre de maneira assimétrica para as frequências espaciais grades-senoidais baixas, médias e altas. Eles também sugerem que a FSC das crianças de 10-11 anos é diferente da FSC de adultos e que a sensibilidade do sistema visual se estende além das faixas etárias estudadas.

Palavras-Chave: crianças; percepção visual da forma; função de sensibilidade ao contraste; frequências espaciais.

Apoio Financeiro: CNPQ

A.05.010

Influência do Consumo Materno de Semente de Linhaça (*Linum usitatissimum*) na Distribuição de Ácidos Graxos Cerebrais e Crescimento Intra-uterino de Ratos

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OBJETIVOS: A semente da linhaça (*Linum usitatissimum*) é considerada uma das melhores fontes vegetais de ácidos graxos (AG) n-3 (alfa-linolênico). Este estudo objetivou avaliar o efeito do consumo da linhaça desde o período pré-gestacional, gestação e lactação sobre o crescimento e o perfil de ácidos graxos cerebrais da prole. **MÉTODOS:** Ratas Wistar recém desmamadas formaram três grupos: Grupo Controle (GC), com ração à base de caseína; Grupo Linhaça (GL), com ração à base de caseína e semente de linhaça como fonte parcial de proteínas e total de lipídeos e fibras; e Grupo Controle Modificado (GCM), com ração à base de caseína adicionada de fibra e óleo de soja de forma equivalente ao GL. As fêmeas acasalaram aos 90 dias e seus filhotes foram sacrificados no pós-parto imediato. As massas corporal e cerebral foram aferidas e o perfil dos AG cerebrais avaliado por cromatografia gasosa. Os resultados foram expressos em percentuais do total de AG. Com o software SPSS10 foram aplicados os testes de Mann Whitney e de Kruskal Wallis, já que a distribuição dos dados não foi normal. Foi considerado significativo $p < 0,05$. **RESULTADOS:** O peso dos filhotes ao nascer foi inferior no GL ($5,00 \pm 0,33$ g $p < 0,05$) em relação ao GC ($5,45 \pm 0,33$ g) e GCM ($5,45 \pm 0,33$ g). Já o peso cerebral do GL ($0,26 \pm 0,02$ g) foi superior ($p < 0,05$) ao GC ($0,18 \pm 0,01$ g) e ao GCM ($0,19 \pm 0,01$ g). O peso cerebral relativo do GL ($5,14 \pm 0,34$ g) foi superior ($p < 0,05$) aos GC ($3,38 \pm 0,11$ g) e GCM ($3,57 \pm 0,07$ g). Teores de ácido araquidônico (AA) e docosahexaenóico (DHA) foram insignificantes na dieta, no entanto a incorporação desses AGs foi expressiva no cérebro de todos os grupos. O percentual de AA foi significativamente menor ($7,91 \pm 0,3$) no GL que nos GC ($12,6 \pm 0,2$) e GCM ($12,87 \pm 0,11$) ($p < 0,05$), enquanto o percentual de DHA foi significativamente maior ($p < 0,05$) no grupo GL ($14,3 \pm 0,2$) em comparação ao GC ($10,4 \pm 0,06$) e GCM ($10,85 \pm 0,53$). **CONCLUSÃO:** O consumo materno da semente de linhaça aumentou o peso cerebral da prole e ocasionou maior incorporação de DHA, sugerindo melhor desenvolvimento do cérebro. No entanto, observou-se menor proporção de AA que pode ter contribuído para a menor taxa de crescimento dos filhotes.

Referências bibliográficas: Acta Paediatrica, 89 (2): 142, 2000; Early Hum Dev. 83:761, 2007.

Palavras-Chave: ácido graxo poliinsaturado, crescimento cerebral, DHA, AA

Apoio Financeiro: Capes

A.05.011

Implementação de um sistema de localização espacial de regiões cerebrais em tempo real para aplicação de TMS por co-registro com fMRI

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Objetivos

A estimulação magnética transcraniana (TMS) é uma técnica que utiliza fortes pulsos de campo magnético capazes de despolarizar neurônios do córtex. No entanto essa técnica tem uma resolução espacial baixa e ainda hoje se utiliza de parâmetros subjetivos para estimar o local a ser estimulado. No intuito de reduzir essa subjetividade, foi desenvolvido um software de neuronavegação que utiliza informações espaciais de imagens funcionais por ressonância magnética (fMRI), assim determinando com maior precisão o local que será estimulado.

Métodos

Realizamos exames de fMRI em 10 voluntários assintomáticos seguindo um protocolo motor simples das mãos direita e esquerda. As imagens foram adquiridas por um equipamento de 1.5 T (Siemens, Magnetom Vision), usando uma sequência de aquisição rápida, do tipo EPI para a geração dos mapas estatísticos e sequências do tipo MPR para co-registro dos mapas estatísticos com imagens estruturais. As imagens foram processadas usando o programa Brain Voyager QX 1.8 (Brain Innovation, Maastricht, Holanda).

O neuronavegador foi desenvolvido em ambiente Matlab® 6.5 (Mathworks, Sherborn, MA, EUA), e faz uso de informações provenientes de um sistema de localização espacial (Polhemus Fasttrack, Colchester, VT, EUA) que permite rastrear em tempo real as respectivas posições na fMRI.

Com o auxílio do neuronavegador estimulou-se usando o TMS o córtex desse grupo de indivíduos sobre a região motora localizada pela fMRI. A eficiência do estímulo foi monitorada por meio de um eletromiógrafo (EMG) posicionado no músculo abdutor do polegar da mão.

Resultados

Os resultados indicam que com o uso da neuronavegação, foi possível estimular a região motora em 100% dos voluntários estudados quando guiados por suas respectivas fMRIs. Um desvio do máximo de atividade entre as leituras de Potencial Evocado pelo EMG e os mapas funcionais foi de (7.5 ± 5) mm.

Conclusão

Acreditamos que o sistema é uma opção importante para auxiliar a realização de exames neurofuncionais mais reprodutíveis e tem condições de ser aplicado em pesquisas clínicas, não apenas como contra prova de exames já bem estabelecidos, mas também em outras áreas de aplicação como o rTMS.

Palavras-Chave: TMS, fMRI, Neuronavegação.

Apoio Financeiro: Fapesp

A.05.012

ACTIVATION OF cAMP-RESPONSIVE ELEMENT BINDING PROTEIN (CREB) IS MODULATED IN THE DEVELOPING RAT SUPERIOR COLLICULUS

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Objectives: The superior colliculus (SC) is an excellent experimental model for studying developmental and plastic processes in the CNS. Recently we have shown that the ERK 1/2 and p38^{MAPK} mitogen-activated protein kinases are activated in the SC in temporal correlation with the period of retino-collicular plasticity (1). Long-lasting changes in neuronal circuitry require the pattern of kinase activation to be translated into a pattern of gene expression, probably through activation of transcription factors. A good candidate is the cAMP-responsive element binding protein (CREB), whose activation depends upon ERK1/2 (2). Here, to further characterize the molecular framework required for SC developmental plasticity, we analyze the expression of CREB during development and following manipulation of the visual input.

Methods: Antibodies against phospho-CREB, total-CREB and beta-actin (Cell Signaling, 1:1000) were used for western blot analysis. SC were obtained from Long-Evans hooded rats at different postnatal ages (P0-P45). Visual deprivation was performed at P3 or P21, and animals sacrificed one week later.

Results: Our results show that the ratio between the phosphorylated and the total form of CREB (activation level index) increases in the SC between P4 and P9 (approx. 60% vs. P0, n=4, p<0.05). This increase occurs in temporal correlation with the critical period for plasticity of the retino-collicular projections. To test the involvement of CREB on plastic processes we used the monocular deprivation protocol, known to induce strong plasticity in the young but not in the adult SC. Our preliminary results indicate that CREB is modulated by deprivation in the young animal but not in the adult.

Conclusions: These results show that CREB is highly regulated during development and following plasticity-inducing manipulation. Altogether, suggest that the ERK1/2-CREB signaling pathway may play a fundamental role in directing the refinement of retino-collicular projections.

References: (1) Oliveira et al., *Int.J.Devl.Neurosci.* 26: 355, 2008. (2) Cancedda et al., *J.Neurosci.* 23: 7012, 2003.

Palavras-Chave: CREB, superior colliculus, plasticity

Apoio Financeiro: Supported by CNPq, FAPESC and FINEP (IBN-Net #01.06.0842-00).

A.05.013

SENSIBILIDADE AO CONTRASTE DE CRIANÇAS PARA FREQUÊNCIA ESPACIAL E RADIAL

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Objetivo: Estudos neurofisiológicos mostram que frequências espaciais (grades senoidais) e frequências radiais são processados por vias visuais diferentes. O objetivo deste estudo foi mensurar e comparar curvas de sensibilidade ao contraste para estímulos radiais (FSCr) e grades senoidais (FSC) em crianças.

Método: Participaram da pesquisa 15 crianças de ambos os sexos, com idade de 6 a 11 anos. Todas tinham acuidade visual normal ou corrigida e não possuíam doenças oculares identificáveis. Os estímulos de frequências espaciais e radiais de 0,25; 1; 2 e 8 ciclos de grau por ângulo visual (cpg) eram circulares, tendo um diâmetro de 7,2 graus de ângulo visual. Os mesmos foram apresentados em um Monitor LG de 19 polegadas com resolução de 1024 x 768 pixels. As medidas foram realizadas binocularmente, utilizando o método psicofísico da escolha forçada, e com os participantes a uma distância de 150 cm da tela. Foram apresentados pares de estímulos, sendo que um deles continha a frequência teste espacial ou radial acima, enquanto o outro era um padrão homogêneo com a luminância média (42,6 cd/m²). A tarefa dos participantes foi escolher sempre o círculo com a frequência de teste. O contraste do estímulo teste variava segundo acertos e erros. A cada três acertos consecutivos uma unidade de contraste era decrescida, e a cada erro era acrescida da mesma unidade (20%).

Resultados: A análise de variância mostrou diferenças significantes entre a FSCr e a FSC [$F(1, 358) = 140,5$; $p < 0,001$]. O teste *post-hoc* Tukey HSD também revelou diferenças significantes entre todas as frequências testadas ($p < 0,001$). Os resultados mostraram que as crianças foram da ordem de 2,0; 2,4; 4,0 e 2,0 vezes mais sensíveis para grades senoidais do que para estímulos radiais nas frequências de 0,25; 1; 2 e 8 cpg respectivamente.

Conclusão: Estes resultados revelam que o sistema visual de crianças de 6 a 11 anos é mais sensível para grade senoidal do que para estímulo radial, sugerindo que estes são processados por mecanismos sensoriais diferentes.

Palavras-Chave: função de sensibilidade ao contraste; frequências espaciais radiais; frequências espaciais grades senoidais; crianças.

Apoio Financeiro: CNPQ

A.05.014

DETECÇÃO DE FREQUÊNCIAS RADIAIS EM CRIANÇAS E ADULTOS JOVENS

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Objetivo: A função de sensibilidade ao contraste (FSC) tem sido utilizada para caracterizar o desenvolvimento da percepção visual da forma, entretanto estudos com padrões radiais simétricos são raros. O objetivo deste estudo foi determinar a FSC de crianças e adultos jovens para estímulos radiais e avaliar o processo de desenvolvimento.

Método: Participaram da pesquisa 16 crianças com idade de 6 a 11 anos (6-7 anos, N= 5; 8-9 anos, N= 5; 10-11 anos, N= 6) e cinco adultos jovens com idade entre 20 e 30 anos. Todos os voluntários estavam livres de doenças oculares e tinham acuidade visual normal ou corrigida. Foram utilizados estímulos de frequências radiais de 0,25; 1; 2 e 8 ciclos por grau de ângulo visual (cpg). Estes eram circulares com um diâmetro de aproximadamente 7,2 graus de ângulo visual e foram gerados em tons de cinza em um Monitor LG de 19 polegadas, com resolução de 1024 x 768 pixels, controlado por um microcomputador. As medidas foram obtidas, binocularmente, a uma distância de 150 cm da tela do monitor, usando um delineamento experimental com medidas repetidas e o método psicofísico da escolha forçada. Nesse paradigma, apresentaram-se pares de estímulos na tela, um com uma das frequências acima e o outro homogêneo com a luminância média de 42 cd/m². A tarefa do participante foi escolher, entre os dois estímulos, aquele que continha a frequência radial.

Resultados: A ANOVA mostrou diferenças significantes entre as idades [$F_{(3, 224)} = 49,76$; $p < 0,001$]. Entretanto, o teste *post-hoc* Tukey HSD revelou diferenças entre todas as idades apenas na frequência radial de 8 cpg ($p < 0,001$).

Conclusão: Estes resultados sugerem que a FSC de crianças de 8-9 anos já se assemelha a dos adultos nas frequências radiais baixas e médias (0,25; 1 e 2 cpg). Os resultados também sugerem que o desenvolvimento da FSC de frequência radial melhora com o passar da idade e se estende para faixas etárias além da estudada.

Palavras-Chave: Função de sensibilidade ao contraste; crianças; frequências espaciais radiais.

Apoio Financeiro: CNPQ

A.05.015

ENRIQUECIMENTO AMBIENTAL E MANIPULAÇÃO TÁCTIL REVERTEM AS ALTERAÇÕES CAUSADAS PELA DESNUTRIÇÃO PROTÉICA NA EXPLORAÇÃO DOS BRAÇOS ABERTOS DO LABIRINTO EM CRUZ ELEVADO

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O déficit na ingestão de proteína durante o período de rápido desenvolvimento do sistema nervoso central resulta em atrasos no desenvolvimento físico e cerebral, com conseqüências, para o comportamento de ratos. Pesquisas mostram que prejuízos causados pela desnutrição podem ser parcialmente revertidos pelo enriquecimento ambiental e a estimulação tátil. O enriquecimento ambiental aumenta a exploração no labirinto em cruz elevado (LCE), além de reverter alguns danos no cérebro de ratos desnutridos.

Objetivos: O objetivo deste trabalho foi comparar em ratos desnutridos (D) e bem nutridos (C), os efeitos do enriquecimento ambiental (E) e da estimulação tátil (H) durante o período de formação do SNC (8 a 35 dias), através do desempenho no LCE aos 36 dias de idade.

Métodos: os ratos foram divididos em dois diferentes grupos de acordo com a dieta: desnutridos (dieta com 6% de proteína) e controles (dieta com 16% de proteína). Também foram subdivididos em grupos conforme a manipulação ambiental: sem estimulação (N), ambiente enriquecido (E), e tátil (H). A manipulação ambiental foi realizada no período de 0 a 35 dias, sendo que os ratos foram expostos ao LCE no 36º dia.

Resultados: a partir da exposição ao LCE, os resultados mostram que D permanecem uma maior porcentagem de tempo nos braços abertos ($18,88 \pm 0,02$) que C ($9,16 \pm 0,01$; $p < 0,001$). Com relação as diferentes estimulações. Já os animais DE apresentaram uma menor porcentagem de tempo nos braços abertos ($11,68 \pm 0,04$) quando comparados aos animais DH ($21,82 \pm 0,03$; $p < 0,005$) e animais DN ($28,48 \pm 0,04$; $p < 0,005$).

Conclusões: Os dados do presente estudo sugerem que tanto a manipulação tátil como o enriquecimento ambiental, amenizam as alterações produzidas pela desnutrição no comportamento de exploração dos braços abertos do LCE. **Palavras-Chave:** ratos, desnutrição, estimulação tátil, enriquecimento ambiental, períodos críticos de formação do SNC e labirinto em cruz elevado

Apoio Financeiro: FAPESP e CAPES

DESENVOLVIMENTO DA SENSIBILIDADE AO CONTRASTE EM CRIANÇAS PARA ESTÍMULOS ANGULARES

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Objetivo: A Função de Sensibilidade ao Contraste (FSC) é uma medida clássica que possibilita descrever atributos ópticos e neurais da visão. O objetivo do estudo foi mensurar e comparar a FSC de frequências angulares em crianças de 6 a 11 anos e adultos.

Método: Participaram da pesquisa 14 crianças, distribuídas em três faixas etárias: quatro crianças de 6 a 7 anos, quatro de 8 a 9 anos e seis de 10 a 11 anos e cinco adultos de 20 a 30 anos. Todos tinham acuidade visual normal ou corrigida e não possuíam doenças oculares identificáveis. Os estímulos com frequências angulares de 3, 24, 48 e 96 ciclos/360° eram circulares, tendo um diâmetro de 7,25 graus de ângulo visual. Os mesmos foram apresentados em um Monitor LG 19 polegadas com resolução de 1024 x 768 pixels. As medidas foram realizadas binocularmente, utilizando o método psicofísico da escolha forçada com os participantes a uma distância de 150 cm dos estímulos. Foram apresentados pares de estímulos, sendo que um deles continha a frequência angular, enquanto o outro era um padrão homogêneo com a luminância média (42,6 cd/m²). A tarefa dos participantes foi escolher sempre o círculo com a frequência angular. O contraste do estímulo teste variava segundo acertos e erros. A cada três acertos consecutivos uma unidade de contraste era decrescida e a cada erro era acrescida da mesma unidade (20%).

Resultados: As análises com a ANOVA para medidas repetidas mostraram diferenças significantes entre os grupos etários [$F(3, 224) = 44,07$; $p < 0,001$]. O teste *post-hoc* Tukey HSD revelou diferenças significantes entre crianças de 6-7, 8-9 e 10-11 anos ($p < 0,001$) apenas na frequência angular de 3 ciclos/360°. Não foi encontrada diferenças significantes entre crianças de 10-11 anos e adultos.

Conclusão: Estes resultados sugerem que a FSC para estímulos angulares melhora gradativamente nas frequências angulares médias e altas até os 10-11 anos, quando atinge o desempenho dos adultos.

Palavras-Chave: Função de Sensibilidade ao Contraste; frequências espaciais angulares; crianças.

Apoio Financeiro: CNPq

STRESS INDUCIBLE PROTEIN-1 AS AN IMPORTANT PLAYER IN SUBVENTRICULAR ZONE NEUROBLAST MIGRATION

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Objectives: Subventricular zone (SVZ) neurogenesis and migration persist throughout lifetime in mammals. Previous data have shown that cellular prion protein (PrP^c) has a role in SVZ neurogenesis. PrP^c-stress inducible protein 1 (STI1) interaction was shown to be important for developmental events such as neuritogenesis and cell death. We have previously shown that PrP^c does not have a decisive role in SVZ neuroblast migration *in vitro*, but show here that STI1 itself is an important player for SVZ cell migration independent of PrP^c interaction.

Methods: P4 mice brains were fixed and vibratome cut parasagittally (50 µm). Sections were incubated with anti-STI1 (1:500) followed by secondary antibody (Ab). Western Blotting was performed in P1 PrP^c-null (KO) and WT mice SVZ protein extracts, using anti-STI1 Ab (1:5000). For SVZ explant cultures, WT and PrP^c KO mice (P0-P7) brains were cut parasagittally (350 µm), explants were plated, covered with Matrigel and kept 48 h in incubator. Immunocytochemistry was performed in explants using anti-PrP^c (1:500) and double labeling with anti- STI1 (1:500) and PSA-NCAM (1:200). For functional assays *in vitro*, the migratory halos around SVZ explants were measured before and 24h after anti-STI1(1:200) treatment. Migration was analyzed by time-lapse videomicroscopy (TLVM) using similar Ab treatment as above.

Results: STI1 immunolabeled cells were identified in the SVZ of brain sections and explant migratory chains. WB analysis with an anti-STI1 Ab revealed a 66 KDa band in SVZ extracts. Most of the STI1 labeled cells are neuroblasts, as they also express PSA-NCAM. After anti-STI1 treatment, halos from WT and PrP^c KO explants decreased compared to control explants treated with pre-immune serum (p<0,001 and p<0,05, respectively, non-parametric one way ANOVA). TLVM showed that anti-STI1 Ab treatment causes retraction of migratory chains which exhibit syncytium-like behavior. Average cell speed decreases (p<0,001, unpaired t Test) showing cell motility inhibition.

Conclusion: These results suggest a role for STI1 in SVZ neuroblast migration participating in cell-cell, cell-ECM adhesive balance crucial for neuronophylic migration.

Palavras-Chave: Subventricular zone, PrPc, STI1, migration

Apoio Financeiro: FAPESP, PRONEX-FAPERJ, CNPq.

A.05.018

The Shh/Gli pathway modulates neogenin1 gene expression during neural development in the mouse embryo

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The Sonic hedgehog (Shh) pathway plays critical instructional roles during vertebrate embryonic development in different tissues. It participates in many biological processes such as stem cell and progenitor cell proliferation, tissue patterning and organogenesis, among others. Considering the variety of developmental processes that are regulated by Shh signaling, we hypothesized that there are far more Shh target genes than have been characterized to date that allow the reiterative deployment of the Shh pathway to elicit such diverse cellular responses. We used molecular biology-based assays (Reverse one-hybrid, and Microarrays) to obtain possible Shh direct target genes. Here we report a previously unknown Shh/Gli direct target gene, neogenin 1 (neo1). Since it has been recently shown that neo1 is expressed in neural stem cells, we studied the possible functional link between Shh and neo1.

Objectives

Isolate novel genes possibly regulated by the Shh/Gli pathway.

Demonstrate the relationship between this pathway and the neo1 gene.

Methods

Microarrays

Reverse one hybrid screening assay

Neurospheres culture

Chromatin Immunoprecipitation assays (ChIP)

In situ hybridization

Results

We found many genes previously unrelated with the Shh/Gli pathway.

One of these genes is neo1, involved in axon guidance and stem cell growth. The expression of neo1 was tested in gain and loss of Shh/Gli pathway function approaches. We also performed ChIP assays to confirm putative Gli binding sites in neo1 promoter.

Conclusion

The Sonic Hedgehog/Gli pathway regulates the expression of the neo1 gene during embryonic neural development

Palavras-Chave: Hedgehog Development neogenin genomics

Apoio Financeiro: Iniciativa Científica Milenio P0639F and Fondecyt 1070248

A.05.019

Possible interactions among 9-O-acetyl GD3, integrins and tetraspanins during development of cerebellum.

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It is known that integrins can function associated to gangliosides and tetraspanins in specific membrane microdomains called glycosynapses, and this association can lead to the modulation of cell motility. The 9-O-acetyl GD3 ganglioside (9-O-acGD3) is a marker of migration in nervous system and its immunoblockade by the antibody Jones arrests the migration of granular cell precursors in the developing cerebellum. We have previously shown that the ganglioside 9-O-acetyl GD3 co-localizes with beta 1 integrin subunit and with FAK-P in cerebellar explants cultures. The aim of this work is to evaluate the possible signaling molecules that participate along with 9-O-acGD3 in the control of neuronal migration in the developing cerebellum.

Cerebellar explants were plated on glass coverslips coated with poly-L-lysine and laminin. In some set of experiments the Jones antibody (1:100) was used to block the granule cells migration *in vitro*. Antibodies against beta 1 integrin subunit, c-src, CD9, P-FAK were used in immunocytochemistry and/or immunoblotting assays. The tissue samples were submitted to sucrose gradient fractionation, HPTLC (high performance thin layer chromatography) and overlay with Jones antibody to detect 9-O-acGD3.

9-O-acGD3 and integrin beta 1 subunit were found in the same fraction obtained from the sucrose gradient fractionation, however integrin beta 1 subunit was also found in another fraction alone. There was co-localization between 9-O-acGD3 and CD9, a tetraspanin present in some types of glycosynapses. In Jones treated explants there was no co-localization between 9-O-acGD3 and the proteins FAK-P e beta 1 integrin. After immunoprecipitation (IP) of beta 1 integrin subunit we detected 9-O-acGD3 band resolved by HPTLC and overlay with Jones antibody. Finally, src-c and FAK-P were found in the IP for beta 1 integrin.

Based on these data, we propose that 9-O-acGD3 could control neuronal motility by association with CD9 which in turn modulate the integrin activity and signaling, however immunoprecipitation assays are necessary to ensure this hypothesis.

Palavras-Chave: 9-O-acetyl GD3, integrin, cerebellum, neuronal migration, tetraspanins

CARACTERIZAÇÃO DA MOVIMENTAÇÃO REFLEXA E ESPONTÂNEA EM LACTENTES COM MIELOMENINGOCELE

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Objetivos: A mielomeningocele (MMC) é uma malformação da medula espinal que causa perdas motoras, dentre elas, da marcha. A atividade muscular, mesmo na ausência de movimentos dos membros inferiores (MMII), sugere a preservação de motoneurônios inferiores ao nascimento. O objetivo deste trabalho é avaliar a movimentação reflexa e espontânea em lactentes com MMC, de forma a elucidar o controle motor nestes pacientes, fundamentando a estimulação da função.

Métodos: Trata-se de um estudo observacional com 7 lactentes com MMC do Hospital das Clínicas/FMUSP. Foram avaliados peso, índice de Apgar, reflexos tendíneos e primários, frequência da movimentação espontânea de MMII (filmada por 10 minutos) e presença de comorbidades ao nascimento e 30 dias após.

Resultados: A avaliação inicial foi feita no 12º dia de vida em média ($12,57 \pm 17$) na UTI (peso ao nascimento = $2941,4 \text{ g} \pm 348,0$; Apgar 10 = $9,1 \pm 0,4$; pé torto congênito = 57% dos casos e hidrocefalia = 85% dos casos. Apenas um (14,2%) apresentou reflexos e movimentos espontâneos abaixo da lesão, sendo, por minuto, $4,5 \pm 0,17$ deles associados a padrões motores globais (considerados de origem supra-espinal) e $1,1 \pm 0,08$ isolados (considerados de origem espinal; Early Hum Dev. 82:227, 2006). Dos quatro pacientes reavaliados ($47,75 \pm 18,89$ dias de vida), três (75%) apresentaram o reflexo magnético, um (25%), a marcha reflexa e um (25%), reflexos tendíneos abaixo da lesão.

Conclusões: Os movimentos espontâneos dos MMII associados a padrões globais de movimentos são mais frequentes do que os isolados, corroborando a hipótese de preservação da modulação supra-espinal em um caso. A presença de respostas reflexas em MMII indica a existência de integração sensório-motora nos níveis medulares abaixo da lesão na maioria dos casos reavaliados. Estas reações não estavam presentes na avaliação inicial, sugerindo que pode haver alguma preservação dos segmentos medulares lesados, mesmo na ausência de respostas detectáveis ao exame clínico. Assim, a estimulação da região plantar pode deflagrar respostas motoras nestas crianças, revelando uma nova possibilidade de intervenção terapêutica para estimulação da independência funcional.

Palavras-Chave: desenvolvimento, sistemas motores, avaliação, função, mielomeningocele

A.05.021

Thyroid hormones in rat visual system development

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During development ipsilateral retinotectal axons reach a mature topographic pattern at the second postnatal week. This process involves elimination of transient axons and density elevation of terminal arbors at correct topographic sites. Congenital Hypotireoidism in humans is associated with both motor and mental deficiency, that might reflect central nervous system connectivity alterations with repercussion over topographic development and use-dependent plasticity. We studied the role of thyroid hormones blockade in the exploratory behavior and development of retina and ipsilateral retinotectal pathway *in vivo*. Lister Hooded rats ranging from PND2 to PND28 were used in this study. Animals were treated with antithyroid agent, Methimazole from GD10 until perfusion at PND2, 10, 21 and 28. Control groups were treated with tap water. Retinotectal projections were traced with intraocular injection of horseradish peroxidase and retinal cells marked within Nissl for analysis. In group treated until PND2, animals presented a reduction of density in terminal labeling at superior colliculus. Animals treated from GD10 to PND10 showed a reduction of 45% from total area labeling and 15% from cluster area, relative to control group. Animals treated until PND21, has presented a expansion in topographic patterns with a reduction in clusters density. Post-natal treatment until PND28 did not induced alterations in topographic densities, but surprisingly induced a 20% elevation on terminal labeling density at disrupted sites. Also, retinal morphologic studies, revealed a shrink in nuclear internal and external layers, and a reduction in total cell number at ganglion cell layer in Metimazol treated animals. Together, our results suggests that hypothyroid animals present a reduced innervation density of retinal axons at the superior colliculus and an abnormal topographical distribution during development. These data are consistent with increased apoptosis, reduced synaptogenesis and disturbed retinotectal synaptic connectivity.

Palavras-Chave: Plasticidade Neural, Hormônios tireoidianos, retinotectal, sistema visual

Apoio Financeiro: CAPES, FAPERJ, CNPQ, PRONEX

Imagética Motora e Desnutrição

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Objetivos: A imagética motora (IM) é a simulação mental de ato motor, sem que ocorra movimentação de quaisquer segmentos corpóreos associados (1). Vários estudos têm demonstrado que a imagética motora pode ser útil no tratamento de distúrbios do desenvolvimento da coordenação motora (DCD). Os estudos de Parsons (1994, 2001) deram base ao conceito de que testes de discriminação de lateralidade utilizando desenhos da mão humana evocam a imagética motora(1,2) O objetivo deste estudo-piloto é avaliar se o grau nutricional compromete o desempenho imagético motor de crianças.

Métodos: Os testes de imagética motora foram aplicados em 43 crianças residentes na vila residencial de uma madeireira no município de Portel, região de Marajó, no Pará. Os testes consistiram na apresentação de um desenho representativo de mãos humanas (direita e esquerda) em quatro inclinações: zero, 90, 180 e 270 graus (cinco repetições para cada posição). Foram apresentadas as mesmas 40 posições para cada criança e observou-se a capacidade destas de identificarem se a mão era esquerda ou direita, aferindo-se o escore em erros. Participaram da avaliação 16 crianças de 6 a 8 anos, 16 crianças de 9 a 10 anos, 6 crianças de 11 a 12 anos e 5 crianças de 13 a 14 anos.

Resultados: As médias de erros nestes quatro grupos etários foram de 17,8, 18,5, 15,2 e 10,8 respectivamente ($p = 0,56$; teste não paramétrico de Kruskal-Wallis). As médias do total de erros para as mãos direita e esquerda foram de 8,5 e 8,3 respectivamente. Comparando-se o grupo de cinco crianças portadoras de desnutrição crônica (escore de desvio-padrão para índice antropométrico altura/idade < -2) com o grupo 38 de crianças eutróficas, as médias de erros foram de 12 e 17,4 respectivamente ($p = 0,19$; teste não paramétrico de Mann-Whitney). As médias de erros para as posições da mão esquerda a zero, 90, 180 e 270 graus foram de 1,7, 2,5, 2,2 e 1,9 respectivamente e, para a mão direita a zero, 90, 180 e 270 graus foram de 2,1, 1,7, 2,4 e 2,3, respectivamente (escore de 0 a 5).

Conclusão: Concluiu-se que os testes são aplicáveis a populações rurais e que o índice de erros diminuiu com o aumento da faixa etária. Não foi possível detectar diferenças nas performances de crianças com desnutrição crônica e eutróficas, observando-se inclusive uma média de erros menor entre as desnutridas, porém sem significância estatística. Apesar do número reduzido de indivíduos desnutridos, os resultados sugerem que o cérebro humano é extremamente resiliente e, mesmo confrontado com um aporte energético comprometido, é capaz de desviar recursos de outros sistemas para garantir a preservação dos processos cognitivos. **Palavras-Chave:** Imagética motora, desnutrição, rotação mental, lateralidade

Apoio Financeiro: CNPq

A.06.001

ANTIOXIDANT EFFECTS OF PHENOLIC COMPOUNDS AGAINST DIFFERENT PROOXIDANT AGENTS IN BRAIN OF RATS

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Considering the important role of oxidative stress in the pathogenesis of several neurological diseases, and the growing evidence of the presence of compounds with antioxidant properties in the plant extracts composition, the aim of the present study was to investigate the antioxidant capacity of different isolated compounds (quercetin, quercitrin, gallic acid and rutin), commonly found in plants, in rat brain homogenates. Cerebral lipid peroxidation (assessed by TBARS method) was induced by iron sulfate (10mM), sodium nitropruside (SNP-5mM) and 3- nitropropionic acid (3-NPA-2mM). Iron, SNP and 3-NPA -induced TBARS production in brain preparations was significantly decreased by Quercetin ($p < 0.001$), Gallic Ácid ($p < 0.001$), Quercitrin ($p < 0.001$) and Rutin ($p < 0.001$). However, the inhibitory potency was distinct depending on the type of pro-oxidant agent used. In fact, they were more efficient in inhibiting TBARS production produced by 3-NPA followed by SNP and Iron, except for rutin, that the inhibitory potency was in the order $SNP > 3-NPA > Iron$. Quercetin is the most potent among tested compounds, presenting lowest IC_{50} values, followed by gallic acid, quercitrin and rutin. This could be explained by the fact that quercetin is more liposoluble than the other tested compounds, so it is capable to protect more effectively against lipid peroxidation. Rutin was the less effective, maybe because of the glycoside group present in its structure that makes this flavonoid unable to penetrate the plasmatic membrane, by lowering its liposolubility, probably preventing its access to the lipid membranes (Saija, 1995 free radical biology medicine). Thus our study indicates that phenolic compounds could be considered effective agents in the prevention of various neurological diseases, which are associated with oxidative stress. Finally we demonstrate that quercetin could be considered a better choice to be used in a future antioxidant therapy with isolated compounds from plant extracts.

Palavras-Chave: oxidative stress, antioxidants, plants, brain

HUMAN MESENCHYMAL STEM CELLS TRANSPLANTED INTO THE LESIONED SUBSTANTIA NIGRA IMPROVE MOTOR SYMPTOMS IN AN EXPERIMENTAL RAT MODEL OF PARKINSONISM

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Cell transplantation into the damaged brain to replace neurons in neurodegenerative diseases is one of the approaches used in the treatment of Parkinson's disease (PD). The effect of the transplant depends on the type of cells used and the brain region where they are implanted. The aim of this work was to evaluate the neuroanatomic and functional effects of intranigral transplants of human mesenchymal stem cells (hMSC), previously exposed to a neural induction medium, in a rat model of unilateral induced parkinsonism.

Rats were lesioned 1 week before the transplant with 6-hydroxydopamine (6-OHDA) in 3 deposits placed in the lateral right striatum, to get a partial lesion of the dopaminergic nigrostriatal system. All rats received dexamethasone, starting 1 day before transplantation until the end of the experiment. Some animals received human cells (hMSC) while others were injected without cells (sham) ventral to the right substantia nigra (SN). Two motor behavioral tests were used to assess both lesion deficits and graft-derived recovery: the amphetamine-induced rotational test and the test of asymmetry of forelimbs. Detection of grafted cells and dopaminergic neurons were evaluated by immunohistochemistry for a specific human nucleoprotein (hRNP) and tyrosine hydroxylase (TH), respectively. Although in SN, dopaminergic cell loss was similar in sham (n=6) and grafted rats (n=7) at the end of the experiment, we observed during the 3 weeks of transplant a major use of the left forelimb instead of the right side in lesioned-grafted animals. Furthermore, the increment of amphetamine-induced turns was reduced in grafted rats with respect to sham rats at 3 weeks post-grafting. Additionally, we detected the human cells integrated into the rat brain in a nondisruptive manner near the SN, even 3 weeks after implantation.

In conclusion, hMSC transplanted at the SN level can improve some of the parkinsonism-related symptoms in a rat model of PD. As we did not find evidence of cell replacement in SN, we suggest that hMSC can promote functional recovery of the remaining dopaminergic neurons of the lesioned SN possibly due to a neurotrophic effect.

Palavras-Chave: human mesenchymal stem cells; transplant; parkinsonian rat model; motor symptoms

Apoio Financeiro: Fundación Andes C-14060/60 and UDD 80.11.037

HUMAN DENTAL PULP CELLS INFLUENCE POSITIVELY FUNCTIONAL RECOVERY AFTER CHRONIC SPINAL CORD INJURY

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Functional deficits following spinal cord injury result from damage to axons, loss of neurons and glia and demyelination. Cell transplantation is a therapeutic strategy that promotes axonal growth and replaces lost cells. The aim of this study was to evaluate the efficacy of the transplant of human dental pulp cells (HDPC) on the recovery of chronic spinal cord injury in C57/Black6 mice. After anesthesia, animals were submitted to laminectomy at T9 level followed by extra dural spinal cord compression with a 30g vascular clip (KENT Scientific Corporation, code INS 14120) for 1 minute. The animals were divided into two groups: HDPC-treated animals and untreated animals. HDPC were labeled with Cell Tracer before transplantation. Cells were injected into the lesion epicenter (8×10^5) in 2 μ L of culture medium (DMEM) four weeks after injury. Behavior testing (BMS and global mobility) was performed weekly up to 8 weeks after transplantation. After this period, the spinal cords were processed for fluorescence microscopy to evaluate the survival of transplanted cells into the host tissue. Our results showed that HPDC were present in the host tissue after 2 months. All animals showed total paralysis 24 hours after the injury. At 8 weeks, HPDC-treated animals, reached the intermediate phase of locomotor recovery, whereas the untreated control group achieved the initial phase, according to BMS score. Video analysis of the global mobility test showed that HPDC-treated animals presented higher speed in locomotion than untreated animals. Our results suggest that transplantation of human dental pulp cells into the spinal cord improves locomotor recovery and also that these cells are able to survive in the host tissue and may contribute to the regenerative process.

Palavras-Chave: spinal cord injury, human dental pulp cells, BMS

Apoio Financeiro: FAPERJ, CNPq, CAPES

A.06.004

Interferon beta and Glatiramer Acetate induce proliferation of Schwann cells *in vitro*

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The use of bioactive conduits seeded with Schwann cells (SC) is considered a promising approach to replace the need for autografting following peripheral nerve injury. However, techniques as well as substances capable of stimulating cultured Schwann cell proliferation are needed, in order to allow for future therapeutic attempts. In the present work the effects of interferon beta (IFN beta) and glatiramer acetate (GA) on Schwann cell cultures were tested, with special interest on the growth curve and potential hyperplasic effects of such substances. Cultures of Schwann cells were prepared from the sciatic nerves of 1 to 2 day-old Sprague Dawley rat pups, which were dissected free from epineurium and processed to cell isolation. Following this procedure, the cells were seeded onto culture plates and treated with different doses of IFN beta (100, 500 and 1000 µl/ml) and of GA (1.2, 2.5 and 5.0 µg/ml) after 24h for five consecutive days. Both treatments showed an important mitogenic effect in comparison with control cultures. Percentage of cell growth presented in control cells was $199.9\% \pm 36$ while IFN beta treated cells demonstrated an increase of $402.4\% \pm 43$ in 100µl/ml dose ($p=0.003$), $351.3\% \pm 27$ in 500µl/ml dose ($p=0.01$) and $384.3\% \pm 13$ in 1000µl/ml dose ($p=0.003$). In GA treatment, the lowest dose of 1.2µg/ml had no difference in relation to control culture ($p=0.2$), however, dosages of 2.5µg/ml and 5.0µg/ml revealed a significant rate of cell growth ($352.4\% \pm 39$, $p=0.03$ and $420.5\% \pm 47$, $p=0.02$ respectively). Overall, the present results provide evidence that both drugs enhance SC proliferation and activity, which may in turn positively influence the peripheral nerve regeneration process.

Palavras-Chave: schwann cell, cell culture, regeneration

Apoio Financeiro: CNPq

Evaluation of human umbilical cord blood mononuclear cell transplantation in neonatal hypoxic-ischemic brain damage

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Neonatal hypoxic-ischemic (HI) encephalopathy remains an important cause of chronic neurologic morbidity in the world. In this work we evaluated the possible therapeutic potential of human umbilical cord blood mononuclear cells (HUCBCs) in rats submitted to a model of neonatal HI brain damage. P7 Lister-Hooded rats had the right common carotid artery ligated and, after a 2-hour recovery period, were placed in a chamber with a flow of oxygen (8%) and nitrogen (92%) for 90 minutes. After 3h, the pups received a single intraperitoneal injection of 2×10^6 cells prelabeled with CellTrace[®] (cell-treated group) or saline (untreated).

Few cells were found in the cerebral cortex ipsilateral to the ischemia, but not in the contralateral hemisphere, 2 days after the injection. Immunohistochemical analysis revealed an increase in the expression of the chemokine stromal-derived factor 1 (SDF-1) in the ipsilateral hemisphere, 2 days after HI. Flow cytometry analysis showed that 31.16% of the HUCBCs expressed the CXC chemokine receptor 4 (CXCR4), the receptor for SDF-1.

The animals were evaluated for weight gain and functional outcome by 3 neonatal reflexes (gait, negative geotaxis and cliff aversion) at 2, 4, 7, 10 and 14 days after the insult. HI animals (untreated) displayed retarded performance in cliff aversion and negative geotaxis reflexes and also a lower weight gain, when compared with sham-operated animals. The cell-treated group showed a tendency to have a performance similar to the sham group at P9 and P11 in the negative geotaxis reflex (sham: $11,90s \pm 2,48$ SE; HI: $20,46s \pm 3,78$ SE; HI+ cells: $11,20s \pm 1,24$ SE at P11), and at P11, P17 and P21 in the cliff aversion reflex (sham: $6,62s \pm 1,73$ SE; HI: $8,69s \pm 1,17$ SE; HI+ cells: $7,10s \pm 1,45$ SE at P17). At P21, the cell-treated group showed a tendency to have a body weight similar to the sham group (sham: $35,81g \pm 0,78$ SE; HI: $31,92g \pm 0,95$ SE; HI+ cells: $34,41g \pm 1,70$ SE).

Our data indicates that HUCBCs migrated to areas of HI brain damage. We suggest that the SDF-1/CXCR4 system may be involved in this migration. Moreover, we showed a tendency for better functional outcome in the cell-treated group.

Palavras-Chave: stem cells; umbilical cord blood; cerebral palsy

Apoio Financeiro: FAPERJ, CNPq, CAPES

A.06.006

INVESTIGAÇÃO DOS EFEITOS DO EXERCÍCIO TERAPÊUTICO SOBRE A REGENERAÇÃO NERVOSA PERIFÉRICA.

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Objetivo:

Apesar de o sistema nervoso periférico apresentar relativa capacidade de regeneração, é comum, após diferentes graus de acometimento, que suas funções se restabeleçam deficitariamente, mesmo depois de submetidos a intervenções apropriadas. Nesse contexto, objetivamos averiguar se o exercício terapêutico é capaz de interferir no processo de regeneração nervosa periférica. **Métodos e resultado:** Para a condução dos experimentos foram utilizados 40 ratos machos *Wistar*, previamente treinados nas condições de experimentação, submetidos a estrangulamento do nervo ciático direito, e separados aleatoriamente em quatro grupos distintos conforme período de início dos exercícios (24hs: n=10; 7 dias: n=10; 14 dias: n=10; Controle: n=10). Durante 21 dias consecutivos, os animais foram submetidos ao nado forçado durante de 20 minutos por dia, exceto o grupo controle (sedentário), e submetidos aos procedimentos de coleta de Índice Funcional do Ciático (IFC), vinte e quatro horas, 7, 14, 21 dias após o estrangulamento do nervo ciático. Após avaliação estatística do IFC (ANOVA de uma via e/ou teste t de Student, $p < 0,05$) não foi possível observar diferenças significantes entre os diferentes grupos estudados. **Conclusões:** Diferente do que foi encontrado na literatura pertinente, o exercício terapêutico, como empregado no presente estudo, foi incapaz de interferir positivamente no processo de regeneração do nervo ciático estrangulado de ratos *Wistar*. **Palavras-Chave:** regeneração, ciático, ratos, exercícios

Apoio Financeiro: PIBIC- CNPQ, PIBIC-FURB

A.06.007

Retinoic acid reduces the inflammatory production of prostaglandins, chemokines and cytokines in cortical astrocytes

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Objectives. Spinal cord contusion injury causes a delayed, local increase of retinoic acid (RA) synthesis [Mey et al., *Eur. J. Neurosci.* 21 (2005): 1555-1568]. Retinoic acid receptors RAR α and RXRs were found in microglia/macrophages in the area of the lesion, in spared neurons and in astrocytes surrounding the site of injury [Schrage et al., *Eur. J. Neurosci.* 23 (2006): 285-295]. Based on these data and on observations from cell culture experiments we suggested that RA signaling is part of an anti-inflammatory feedback loop in the CNS [Mey, *J. Neurobiol.* 66 (2006): 757-779; Mey et al., *Glia* 55 (2007): 152-164]. This hypothesis was tested with astrocyte primary cultures from cerebral cortex of perinatal mice.

Methods. Using a gene chip analysis we assessed the inflammatory effects elicited by lipopolysaccharides (LPS) and the influence of RA on this response. Effects of RA were then investigated and quantified with real time RT-PCR, ELISA and Western blotting.

Results. RA reduced mRNA expression and protein release of specific pro-inflammatory mediators including chemokines CCL2 (MCP-1), CCL3 (MIP-1 α), CCL5 (RANTES), CXCL1 (KC), CXCL2 (MIP-2) and cytokines IL-1 β , IL-6, IL-12, and TNF α . In addition, we found that RA influenced metabolism of prostaglandins, which are important mediators of inflammation and pain signaling. In the astrocytes LPS treatment induced the expression of enzymes for the production of arachidonic acid and prostaglandins but caused down-regulation of a prostaglandin degrading enzyme and of leukotriene synthesizing enzymes that compete with prostaglandin synthesis. Consequently, the secretion of the prostaglandin E₂ was highly increased after LPS exposure. RA counteracted the inflammatory regulation of COX-2 mRNA and protein and thereby reduced the synthesis of prostaglandin E₂ by approximately 60% (controls: 8 +/- 1 pg/ml, LPS: 899 +/- 28 pg/ml, LPS+RA: 313 +/- 35 pg/ml, means, SEM, n=3).

Conclusion. Astrocytes may be a physiological target of endogenous RA signals after CNS injury. The influence of RA on inflammatory reactions appears to be mediated by direct and indirect molecular mechanisms, which are now being investigated. **Palavras-Chave:** retinoic acid, neuroinflammation, prostaglandin, astrocyte, cytokine

Apoio Financeiro: EURON Graduate School of Neuroscience

EMBRYONIC STEM CELLS PROMOTE FUNCTIONAL RECOVERY AFTER MODERATE SPINAL CORD COMPRESSIVE INJURY

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Objectives: Experimental models of compressive spinal cord injury (SCI) simulate the clinical situation that is commonly observed in humans and allow studies of functional and morphological recovery following regenerative approaches. Several types of stem cells have been utilized in animal models of SCI with different outcomes, with no consensus regarding the results. In this work, we tested the hypothesis that grafts of pre-differentiated mouse embryonic stem cells (ES) could improve functional and morphological recovery after SCI in mice. **Methods:** C57/Black6 mice were subjected to laminectomy at T9 level and extradural compression of the spinal cord with a vascular clip (30g force, 1 minute) to promote an acute injury. Two groups were analyzed: ES-treated (EST) and control (CT), n = 8 each. ES pre-treated with retinoic acid (2 μ M) for 4 days were injected (8×10^5 cells/2 μ l) into the epicenter of the lesion 10 minutes after SCI in EST animals. During 8 weeks, animals' performance was analyzed in open-field using Basso Mouse Scale (BMS), and videotaped for quantitative evaluation of global mobility improvement. Spinal cord serial cross sections were obtained rostrally and caudally from the epicenter, and stained with Luxol Fast Blue (LFB). The spared white matter was calculated as total cross-sectional area minus LFB not stained area and reported as a percentage of the total cross-sectional area. For statistic analysis Mann-Whitney and T tests were applied. **Results:** All animals showed complete flaccid paralysis 24 h after injury with improvement afterwards. While CT improved to BMS intermediate phase (2.037 ± 0.399), EST animals reached the final phase of locomotive recovery (3.872 ± 0.711), $p < 0.05$. In the videotape, EST animals presented a higher speed of locomotion (10.73 ± 0.907) than CT animals (5.581 ± 0.290), $p < 0.05$. In morphological analyses, EST group showed an increase in white matter sparing (46.80 ± 1.24) when compared to CT group (39.97 ± 0.925), $p < 0.05$. **Conclusions:** Our results indicate that acute transplantation of pre-differentiated embryonic stem cells into the spinal cord increases the spared white matter and improves locomotor function after SCI in mice.

Palavras-Chave: spinal cord injury, compressive experimental model, embryonic stem cell, functional analysis

Apoio Financeiro: CNPq, FAPERJ, Ministério da Saúde, TWAS.

A.06.009

Vascular endothelial growth factor stimulates sciatic nerve regeneration and improves functional recovery

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Peripheral nerve injuries are a commonly found clinical problem and often result in long-term functional deficits. Treatment of lesions causing complete transection of peripheral nerve is primarily aimed by recreating continuity of the nerve to allow axonal regrowth. Reconstruction of peripheral nerve defects is currently undertaken with nerve autografts. However, there is a limited availability of nerves that can be sacrificed and, in general, the functional recovery is not satisfactory. Extensive investigation towards the development of new methods to improve regeneration following nerve injury is ongoing. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor, which stimulates proliferation and migration of endothelial cells and formation of new blood vessels. Increased rates of axonal regeneration in the vicinity of a better vascularised area suggest a positive interaction between tissue regeneration and angiogenesis. The aim of this study is to investigate the effects of VEGF on regeneration of the sciatic nerve "in vivo". For that the left sciatic nerves and the adjacent muscle groups of female adult C57Bl/6 mice were exposed, and a 50 ug vector containing hVEGF165 gene (50 ul PBS) was injected in the thigh just under the sciatic nerve followed by application of 3 pulses of 150 V/cm with 50 ms of duration with 1s of interval. After, the left sciatic nerve was transected and the proximal and distal stumps were placed in a resorbable collagen tube (6mm long). After 6 weeks mice were perfused and the sciatic nerves were processed for light and electron microscopy. Analysis of semithin sections showed signs of regeneration with a well-organized nerve tissue. Motor function was tested at 2, 4 and 6 weeks after surgery and showed that the sciatic functional index (SFI) was significantly ($p = 0.01$) improved in animals that received the VEGF administration (-99.00 ± 9.59) as compared with control group (-131.55 ± 3.96). These results demonstrate a direct positive relationship between increased vascularisation and enhanced nerve regeneration, indicating that VEGF administration can support and enhance the growth of regenerating nerve fibers.

Palavras-Chave: nerve injury, VEGF, motor function

Apoio Financeiro: CNPq, CAPES, FAPERJ.

A.06.010

Expression of chondroitinase AC by adult bone marrow-derived mononuclear cells promotes GAP-43 and synaptophysin expression at injury sites in the motor cortex

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Objectives: Adult mammalian CNS is unable to regenerate axons and to reestablish functional connections after an injury due to glial scar formation at the injury site. Glial cells produce high levels of axonal growth inhibitory molecules, including chondroitin sulfate proteoglycans. Chondroitin sulfate (CS) degradation by chondroitinases promotes axonal regeneration after spinal cord and nigrostriatal tract injuries. We have cloned the *Flavobacterium heparinum* chondroitinase AC gene into the expression vector pcDNA3.1(+), transfected adult bone marrow-derived mononuclear cells (aBMC) with the vector and transplanted the transfected cells at injury sites in the motor cortex of BALB/c mice (1). We showed that degradation of CS by chondroitinase AC expressed by the transplanted aBMMC enhances the expression of growth-associated protein GAP-43. We have also observed that GAP-43 and synaptophysin were expressed close to injury sites in all animals that were transplanted with chondroitinase AC-expressing aBMC. The aims of the present work were to construct a vector for controllable chondroitinase AC gene expression, and to evaluate the effect of aBMC transplantation on locomotion of injured animals.

Methods: We have cloned the chondroitinase AC gene into the retroviral system BD RevTetOnTM. This retroviral system consists of a regulatory vector, pRevTetOn, and an expression vector, pRevTRE; the regulatory function of the protein expressed by pRevTetOn is controlled by doxycycline.

Results: The retroviruses containing chondroitinase AC gene were produced by transfecting packaging cell lines, PE501 and PT67, and we have produced viruses with titles of 7×10^5 cfu/ml (RevTRE-cAC) and 5×10^5 cfu/ml (RevTetOn). Locomotion analysis showed that transplantation of chondroitinase AC-expressing aBMC did not improve the walking deficiency caused by the injury, and this could be related to the disorganization in growing axons observed.

Conclusions: In conclusion, although degradation of CS by chondroitinase AC expressed by exogenous aBMC promotes expression of GAP-43 and synaptophysin, neurons might not be making the right connections.

References: 1- J. Neurosci. Meth, 171:19-29, 2008

Palavras-Chave: chondroitinase, central nervous system, injury, regeneration

Apoio Financeiro: CNPq, FAPESP, FADA-UNIFESP.

A.06.011

Study of the functional recovery after treatment in the chronic phase of the sensorimotor cortical ischemia with bone marrow mononuclear cells

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Introduction: Our group has been observed therapeutic effect of the treatment with bone marrow mononuclear cells (BMMCs) when administered one day after sensorimotor cortical ischemia. In the present work we postponed the time of treatment to one month after ischemia to investigate its efficacy when performed in the chronic phase of the disease.

Methods: Ischemic lesion was induced by thermocoagulation in the left sensorimotor cortex. BMMCs were obtained from donor rats and injected into the femoral vein thirty days after the ischemia. In the BMMC group, animals received 3×10^7 cells. In the control group, animals received PBS. Animals were tested for behavior at post-ischemic days (PID) 2, 14 and 28, and at post-treatment days (PTD) 7, 14, 21, 28 and 35. Two behavioral tests were used: the cylinder test and the adhesive-removal test.

Results: In the cylinder test, no significant difference in the functional performance was observed between the groups, although the BMMC group presented a clear tendency of recovery after the treatment. In the adhesive test, the analysis of the side-preference of first removal in the forelimbs showed no functional recovery in both groups, with no significant difference between them, although a slight tendency of recovery was observed in the BMMC group after the treatment. The analysis of the time of removal in the impaired forelimb showed spontaneous recovery before treatment in both groups, with no significant difference between them.

Conclusions: Our results have suggested that the treatment with BMMCs in the chronic phase of the sensorimotor ischemia has no significant effect in the functional recovery. However, the tendency of increased recovery observed after the treatment with the BMMCs suggests that this treatment might be effective, and changes in some parameters of this treatment should also be tested to verify with more accuracy the real therapeutic potential of these cells and the best protocol. For example, increased doses of BMMCs might convert the observed tendency in a significant difference, as well as the beginning of the treatment inside the chronic phase but closer to the day of induction of the ischemia. **Palavras-Chave:** ischemia, functional tests, bone marrow mononuclear cells

Apoio Financeiro: Capes

ESTUDO *IN VIVO* E *IN VITRO* DOS EFEITOS DAS GTPASES RHO NAS CÉLULAS-TRONCO RETINIANAS

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Objetivos: A estrutura do olho denominada Corpo Ciliar (CC) contém células-tronco retinianas e é capaz de formar neuroesferas em cultura, sendo relacionada ao potencial regenerativo da retina de mamíferos. O objetivo foi avaliar a participação das GTPases Rho, proteínas importantes na proliferação, diferenciação e morte neuronal, sobre a proliferação das células do CC.

Métodos e Resultados: *In vivo*-Camundongos adultos receberam 4 injeções intraoculares do inibidor das GTPases (ToxinaA), ativador (ácido lisofosfatídico, LPA) ou PBS (controle), em conjunto ou não com mitógenos (FGF, insulina). Após fixação e corte em criostato, foi feita análise por imunofluorescência com anticorpos contra as proteínas RhoA, RhoB, Rac1 (Sta Cruz), marcadores de proliferação Ki67 (Vector) e BrdU (Accurate) e de progenitores Pax6 (Babco) e Chx10 (Chemicon). *In vitro*- Após tratamento enzimático (colagenase/hialuronidase), células do CC foram dissecadas e cultivadas em meio DMEM-F12 com mitógenos FGF+EGF+heparina. Após 7 dias, as neuroesferas foram diferenciadas com BDNF+Ácido Retinóico+SFB e tratadas com ToxinaA. *In vivo*, a toxinaA foi capaz de aumentar o número de células Ki67+ no CC em 7 vezes e apresentou maior proliferação em conjunto com os mitógenos (aumento de 11 vezes). O mesmo foi visto *in vitro*, onde neuroesferas diferenciadas apresentaram mais células BrdU+ que o controle (10%). Não foram detectadas células Ki67+ no CC após a injeção de LPA *in vivo* e neuroesferas apresentaram diminuição de 30% no número de células marcadas com BrdU após toxinaA.

Conclusões: Os dados indicam que as GTPases Rho podem atuar como controladores negativos da entrada no ciclo celular nas células do CC adultas e diferenciadas, pois o bloqueio foi capaz de induzir a proliferação no CC de animais adultos e das neuroesferas em diferenciação. Em contrapartida, a ativação destas proteínas promove aumento de progenitores não proliferantes e ainda inibe a proliferação existente. **Palavras-Chave:** GTPases Rho, retina, regeneração

Apoio Financeiro: FAPESP, CNPq

TRANSPLANTATION OF MOTOR NEURONS DERIVED FROM MOUSE EMBRYONIC STEM CELLS PROMOTE A TRANSIENT RECOVERY IN A RAT MODEL OF FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Objectives The aim of this work was differentiate to motor neurons (MN) from mouse embryonic stem (ES) cells and to transplant them in the spinal cord (SC) of adult transgenic rats that express the mutated G93A human superoxide dismutase 1 (hSOD1^{G93A}), a model of familial amyotrophic lateral sclerosis (ALS), to evaluate their integration and therapeutic capacity.

Methods HBG3 ES cells that express green fluorescent protein (GFP) under the control of the MN-specific promoter *hb9* were differentiated as described previously (Cell 110: 385, 2002). The hSOD1^{G93A} transgenic animals were genotyped by PCR; we transplanted 100,000 differentiated cells in 1 µl into the L5 ventral gray matter after laminotomy (0.5 mm lateral to the midline, and 1.5 mm deep from the dorsal surface of the SC) in adult transgenic rats at 3 months of age. In order to assess motor function, we performed the rotarod test. This trial allowed us to establish the onset of motor deterioration in non-transplanted animals after 4 months. At the end of the experiments we analyzed the SC by cresyl violet staining and immunohistochemistry for choline acetyltransferase (ChAT).

Results We examined, by immunocytochemistry, that after stimulation of ES cells with retinoic acid and sonic hedgehog, cells expressed GFP and the neuronal markers β-tubulin III, Islet 1 and ChAT, indicating their differentiation to MN. We analyzed the SC of transplanted animals after 7 days and observed cells positive for GFP and ChAT. In transgenic non-transplanted rats, the paralysis started after 16 weeks of age accompanied by a marked reduction in the number of MN in the SC, which correlated with the decreased performance in the rotarod test. On the other hand, the transplanted group showed a transient significant recovery of motor function in weeks 16 and 17 of age. However, afterwards the animals showed a decreased performance in the rotarod test, similar to the non-transplanted animals and finally they became paralyzed. We didn't find MN in the SC of grafted animals at this terminal stage. These results suggest that the transient recovery could be due to the transplanted population, but the MN didn't survive for long periods of time.

Palavras-Chave: motor neurons, embryonic stem cells and amyotrophic lateral sclerosis

Apoio Financeiro: DGAPA (IN224207), CONACyT 14285 and TWAS

A.06.014

Human Immature Dental Pulp Stem Cells in the Reconstruction of Corneal Epithelium with Total Limbal Deficiency.

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Objective: To evaluate the similarities between human Immature Dental Pulp Stem Cells (hIDPSC) and Limbal Stem Cells (LSC) and the potential of hIDPSC in the reconstruction of the cornea epithelium after induction of Total Limbal Deficiency (TLD) in rabbit eye.

Methods: The hIDPSC were cultivated according to literature (1). The similarities between hIDPSC were available by RT-PCR and immunocytochemistry analysis. TLD was induced by chemical burn in one eye of rabbits, in accordance with the Instituto Butantan Ethics Committee (protocol nº 250/06). Experimental group (n=9) received undifferentiated hIDPSC cultivated on the scaffold that was transplanted in the injured eyes were covered by amniotic membrane (AM), while control group (n=3) only received AM. After 3 months both groups were sacrificed and the tissues formed onto the rabbit eyes were submitted to immunohistochemistry and FISH analyses, besides histological study. Transmission electron microscopy (TEM) was also performed.

Results: RT-PCR results showed that undifferentiated hIDPSC and LSC present similar pattern of expression of LSC markers. After hIDPSC transplantation into injured rabbit eye, the transparency of the cornea was improved indicating differentiation of hIDPSC into corneal tissue, which was demonstrated by immunohistochemistry analysis with antibodies against corneal epithelium, and other human specific markers. In control group, the conjunctiva fully covered the cornea and was strongly vascularized, while reacting negatively for these markers. Histological and TEM analyses confirmed the formation of corneal epithelium, while in control group the cornea structure was completely disorganized.

Conclusions: We observed the similarities in gene expression pattern between undifferentiated hIDPSC and LSC. Moreover, successful improvement the transparency of the new-formed cornea was demonstrated after transplantation of hIDPSC into the TLD model. Our data suggest that hIDPSC are a strong candidate to be used in corneal epithelium reconstruction.

Reference: 1. *Cells Tissue and Organs*. 184:05-16, 2006.

Palavras-Chave: dental pulp stem cells, tissue engineering, corneal epithelium reconstruction

Apoio Financeiro: Capes, CNPq, Fapesp

UP-REGULATION OF 9-O ACETYL GD3 GANGLIOSIDE AFTER CEREBELLAR LESION IN ADULT RAT.

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Gangliosides are glycosphingolipids located in the cellular membrane of mammals, specially in the nervous system (Lloyd and Furukawa, 1998). The ganglioside 9-O-acetyl GD3 is expressed at high levels during the development and it has been related to radial migration, tangential migration and axonal extension (Mendez-Otero et al., 1988; Santiago et al., 2001; Santiago et al., 2004). In the adulthood, this ganglioside expression persists only at the subventricular zone (SVZ), the rostral migratory stream (RMS), the cerebellum and in the retina (Mendez-otero and Cavalcante, 1996; Mendez-Otero et al., 1988). It was also been described the increase of the ganglioside 9-O-acetyl GD3 expression in a model of sciatic nerve crush (Ribeiro-Resende et al., 2007).

In this study we intend characterize this ganglioside expression in a model of cerebellar injury. In this model, we used Lister-hooded adult rats and the cerebellar lesion was made through an incision of 0,7 cm depth, trespassing the vermis through an opening at the rat skull delimited by estereotaxic coordinates. The animals were sacrificed seven days after the surgery. The expression of the ganglioside 9-O-acetyl GD3 was analyzed by immunohistochemistry with Jones monoclonal antibody and posterior analysis through confocal microscopy. We also separated the cerebellum gangliosides by HPTLC (high performance thin layer chromatography) and immunostained the bands relative to the ganglioside 9-O-acetyl GD3 in injured and control animals. We observed the increase of the ganglioside 9-O-acetyl GD3 expression after the cerebellar lesion, specially on the molecular and the Purkinje cell layers. Furthermore, the HPTLC analyses confirm this up-regulation, showing an increase of 1,5x in comparison with the control. This change on the 9-O-acetyl GD3 ganglioside expression after injury could suggest a possible influence of this molecule on regenerative processes in the central nervous system.

Palavras-Chave: 9-O acetyl GD3 ganglioside; Cerebellum; Regeneration

Apoio Financeiro: FAPERJ, CNPq, CAPES

A.06.016

Electromagnetic fields effects on peripheral nerve regeneration.

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Objectives: After a peripheral nerve crush lesion there is a variable possibility of regeneration, depending on many factors including type of lesion, animal specie, metabolic characteristics and others. The proximal stump, connected with the cell body, will generate axon sprouts that will try to achieve the target-organ and reestablish normal function. This process needs, sometimes, to be reinforced by artificial methods. The use of electromagnetic fields can enhance regeneration but its diversity of parameters must be better understood. This study aimed to identify the effects of a prolonged protocol of low frequency pulsed electromagnetic field (PEMF) on peripheral nerve regeneration and its influence on associated oxidative stress. **Methods:** We used 34 male Swiss mice (*Mus musculus*) divided into PEMF (n=17) and control (n=17) groups. All animals of them were submitted to an unilateral sciatic crush lesion and PEMF group was exposed to a 72 Hz, 2 Gauss electromagnatic field, 30 minutes, five days a week, for three weeks. Functional analysis was carried out weekly. At the end of the three week period of stimulation animals were euthanized and histological, morphometric and oxydative stress analyses were proceeded. **Results:** Functional analysis showed no differences between PEMF and control groups. Histological appearance was similar between crushed and treated or control nerves, except for the presence of larger vessels in PEMF group, but without significant statistical differences. Morphometric assessment did not show any significant difference between pemf and control groups. The expression of free radicals was more pronounced on PEMF nerves. **Conclusion:** Except for the oxidative stress analysis, a prolonged regimen of exposition to a low frequency and amplitude electromagnetic field did not show any outcome on nerve regeneration. **Palavras-Chave:** Sciatic nerve lesion, electromagnetic fields, regeneration, mouse

Apoio Financeiro: Fiocruz, UFRJ, Cnpq

B.01.001

EXPOSIÇÃO CRÔNICA DE CAFEÍNA NA ADOLESCÊNCIA INDUZ SENSIBILIZAÇÃO CRUZADA AO METILFENIDATO EM RATOS WISTAR: PARTICIPAÇÃO DE DARPP-32

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Objetivos: Avaliar se a administração crônica de cafeína em ratos adolescentes altera a hiperlocomação induzida metilfenidato (MPD) na fase adulta e investigar a participação da proteína DARPP-32 neste modelo.

Métodos: Ratos Wistar jovens machos com acesso a água ou cafeína (0,3 g/L) *ad libitum* por 28 dias. O consumo de cafeína foi interrompido por 14 dias, quando os ratos foram tratados com salina ou MPD nas doses de 1 ou 2 mg/kg i.p. A atividade locomotora foi avaliada usando o teste de campo aberto. Após a avaliação comportamental, os animais foram mortos, o cérebro removido e o córtex cerebral e estriado dissecados. As proteínas foram separadas por SDS-PAGE e por Western-blotting realizou-se a detecção do conteúdo da proteína DARPP-32. Os complexos imunes foram quantificados e estatisticamente comparados com One Way ANOVA ($P < 0,05$); seguido por Tukey para análise comportamental ($n=12$) e Duncan para análise bioquímica ($n=5$). Os dados estão expressos como média $\pm$ erro padrão.

Resultados: Os animais expostos à cafeína e tratados com salina não tiveram alteração na atividade locomotora (n° de cruzamentos: controle = $70,6 \pm 4$; CAF = $85,6 \pm 5,5$). Porém, quando estes receberam MPD (1 mg/kg) na fase adulta tiveram um aumento significativo da atividade locomotora (CAF+MPD = $95,6 \pm 3$). Houve um aumento na locomoção dos animais tratados com MPD 2mg/kg (MPD2 = $90,4 \pm 4,8$). A análise bioquímica demonstrou um aumento do conteúdo da proteína DARPP-32 no córtex cerebral quando comparado ao grupo que recebeu somente cafeína (D.O.:controle = $1,4 \pm 0,1$; CAF = $0,75 \pm 0,1$; MPD1 = $0,9 \pm 0,1$; CAF+MPD1 = $0,95 \pm 0,1$). No estriado, a associação causou aumento do imunoconteúdo quando comparada com o grupo que recebeu somente cafeína ou 1 mg/kg de MPD (D.O.:controle = $0,5 \pm 0,07$; CAF = $0,8 \pm 0,07$; MPD1 = $1,1 \pm 0,08$; CAF+MPD1 = $1,4 \pm 0,1$).

Conclusão: Os dados demonstram importante relação entre cafeína e MPD em doses que não teriam efeito *per se*, indicando uma sensibilização cruzada com participação da proteína DARPP-32. A interação entre os sistemas adenosinérgico e dopaminérgico é conhecida, mas um efeito em longo prazo induzido por cafeína, como o observado neste estudo, é inédito.

Palavras-Chave: Cafeína, metilfenidato, TDAH, sensibilização cruzada.

Apoio Financeiro: UNESC e CNPq.

B.01.002

DOPAMINE MODIFIES STRIATAL MITOCHONDRIAL FUNCTION

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Dopamine (DA) is able to induce neurotoxic effects in several neurological and psychiatric diseases. It has been postulated that dopamine may generate changes on mitochondrial function by inhibition of respiratory chain. The aim of this work was to determine the *in vitro* effects of dopamine on striatal mitochondrial function. Dopamine (DA) and oxidation products have been related to mitochondrial dysfunction. Striatal submitochondrial membranes and intact mitochondria were incubated with different DA concentrations for 5 minutes. Significant changes were observed in state 4 oxygen uptake (resting respiration) after 1 mM dopamine incubation. A 35% decrease in state 3 oxygen uptake (active respiration state) was found after 1 mM dopamine incubation. Incubation of submitochondrial membranes with 0.5-0.75-1 mM dopamine inhibited cytochrome oxidase by 38%, 46% and 53% respectively showing the possible inhibition of mitochondrial respiration through the inhibition of cytochrome oxidase. Complex I activity was significantly inhibited by incubation of striatal submitochondrial membranes with 1 mM dopamine. Hydrogen peroxide production significantly increased by 91% 1 mM DA incubation. Also, DA was able to induce striatal mitochondrial membrane depolarization.

Our results suggest that in our study conditions, DA modifies striatal mitochondrial function through inhibition of oxygen consumption, inhibition of cytochrome oxidase activity, increase in hydrogen peroxide production and mitochondrial membrane depolarization.

Palavras-Chave: Dopamine, Striatal mitochondria, Nitric oxide

LIBERAÇÃO DE GABA ESTIMULADA POR RECEPTORES METABOTRÓPICOS DE GLUTAMATO EM CÉLULAS AMÁCRINAS DA RETINA DE GALINHA

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Introdução e objetivo: As interações entre os sistemas de neurotransmissores glutamatérgico e GABAérgico são muito importantes para o pré-processamento da informação visual feito pela retina. A ativação de receptores ionotrópicos de glutamato determina liberação de GABA de células horizontais e amácrinas da retina de galinha. Os receptores metabotrópicos para glutamato estão presentes em diversos tipos celulares retinianos, inclusive nos neurônios GABAérgicos. O objetivo deste trabalho foi investigar os efeitos da ativação de receptores metabotrópicos de glutamato na liberação de GABA de células amácrinas da retina de galinha.

Métodos e Resultados: Retinas de *Gallus domesticus* de P1 a P7 foram dissecadas e tratadas com drogas agonistas e antagonistas de receptores de glutamato. O material foi fixado, crioprottegido, seccionado e processado imunohistoquimicamente para GABA. O tratamento com o agonista do grupo I e II dos mGluRs, T-ACPD, 100mM, induziu a uma diminuição no número de células amácrinas GABA-positivas em relação ao controle (média da % do controle $\pm$ erro padrão; $54,0 \pm 5,5$, $p < 0,001$). O antagonista do grupo II, MCCG, 200mM, parece reverter parte da liberação pelo T-ACPD, mas seu efeito não foi estatisticamente significativo ($77,8 \pm 4,2$). O antagonista do grupo I, AIDA, 200mM, bloqueou parcialmente o efeito do T-ACPD ($84,3 \pm 6,9$; $p < 0,05$). Os antagonistas dos receptores ionotrópicos NMDA e KA, MK-801 e DNQX, respectivamente, quando aplicados juntos, preveniram a diminuição do número de células GABA-positivas, aumentando a marcação dessas células além do controle ($148,9 \pm 11,1$; $p < 0,01$). MK, 5mM, e DNQX, 200mM, aplicados separadamente, elevam a quantidade de células ao nível do controle ($91,3 \pm 7,1$; $104,7 \pm 3,6$). O agonista do grupo III, L-SOP, 100mM, produz uma liberação de GABA menos acentuada ($69,8 \pm 3,2$; $p < 0,05$).

Conclusão: A ativação de receptores metabotrópicos de glutamato de todos os grupos é capaz de induzir a liberação de GABA, e é provável que este efeito resulte de uma ação indireta em receptores ionotrópicos de glutamato. Em experimentos posteriores, investigaremos a via através da qual ocorrem tais interações entre os receptores.

Palavras-Chave: retina, GABA, glutamato

Apoio Financeiro: CNPq, FAPERJ, UFF, UFRJ, PRONEX

B.01.004

REGULAÇÃO DO DESENVOLVIMENTO DOS SISTEMAS GLUTAMATÉRGICO E GABAÉRGICO EM RETINAS DE EMBRIÃO DE GALINHA.

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Objetivo: A adenosina é um neuromodulador que age como neuroprotetor ao ativar seus receptores (A₁ ou A_{2A}). Temos indícios de estudos *in vitro* que o efeito via A_{2A} envolve a regulação de receptores glutamatérgicos. Objetivamos avaliar a influência de adenosina no desenvolvimento dos sistemas glutamatérgico e GABAérgico.

Métodos: Ovos embrionados de *Gallus domesticus* com 14 dias (E14) foram injetados com agonistas e antagonistas de adenosina (concentração final estimada de 100nM) ou DMSO (controle). Em E16, as retinas foram fixadas ou estimuladas por 30 minutos com NMDA (100mM). Então, as retinas foram crioprotegidas, cortadas em criostato e processadas por imunohistoquímica para GABA ou GAD (enzima que sintetiza o GABA). As células amácrinas imunorreativas para GABA ou GAD foram quantificadas e os dados obtidos analisados (One-way ANOVA). Os valores apresentados correspondem à média ± desvio padrão da porcentagem de células amácrinas imunorreativas para GABA ou GAD (%IR-GABA ou %IR-GAD) com relação ao controle.

Resultados: A exposição ao agonista A₁ (CHA) ou ao antagonista A_{2A} (SCH) de adenosina aumentou a %IR-GABA. O efeito de CHA foi bloqueado por SCH e ZM241385, antagonistas de A_{2A} (CTR=100 n=12; CHA=142±3,90 n=12 p<0.001; SCH=128±11,4 n=3 p<0.01 ZM=128±4,6 n=3 p<0.001; CHA+ZM=108±6,9 n=2 p<0.001; CHA+SCH=98±18,2 n=2 p<0.001). O antagonista A₁ (DPCPX) aumentou a %IR-GABA tanto sozinho quanto na presença de CHA, onde percebe-se uma potencialização do efeito (DPCPX=127±12,7 n=5 p<0.05; CHA+DPCPX=167±13,7 n=4 p<0.001). O CHA também aumentou a %IR-GAD, (CTR=100 n=3; CHA=124±9,1 n=3 p<0.05). Como esperado (Neuroch.Res.28,1475, 2003) estimulação com NMDA diminuiu a %IR-GABA (55±2,2 n=2 p<0,05) em retinas tratadas com DMSO. Este efeito foi menor em retinas tratadas com CHA (81±6,3 n=2 p<0,05), o qual não foi bloqueado pelo tratamento simultâneo com DPCPX (80±9,3 n=2 p<0,05).

Conclusão: Os dados, observados *in vivo*, sugerem que a adenosina module o número de células amácrinas GABAérgicas e a funcionalidade do receptor NMDA. O efeito nos receptores NMDA pode ser devido à diminuição da expressão da subunidade NR1, como mostrado *in vitro* (dados não publicados).

Palavras-Chave: Adenosina, GABA, GAD, NMDA

Apoio Financeiro: CAPES, FAPERJ, PRONEX-MCT, CNPq

B.01.005

Nitric oxide signaling induces AKT phosphorylation in cultures of avian retinal neurons

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Objectives: Previous studies have shown that nitric oxide (NO) produced from the NO donor S-nitroso-Acetyl-D-L-Penicillamine (SNAP) inhibits apoptosis of retinal neurons in culture through a cGMP-dependent mechanism and also involving multiple kinase pathways including phosphatidylinositol 3 kinase (PI3K), MAP kinase, cAMP-dependent kinase and calcium/calmodulin-dependent kinases (CaMKs). The PI3K-AKT pathway is an important signaling pathway with neuronal survival-promoting properties and shown to play an important role in plasticity associated with CNS development. Herein, the purpose of this study was to explore whether endogenous NO produced from L-arginine (L-ARG) or exogenous NO from the NO donor SNAP regulates AKT activity in cultures of chick retinal neurons.

Methods: Cultures prepared from 8 day-old-chick embryo retinas were treated or not with SNAP or L-ARG and then processed for detection of AKT phosphorylation levels on Ser 473 sites by western blot using a specific antibody for the phosphorylated enzyme.

Results: Either SNAP or L-ARG lead to enhanced AKT phosphorylation in a concentration and time-dependent manner. The effect was blocked by the soluble guanylate cyclase inhibitors ODQ or LY83583, and mimicked by the cGMP analog 8-Bromo cyclic GMP or YC-1, a guanylate cyclase activator. In addition, the effect of NO was also suppressed by LY294002, wortmannin, KN93 or KN62, indicating the involvement of PI3K and CAMK signaling pathways. Interestingly, pretreatment with the calcium chelator EGTA or the calmodulin inhibitor W-7 also abrogated the effects of SNAP or L-ARG. This revealed that the influx of extracellular calcium and activation of calmodulin mediate AKT phosphorylation. Indeed, NO produced from L-ARG or SNAP promoted Ca²⁺-influx through L-type Ca²⁺ channels.

Conclusions: The results indicate that endogenous or exogenous NO participates in the activation of survival pathways involving the AKT protein kinase in retinal cells.

Palavras-Chave: SNAP, L-ARG, P-AKT, NITRIC OXIDE, RETINAL NEURONS

Apoio Financeiro: CAPES, CNPq, FAPERJ, PRONEX, PROPP-UFF

B.01.006

NEUROTRANSMITTERS AND SYNAPTIC PROTEINS ARE MODIFIED IN THE MEDIAL POSTERO-DORSAL AMYGDALA IN FEMALE RATS ALONG THE ESTRUS CYCLE IN BOTH BRAIN HEMISPHERES

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The function of the medial postero-dorsal nuclei of the amygdala (MePD) is related mainly to sexual behavior mediated by the receptors for estrogens and androgens. The activation of these steroid receptors are capable to alter gene expression in specific sites. To study the synaptic plasticity is useful to understand the molecular mechanisms that direct the function of the nervous system. Electrophysiological results suggest that MePD is different between male and female rats in both brain hemispheres. The aim of the present work is to understand the brain laterality in the sexual dimorphism along the estrous cycle. The mRNA is quantified for the subunits NR1 and NR2B of the glutamatergic NMDA receptor, Narp docking protein for AMPA, and the enzymes GAD65 and GAD67 that synthesize GABA in the MePD of adult male and female rats. Using real time PCR (qRT-PCR), it was possible to identify lower mRNA for the NR1 subunit in the right hemisphere of females in proestrus than in estrus ($p = 0.027$). In the left hemisphere, females in estrus had more mRNA for the NR1 subunit than males ($p = 0.008$). On the left MePD, females in proestrus show higher mRNA for NR2B than males ($p = 0.002$). Interesting enough, MePDs of males, and females in estrus showed more mRNA expression for both NR1 and NR2B in the right than in the left side. The present report provides findings relevant to the knowledge of this forebrain area, and to understand the role of excitatory and inhibitory components in the local modulation and projective circuits in males and females.

Palavras-Chave: medial amygdala, estrus cycle, brain laterality, sexual dimorphism, NMDA, AMPA, GAD

Apoio Financeiro: FAPESP, CNPq, FAEPA

B.01.007

ATIVACÃO DO RECEPTOR VANILÓIDE DO TIPO 1 (TRPV1) ALTERA EXPRESSÃO E ATIVIDADE DE ENZIMAS DE SÍNTESE DO ÓXIDO NÍTRICO NA RETINA DE RATOS.

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Objetivo Avaliar a relação protéica e funcional entre o TRPV1 e as enzimas de síntese do óxido nítrico (NOS) na retina de ratos adultos (*Rattus norvegicus*).

Métodos Foram realizados experimentos de imunoistoquímica para avaliar a co-localização dos subtipos neuronal (nNOS), endotelial (eNOS) e induzível (iNOS) da enzima de síntese do óxido nítrico com o receptor TRPV1, bem como com marcadores de células de Müller, de astrócitos radiais, de microglia, e do endotélio. Utilizamos técnicas de *immunoblotting* para avaliar os efeitos da injeção intraocular de diversas concentrações de capsaicina (agonista de TRPV1) sobre o conteúdo protéico das NOS. Também procedemos a avaliação da produção *in-situ* de óxido nítrico (NO) com a utilização da sonda DAF-2 após a aplicação de capsaicina.

Resultados Nossos resultados indicam que o receptor TRPV1 é expresso em células endoteliais da retina que expressam eNOS e em pericitos retinianos, e que a ativação desses receptores leva a aumento da produção de NO em vasos retinianos. O TRPV1 também se encontra expresso em células microgliais retinianas, e sua ativação por capsaicina aumenta a expressão de iNOS. A expressão de TRPV1 em células amácrinas que expressam nNOS é pequena, sendo que encontramos co-localização de TRPV1 e nNOS em apenas 6,2% dessas células. Porém, a aplicação intraocular de capsaicina provocou diminuição da expressão protéica de nNOS *in-vivo*, que é revertida após 14 dias de sobrevivência, ou ainda suprimida pela aplicação conjunta de capsazepina, um antagonista de TRPV1.

Conclusões O aumento da produção de NO produzido por capsaicina em vasos é indicativo de que o receptor TRPV1 pode possuir papel importante nos mecanismos de controle da perfusão sanguínea da retina de ratos. A expressão de TRPV1 em células microgliais e o aumento da expressão de iNOS quando da sua ativação sugerem que esse receptor representa mais uma via imunomodulatória no tecido retiniano. A modulação da expressão de nNOS por capsaicina é indicativo de que a expressão de nNOS pode ser modulada por sinais oriundos de outras células que expressam TRPV1, ou seja, neurônios não-nitrérgicos, microglíócitos, ou ainda células endoteliais.

Palavras-Chave: Retina; Óxido nítrico; Receptor Vanilóide; capsaicina

Apoio Financeiro: FAPESP; CNPq

B.01.008

MEDIADORES LIPIDICOS GENERADOS A PARTIR DE ÁCIDO FOSFATÍDICO EN NÚCLEOS DE CEREBELO

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Se ha demostrado la presencia de lípidos en el núcleo celular formando parte de la cromatina como así también de las enzimas que los modulan.

Objetivos: El propósito del presente trabajo fue evaluar la metabolización de ácido fosfatídico en fracción nuclear de cerebelo.

Métodos: Para este fin cerebelos de ratas adultas (4 meses) eran homogeneizados y la fracción nuclear purificada por ultracentrifugación en gradiente discontinuo de sacarosa. La pureza de la fracción nuclear fue determinada por microscopía electrónica y por tinción de los núcleos con DAPI.

Resultados: Los resultados indicaron que en núcleos la incubación con [³H]ácido fosfatídico (PA) produce diacilglicérido (DAG), ácido lisofosfatídico (LPA), monoacilglicéridos (MAG) y glicerol + glicerol-3-fosfato (PSA). Esto es indicativo de una metabolización de PA por la vía lipido fosfato fosfolipasas (LPP)/ diacilglicérido lipasa (DAGL)/monoacilglicérido lipasa (MAGL) y por otra vía que involucra la acción secuencial de fosfolipasas A (PLA) / lisoPA fosfolipasas. El empleo de distintos moduladores de estas vías enzimáticas nos permitieron determinar que 0,2 mM de Tritón X-100 y 10 mM Ca Cl₂ inhiben la formación de DAG en un 33%. Por otro lado la presencia de NaF (50 mM) disminuye la transformación de PA en DAG (36%), favoreciéndose la producción de LPA (32%). En estas condiciones la formación de glicerol + glicerol-3-fosfato se vio aumentada en 150%. Las LPP metabolizan además de PA, otros lípidos fosforados como LPA, esfingosina-1-fosfato (S1P) y ceramida-1-fosfato (C1P). Para evaluar el grado de competencia entre [³H]-PA y LPA, S1P o C1P se ensayaron las actividades enzimáticas en presencia de concentraciones crecientes de los mismos. La co-incubación de [³H]-PA con LPA (100 μM) disminuyó la formación de DAG en un 27%. S1P (10 μM) disminuyó la degradación de PA en un 18%.

Conclusiones: Los resultados presentados indican que en núcleos de cerebelo el PA puede ser metabolizado por diferentes vías enzimáticas siendo esto indicativo de la existencia de importantes vías de señalización en esta fracción.

Palavras-Chave: Acido fosfatídico, diacilglicerol, nucleo, cerebelo

Apoio Financeiro: PICT 01-14527; PIP 5586

B.01.009

METABOLIZACION DE ENDOCANABINOIDES POR MONOACIL GLICERIDO LIPASA Y ACIDO GRASO AMIDO HIDROLASA EN SISTEMA NERVIOSO CENTRAL

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El “sistema canabinoide” representa un nuevo mecanismo de comunicación celular. Está formado por tres tipos de elementos: 1) ligandos endógenos, 2) receptores de membranas, y 3) mecanismos para la inactivación de la señal. Los ligandos, 2-araquidonoilglicerol (2-AG) y araquidonoiletanolamida (AEA), son moléculas lipofílicas que actúan como neuroprotectores en enfermedades neurodegenerativas. El 2-AG y la AEA son degradados enzimáticamente por la monoacilglicérido lipasa (MAGL) y la ácido graso amido hidrolasa (FAAH), respectivamente.

Objetivos: El propósito de este trabajo fue caracterizar la actividad de MAGL y FAAH en SNC de ratas adultas.

Métodos: Para ello se trabajó con sinaptosomas provenientes de corteza cerebral (CC) purificados por gradiente de Ficoll. Las actividades MAGL y FAAH se ensayaron empleando como sustrato 2-araquidonoil[³H]glicerol y araquidonoil[³H]etanolamida, respectivamente. Las incubaciones se llevaron a cabo empleándose 50 µg de proteína, por 15 min a 37°C y se frenaron con el agregado de Cloroformo:Metanol (1:1). Los productos de la reacción [³H]glicerol y [³H]etanolamina, se evaluaron en la fase acuosa y los sustratos sin reaccionar fueron separados por cromatografía en capa fina a partir de la fase orgánica.

Resultados: El 2-AG fue vehiculizado en acetonitrilo o en BSA, obteniéndose una formación de [³H]glicerol de 4657±200 pmoles y de 928±90 pmoles, respectivamente. La AEA fue agregada en etanol o BSA, en este caso se generaron 190±12 y 311±28 pmoles de [³H]etanolamina, respectivamente. La transformación del 2-AG por la MAGL fue del 55% y la de AEA por la FAAH del 12%. Los resultados son promedios ± DS de 3 muestras individuales.

Conclusiones: Los resultados hallados indican un activo metabolismo de 2-AG y una transformación marcadamente menor de AEA en sinaptosomas.

Palavras-Chave: endocanabinoides, monoacilglicérido lipasa, ácido graso amido hidrolasa, sinaptosomas

Apoio Financeiro: Fundación Florencio Fiorini, PICT01-14527

B.01.010

Liberação de ATP de Células de Retina Embrionária de Pinto Mantidas em Cultura

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Objetivos: Na retina embrionária de pinto, ATP induz a proliferação de células gliais em cultura, através da ativação de receptores P2Y1, PKC e MAP cinases. Por outro lado, astrócitos corticais são capazes de liberar ATP de vesículas acidícas através de um processo dependente de cálcio¹. Neste estudo, investigamos o efeito de glutamato, kainato e de alta concentração de K⁺ sobre a liberação de vesículas de ATP em culturas de células de retina de embrião de pinto.

Metodologia: A exocitose de vesículas contendo ATP foi estimada usando-se o método de Bodin e Burnstock² modificado. Células de retinas de embriões de galinha com 8 dias e cultivadas por 14 dias (E8C14) foram incubadas, por 5 min, a 37°C, com quinacrina 5 mM, composto capaz de marcar vesículas acidícas que contém ATP. Após este período, as culturas foram lavadas em solução salina e a fluorescência analisada por microscopia. O conteúdo de ATP liberado das células foi estimado por luminescência, utilizando o “ATP determination kit” da Invitrogen.

Resultados: A presença de vesículas fluorescentes foi observada principalmente em células gliais, sendo a marcação em neurônios reduzida. Em células gliais, o tamanho e a quantidade de vesículas fluorescentes foram maiores na região perinuclear e a sua densidade nas células aumentou com o desenvolvimento da cultura. A adição de KCl 50 mM, Glutamato 1mM ou Kainato 0.1 mM foi capaz de induzir uma diminuição progressiva na quantidade de vesículas fluorescentes, sendo a diminuição bastante acentuada após 10 min de estimulação. Ensaios de luminescência para o ATP mostraram que KCl 50 mM foi capaz de induzir uma liberação significativa deste nucleotídeo em culturas purificadas de glia (basal = 0.91 ± 0.14 pmol/cultura; KCl = 3.68 ± 0.17 pmol/cultura; $p < 0.001$).

Conclusões: Estes resultados sugerem que células gliais de retina em cultura liberam ATP por despolarização. Estas células apresentam vesículas passíveis de serem coradas com quinacrina e que podem liberar seu conteúdo por estimulação de receptores glutamatérgicos ou despolarização.

Referências: 1- J. Biol. Chem. 282 (39):28749, 2007; 2- J. Cardio. Pharmacol. 38(6): 900, 2001.

Palavras-Chave: ATP, Retina, Exocitose, Glia de Müller

Apoio Financeiro: Faperj, CNPq, MCT-Pronex, Propp-UFF

B.01.011

S100B secretion is stimulated by fluoxetine via a mechanism independent of serotonin

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Objectives: The aim of this study was to evaluate the S100B secretion directly in astrocyte cultures and hippocampal slices exposed to fluoxetine, serotonin and serotonergic agonists and antagonists - DPAT(8-hydroxy-DPAT hydrobromide), NAN-190 (NAN-190 hydrobromide) and Ketanserin.

Methods: Primary astrocytes culture were prepared with hippocampi of newborn Wistar rats maintained in DMEM containing 10% FCS in 5% CO₂/95% air at 37°C and allowed to grow to confluence and used at 21 days, *in vitro*. S100B secretion essays were carried out in serum-free DMEM. Hippocampi slices of 0.3 mm were obtained using a McIlwain Tissue Chopper. The cells and slices were treated with fluoxetine at 100 nM, serotonin and DPAT at 50 nM, serotonin plus ketanserin (5HT₂ receptor antagonist - 10 uM), serotonin plus NAN-190 (5HT_{1A} receptor antagonist). The S100B content was determined by ELISA in the culture medium at 1, 6, 24 h and in the incubation medium of slices at 1 h after treatment.

Results: We observed a significant increment of the S100B release in the presence of fluoxetine (119 ± 3,7 percent of basal values condition P<0,05). Serotonin and DPAT (serotonergic agonist) decrease S100B secretion (67 ± 6,5 and 78 ± percent of basal values conditions respectively P<0,05). In the presence of NAN-190, a 5HT_{1A} receptor antagonist the effect of serotonin was reverted (110 ± 4 percent of basal values condition P<0,05), while in the presence of ketanserin (5HT₂ receptor antagonist) the effect remained the same (78 ± 5 percent of basal values condition P<0,05).

Conclusion: Increment of the S100B secretion in the presence of fluoxetine was PKA dependent and the effect of serotonin and DPAT occurred probably via 5HT_{1A} receptor. These data reinforce the importance of fluoxetine, independently of serotonin and serotonin receptors, for antidepressant activity. Moreover, it may be hypothesized that the blockade of 5HT_{1A} receptors, in combination with fluoxetine or others selective serotonin reuptake inhibitor, may produce a faster onset of the antidepressant effect. In addition an expansion of the concept of blockade of 5HT_{1A}, including astrocyte receptors, reinforces this hypothesis. **Palavras-Chave:** S100B, astrocytes, serotonin

Apoio Financeiro: CNPq, CAPES, FINEP/ Rede IBN 01.06.0842-00, FAPERGS.

ESTUDO DO PRÉ-CONDICIONAMENTO COM NMDA CONTRA DANOS MOTORES INDUZIDOS PELO TRAUMATISMO CRÂNIO-ENCEFÁLICO

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Objetivos: Investigar a coordenação motora de camundongos submetidos a um modelo de traumatismo crânio-encefálico (TCE) e pré-tratados com NMDA.

Métodos: camundongos CF-1 adultos machos foram pré-tratados com solução salina (SAL) ou NMDA (75 mg/kg) 24 horas antes do trauma. Os animais foram anestesiados por inalação com O₂/N₂O (33%:66%) e em seguida submetidos ao TCE pelo impacto de um peso 50 g no crânio. A destreza dos animais foi avaliada pelo teste de Impressão das Pegadas (*Footprint*) e pelo *Rotarod* em 1,5 ou 6h após o trauma. Para avaliação da destreza no *Footprint* as patas dianteiras e traseiras foram pintadas. A performance dos animais foi avaliada antes da administração do NMDA e após o trauma nos dois testes. Os dados foram avaliados por One way ANOVA, post hoc Tukey; estatisticamente significante $P < 0,05$. Os dados estão expressos como média da porcentagem em relação ao grupo controle (SAL) $\pm$ desvio padrão (n = 6-7).

Resultados: Dados preliminares indicam que o pré-condicionamento com NMDA não altera a destreza avaliada no *Rotarod*: “tempo de permanência na roda” e “tempo de latência para a queda”, nos tempos de 1,5 ou 6 horas após o TCE. Contudo, no Teste do *Footprint* a diminuição da medida da “largura da passada” (LP) e da “largura do passo traseiro” (LPT) avaliada 1,5 horas após TCE foi prevenida pelo pré-condicionamento (LP_{SAL} = 100 $\pm$ 10,2%; LP_{TCE} = 68,4 $\pm$ 33,9%; LP_{NMDA+TCE} = 98 $\pm$ 13%; LPT_{SAL} = 100 $\pm$ 20%; LPT_{TCE} = 77 $\pm$ 10%; LPT_{NMDA+TCE} = 97 $\pm$ 13%;). A diminuição da medida da “impressão da pegada” avaliada 6 horas após o TCE também foi prevenida pelo pré-tratamento com NMDA (*Footprint*_{SAL} = 100 $\pm$ 37%; *Footprint*_{TCE} = 56 $\pm$ 49%; *Footprint*_{NMDA+TCE} = 126 $\pm$ 39%).

Conclusão: Os resultados demonstram que o pré-tratamento com NMDA previne contra prejuízos motores induzidos pelo TCE nos tempos de 1,5 ou 6 horas após o trauma. Tendo em vista o papel protetor do pré-condicionamento com NMDA, entende-se que este mecanismo de tolerância celular possa desempenhar funções preventivas ao SNC e/ou de manutenção contra danos que envolvam o sistema glutamatérgico no TCE.

Palavras-Chave: NMDA; TCE; Pré-condicionamento

Apoio Financeiro: UNESC, FAPESC e CNPq.

B.01.013

Efeitos da desnutrição na liberação de ácido g-aminobutírico (GABA) induzida por aminoácidos excitatórios em retina de ratos.

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Glutamato e GABA são os principais neurotransmissores excitatório e inibitório, respectivamente, presentes no SNC. Na retina de rato, o GABA é expresso nas células amácrinas e células presentes na camada de células ganglionares (CCG). Estudos anteriores demonstraram que os aminoácidos excitatórios (AAE's) são capazes provocar a liberação de GABA da retina de aves, gambá e macaco. Vários sistemas de neurotransmissores são alterados durante o desenvolvimento de ratos desnutridos. Neste trabalho, o efluxo de GABA endógeno estimulado pelos AAE's foi estudado em retinas de ratos adultos normais e desnutridos pela técnica de imunohistoquímica. Os animais foram divididos em 2 grupos: normais, alimentados com dieta comercial, e desnutridos, alimentados com a dieta básica regional (DBR), obtida a partir de um inquérito alimentar de uma população da Zona da Mata (PE). As retinas foram mantidas em solução de Ringer e eram estimuladas com agonistas glutamatérgicos (NMDA; cainato, KA; aspartato, ASP). NMDA, em animais normais, reduziu apenas o número de células amácrinas imunorreativas (IR) para GABA (62,5%). NMDA reduziu a imunorreatividade para-GABA tanto entre células amácrinas (25%) quanto na CCG (35%) em animais desnutridos. KA e ASP reduziram o número de células IR-GABA em ambas as populações celulares estudadas dos animais normais e desnutridos. KA provocou a redução de aproximadamente 50% e 20% das células IR-GABA das CNI e CCG, respectivamente em animais normais. Nos animais desnutridos, a redução na imunorreatividade provocada por KA foi de cerca de 50% nas duas populações celulares estudadas. O efeito de ASP foi estudado pela primeira vez em roedores. Em animais normais, o ASP provocou a redução de células IR-GABA em torno de 50% e 23%, e nos desnutridos de 30% e 50% nas CNI e CCG, respectivamente. O efeito dos AEE's foi mais intenso na CCG de ratos desnutridos. Esta foi a primeira análise funcional dos efeitos da desnutrição provocada pela DBR em retinas de roedores.

Palavras-Chave: Glutamato, GABA, Desnutrição, retina

Apoio Financeiro: FAPERJ, CNPq, PRONEX

B.01.014

Efeito proliferativo do Fator de Crescimento Epidermal em células da retina in vitro: Vias de sinalização envolvidas.

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OBJETIVO: O objetivo do nosso trabalho foi investigar o efeito proliferativo do Fator de Crescimento Epidermal (EGF) em células da retina de ratos neonatos mantidas em cultura; bem como caracterizar as vias de sinalização envolvidas.

Materiais e Métodos: Para realização deste trabalho utilizamos cultura de células da retina de ratos da linhagem Lister Hooded. Animais P0-P2 foram sacrificados, as retinas dissecadas, as células dissociadas e plaqueadas em placas de Petri em meio 199. As culturas foram incubadas em estufa a 37°C num ambiente de 5% de CO₂ e 95% de ar por diferentes períodos. Para avaliar a proliferação utilizamos a técnica de incorporação de [³H] – timidina. A expressão proteica foi analisada pela técnica de Western blot.

RESULTADOS: Os resultados mostraram que em 48h, o tratamento com EGF em diferentes concentrações aumenta a proliferação celular (CT 100%; EGF 0,1ng/mL 172,95±20,54%, EGF1ng/mL 319,15±21,34%, EGF5ng/mL 332,73±24,10%, EGF10ng/mL 283,68%±33,67%, EGF100ng/mL 331,43±27,37% n=18). O efeito do EGF 0,1 ng/mL foi abolido pelo AG1478 (CT100%, EGF 174,53±12%, AG1478 1,25mM 93,4±7,1%, AG1478+EGF 101,59±6,09% n=15), pelo cloreto de queleritrina (CT 100%, EGF 186,42±9,9%, CC 1,25mM 100,52±7,14%, EGF+CC 134,64±13,27% n=11), pelo BAPTA-AM (CT 100%, EGF 162,03±9,1%, BAPTA-AM 20mM 83,71±6,06%, EGF+BAPTA-AM 105,05±4,6% n=15), pelo PD98059 (CT 100%, EGF 202,89±16,7%, PD 20mM 76,69±3,6%, EGF+PD 123,9±10,59%; n=14) e pelo SB202190 (CT 100%, EGF 184,7±18,12%, SB 25mM 134,44±4,05%, EGF+SB 134,70±6,59% n=10). Esses resultados mostram o envolvimento do EGFR, de uma PKC dependente de Ca²⁺, da MAP cinase e da p38, respectivamente nas vias de sinalização ativadas por este fator de crescimento. Foi demonstrado também um aumento da expressão da ciclina D1 em 4h e 48h de após o tratamento com EGF (CT 4h 100%, EGF1ng/ml 4h 125.07±1.41%, CT 48h 47.22±2.23, EGF1ng/mL 48h 76.08±11.9 n=3).

CONCLUSÃO: Nossos resultados sugerem que o EGF seja um importante mitógeno para as células da retina, sendo seu efeito mediado pela via da PKC-MAP cinase levando um aumento na expressão da ciclina D1.

Palavras-Chave: EGF, retina, proliferação, vias de sinalização

Apoio Financeiro: PRONEX-MCT, CAPES, FAPERJ e CNPq

B.01.015

Efeito proliferativo do fator de crescimento semelhante à insulina em células da retina de ratos neonatos: Vias de sinalização envolvidas.

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Objetivos: O objetivo do nosso trabalho foi analisar o efeito do fator de crescimento semelhante à insulina do tipo 1 (IGF-1) na proliferação de células da retina de ratos neonatos.

Métodos: Células da retina de ratos Lister Hooded recém nascidos (P0-P1) foram dissociadas e semeadas ($1,25 \times 10^6$ céls) em placas de Petri (40 mm) previamente tratadas com poli-L-ornitina (50mg/mL). Após um período inicial de 1h, para possibilitar a adesão das células às placas, as culturas foram tratadas com IGF-1 e ou drogas a serem testadas e incubadas em meio de cultura completo, à 37°C, num ambiente de 5% CO₂, por diferentes intervalos de tempo. A proliferação celular foi avaliada por incorporação de [³H]-timidina e a radioatividade das amostras foi medida em cintilador de fase líquida.

Resultados: Nossos resultados demonstram que o tratamento com IGF-1 por 24h aumenta a proliferação de células da retina em aproximadamente 50% quando comparado ao controle. Este efeito se dá de maneira dose-dependente, sendo que o tratamento com 10ng/mL induziu a melhor resposta [ct=100%; IGF-1=149,8 ± 6,2% (n=10)]. O tratamento com o inibidor das proteínas Src [PP1-1,0μM] aboliu o efeito do IGF-1 [ct=100%; IGF-1=142,52±8,19%; PP1=120,74±2,89%; IGF-1/PP1=108,68±4,53% (n=5-9)]. A presença do inibidor da fosfolipase C [U73122 (4μM)] bloqueou o efeito do IGF-1 [ct=100%; IGF-1=146,88±7,92%; U=81,51±3,70%; IGF-/U=91,85±3,52% (n=6-10)]. O efeito do IGF-1 também foi abolido pelo tratamento com o inibidor dos receptores de EGF (AG1478) na concentração de 2,5μM [ct=100%; IGF1=139,49±4,02%; AG1478=93,12±6,55%; IGF/AG=107,11±3,82% (n=9-12)]. O bloqueio da metaloproteinase 9 (MMP9 - 20μM) igualmente inibiu o efeito do IGF-1 [ct=100%; IGF-1=145,66±8,48%; MMP9=98,73±5,63%; IGF-1/MMP9=108,91±3,29% (n=6-9)].

Conclusões: Nossos resultados indicam que o efeito do IGF-1 na proliferação de células da retina seja mediado pela transativação dos receptores de EGF e envolva ativação da metaloproteinase 9.

Palavras-Chave: retina, IGF-1, receptor de EGF e proliferação celular

Apoio Financeiro: FAPERJ, CNPq, PRONEX-MCT e CAPES

B.01.016

Glutamine supplementation during brain development facilitates cortical spreading depression propagation in weaned young rats

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OBJECTIVE – The amino acid glutamine (GLN) participates, in the brain, in the so-called “glutamine-glutamate cycle” and is the most abundant amino acid in cerebrospinal fluid, being likely to influence brain excitability. Recently it has been shown in the rat brain that GLN modulates epileptiform activity in vitro, provoking, in elevated concentrations, events resembling spreading depression (SD; Neurobiol. Dis.25:230, 2007). Here we investigated in weaned rats the effects of previous GLN oral supplementation, during the brain development period, on SD.

METHODS – Male Wistar rat pups (n=10) received GLN (500mg/Kg/day) by gavage during postnatal days 7 to 27. At 30-40 days of life, they were submitted to a SD recording session during 4h, on 2 cortical parietal points of the right hemisphere. SD velocity propagation was calculated from the time required for a SD-wave to cross the inter-electrode distance.

RESULTS: GLN rats presented higher ($P<0.05$) SD propagation velocities (ranging, during the 4h-recording period, from 4.14 ± 0.23 to 4.32 ± 0.26 mm/min; mean $\pm$ sd), as compared to water-treated controls (n=10; ranging from 3.71 ± 0.20 to 3.76 ± 0.24 mm/min). This water control group did not differ from a naïve control group (n=10) that was not submitted to the gavage procedure and presented SD-velocities ranging from 3.68 ± 0.06 to 3.76 ± 0.24 mm/min. A fourth group (n=9), treated with a “placebo amino acid” (glycine) that is not involved in the “glutamine-glutamate cycle”, also displayed SD-velocities in the control range (from 3.47 ± 0.15 to 3.71 ± 0.34 mm/min).

CONCLUSION: The results indicate that GLN supplementation during brain development facilitates cortical spreading depression propagation, as judged by the higher SD-velocities, supporting a role for GLN in the brain excitability, probably via neurotransmitters synthesis modulation.

Palavras-Chave: Glutamine, Spreading Depression

Apoio Financeiro: CNPq, IBN-Net, Propesq/UFPE.

B.01.017

DIPEPTIDYL-PEPTIDASE IV ACTIVITY OF RAT HIPPOCAMPAL SYNAPTOSOMES UNDER BASAL, CALCIUM EXCEEDING AND KCL-STIMULATED CONDITIONS

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Objective: To evaluate the relation of dipeptidyl-peptidase IV (DPPIV) enzyme activity with synaptic activity in hippocampus.

Methods: Isolated crude hippocampal synaptosomes were incubated (37°C, 15 min) with artificial cerebrospinal fluid (aCSF), Ca²⁺-containing (control) (aCSF [i]) or Ca²⁺-free (aCSF[ii]), to simulate basal condition; and aCSF+KCl, Ca²⁺-containing (aCSF[iii]) or Ca²⁺-free (aCSF[iv]), to simulate depolarized condition. Synaptosomal fractions were then obtained by the following sequential procedures: *Exocytose (E)* - supernatant of the incubation mixture centrifuged 12,000xg, 20min; *Soluble (S)* - supernatant of pellet of this 1st centrifugation homogenized (1000rpm, 2min) (in Tris-HCl 10mM buffer, pH 7.4) and ultracentrifuged (100,000xg, 35min); and *Membrane (M)* - supernatant of pellet of this last centrifugation homogenized (Tris-HCl 10mM buffer + Triton X-100, pH 7.4) and ultracentrifuged (100,000xg, 35min). Contents of lactate dehydrogenase activity (fractionation quality), protein (Bradford method) and CD26 (canonical DPPIV, EC 3.4.14.5) (sCD26 ELISA; ng/mg protein) were determined by spectrophotometry. DPPIV enzyme activity (H-Gly-Pro-4-methoxy-b-naphthylamidase; pmol hydrolyzed substrate per minute) and its inhibition (residual activity; %) by diprotin A, Ca²⁺ and EGTA were determined by fluorimetry. Results expressed as mean±SEM.

Results: DPPIV activity was higher in S (230.95±34.73, n=12) than in M (123.00±16.10, n=10) (control), in all aCSFs. Ca²⁺ reduced activity of DPPIV-S (aCSFs [i]:54±8, n=5; [ii]:45±8, n=6; [iii]:62±9, n=5; [iv]:56±10, n=7) and has no effect on DPPIV-E or -M (except for DPPIV-M in aCSF[i]:50±8, n=6). Diprotin A reduced activity of DPPIV-E (44±7, n=4), -S (25±6, n=4) and -M (44±4, n=4) in aCSF[i]. EGTA only reduced DPPIV-S activity (55±11, n=4) in aCSF[i]. CD26 -M (1852.2±38.6, n=3) > -E (688.2±258.6, n=2) > -S (273.0±45.8, n=3) in aCSF[i].

Conclusions: The present data demonstrated for the first time that DPPIV-hydrolysing ability is present in synaptosomal fractions, coupled with synaptic activity in the hippocampus. **Palavras-Chave:** DPPIV, NPY, synaptosome, hippocampus

Apoio Financeiro: FAPESP

ESTUDO DA FOSFORILAÇÃO DE MAPks DO HIPOCAMPO DE RATOS SUBMETIDOS AO MODELO DE MANIA

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Objetivos: Avaliar o efeito dos estabilizadores de humor, lítio e valproato, na fosforilação de proteínas de sinalização intracelular, no modelo animal de mania induzido por anfetamina (AMPH).

Métodos: O episódio maníaco foi induzido em ratos Wistar machos (60 dias) tratados com anfetamina 2 mg/Kg, (i.p) por 14 dias. O grupo controle foi tratado com solução salina. No 8º dia de tratamento todos os ratos receberam salina, ou lítio (47 mg/Kg), ou valproato (200 mg/Kg) além da AMPH. Ao todo foram 6 grupos de tratamento com 5 animais cada. Após o tratamento, os ratos foram mortos, o cérebro rapidamente removido e o hipocampo dissecado. As proteínas foram separadas por SDS-PAGE e por Western-blotting realizou-se a imunodeteção das proteínas ERK1/2 e JNK1/2 na forma fosforilada e total. Os complexos imunes foram quantificados com Scion Image e estatisticamente comparados com One Way ANOVA, considerando significativo $P < 0,05$. Dados estão expressos como média da porcentagem em relação ao grupo controle (salina) $\pm$ erro padrão ($n = 3-5$).

Resultados: Observamos que os tratamentos dos animais com anfetamina associada ou não com os estabilizadores de humor não alteraram a ativação das proteínas ERK1/2 (Control = $100 \pm 27\%$ e $100 \pm 24\%$; AMPH = $76 \pm 40\%$ e $70 \pm 25\%$, ERK 1 e 2 respectivamente) e JNK1/2 (Control = $100 \pm 27\%$ e $100 \pm 31\%$; AMPH = $88 \pm 17\%$ e $124 \pm 19\%$, JNK 1 e 2 respectivamente).

Conclusão: Estudos demonstram que o tratamento crônico com AMPH induz a ativação da proteína ERK da via das MAPks (quinases ativadas por mitógenos), em muitas áreas do cérebro, como o núcleo accumbens, estriado e córtex pré-frontal, o que não ocorre com a proteína JNK. No presente estudo foi investigada esta atividade no hipocampo e observou-se que a ativação da via da ERK e da JNK não estão envolvidas nas alterações neuroquímicas induzidas por AMPH e/ou pelos estabilizadores de humor nesta região, em um modelo animal de mania. Sugere-se que o dano celular induzido pelo tratamento com AMPH possa ocorrer independente da ativação das MAPks no hipocampo.

Palavras-Chave: ERK, JNK, modelo de mania, lítio, valproato, anfetamina

Apoio Financeiro: CNPq, UNESC

B.01.019

ÓXIDO NÍTRICO MODULA A LIBERAÇÃO DE GABA DE FORMA BIFÁSICA NA RETINA DE GALINHA.

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OBJETIVO: O óxido nítrico (NO) é um mensageiro que influencia a fisiologia da retina. Objetivamos verificar a modulação do NO endógeno sobre o sistema GABAérgico na retina intacta de galinha.

MÉTODOS: Retinas de animais pós-eclosão foram tratadas semi-*in vivo* com drogas que interferem no sistema nitrérgico: 1) incubadas com L-arginina (L-arg), substrato da produção de NO (100mM ou 1mM), por 25 min; ou com NMDA por 30 min (100mM, agonista de receptor NMDA) que estimula a produção de NO; 2) pré-incubadas com 7-NI (100mM), inibidor da óxido nítrico sintase ou KT(1mM), inibidor da proteína cinase dependente de GMPc, por 10 min, seguidos da incubação com NMDA ou L-arg na presença das mesmas drogas. As retinas foram cortadas (12µm) e imunoprocessadas para o GABA. Os corpos de células amácrinas e células na camada de células ganglionares (CCG) imunoreativos para GABA (GABA+) foram quantificados. Os valores estão expressos como média±desvio padrão da porcentagem de células GABA+ (%IR-GABA) em relação ao controle.

RESULTADOS: A incubação com NMDA reduziu a %IR-GABA na CCG (61±5, P<0,01, N=5) e este efeito foi bloqueado pelo 7-NI (86±2, N=3). De forma semelhante, L-arg (1mM) reduziu a %IR-GABA na CCG (65±7, P<0,001, N=3), sugerindo que o NO estimula a liberação de GABA nesta camada. Entretanto, a incubação com L-arg (100mM) aumentou a %IR-GABA na CCG (134±6, P<0,001, N=9) e em células amácrinas (166±15, P<0,01, N=13), efeito que foi bloqueado pelo 7-NI na CCG (25±2, P<0,001, N=3) e amácrinas (76±12, P<0,01, N=3). O efeito da L-arg (100mM) foi bloqueado pela ausência de Na⁺ na CCG (85±7, N=3) e nas células amácrinas (103±6, N=3), sugerindo que NO inibe a liberação de GABA dependente de Na⁺. O efeito da L-arg (100mM) foi bloqueado por KT em células amácrinas (92±22 P< 0,01, N=3) e parcialmente na CCG (119±21, N=3).

CONCLUSÃO: Os dados sugerem que a produção de NO em alta concentração (possivelmente estimulada por receptores NMDA ou por L-arg 1mM induz liberação de GABA na CCG, enquanto a produção de NO em baixa concentração (estimulada por L-arg 100mM) inibe a liberação de GABA em ambos os tipos celulares por mecanismo dependente de PKG.

Palavras-Chave: 1. Óxido Nítrico 2.GABA 3. Retina

Apoio Financeiro: CNPq, CAPES, FAPERJ, PRONEX.

B.01.020

REGULAÇÃO DA EXPRESSÃO DO RECEPTOR A2a DE ADENOSINA DURANTE O DESENVOLVIMENTO DA RETINA

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Objetivos: O receptor A2a de adenosina (ado), presente em diversos tecidos, está acoplado positivamente à adenilil ciclase. Resultados anteriores mostraram que ado foi capaz de induzir acúmulo de AMPc em retinas e em culturas de retina de embrião de galinha. Em culturas purificadas de neurônios de retina observou-se que ado bloqueava a morte dos neurônios induzida pela troca de meio ou por glutamato. Esse efeito ocorria através da ativação de receptores A2a e era mimetizado por análogos permeáveis de AMPc. Nosso objetivo foi estudar a presença de receptores A2a de adenosina nas retinas durante o desenvolvimento e nos diferentes tipos de culturas de retina.

Métodos: Experimentos de “Western Blotting” foram feitos para estudar a expressão do receptor A2a em retinas intactas ao longo do desenvolvimento e em culturas mistas e purificadas de neurônios e de glia. Para estudar a localização celular desses receptores, foram feitos experimentos de imunocitoquímica para culturas mistas e purificadas de neurônios.

Resultados: O receptor A2a mostrou-se presente em retinas de embrião de 6 dias (E6), não havendo variação de sua expressão em E7. O nível de expressão começou a decair em E9, a diferença tornando-se significativa em E11 ($72,0 \pm 10,4\%$ de E6, $n=4$, $p<0,05$), e se manteve em E14 ($63,1 \pm 6,1\%$ de E6, $n=4$, $p<0,01$), E15 ($73,6 \pm 6,7\%$ de E6, $n=3$, $p<0,05$) e E16 ($72,7 \pm 3,2\%$ de E6, $n=4$, $p<0,05$). Entretanto, em retinas de 1 dia após a eclosão, ocorreu o retorno aos níveis iniciais de expressão ($98,0 \pm 7,7\%$ de E6, $n=3$). Os receptores A2a também estavam expressos em níveis semelhantes ($n=3$) em culturas mistas ou purificadas de neurônios ou células gliais de retina, podendo se observar sua localização imunocitoquímica tanto em neurônios como em células gliais.

Conclusão: Os resultados indicam que o receptor A2a está presente na retina e sua expressão varia de acordo com o desenvolvimento. O receptor mostrou-se também presente em diferentes tipos de culturas de retina, localizando-se tanto em células gliais como em neurônios.

Palavras-Chave: adenosina, receptor A2a, retina

Apoio Financeiro: CNPq, CAPES, FAPERJ, PRONEX-MCT.

B.01.021

Expression and function of muscle Wnt receptors Frizzled at the vertebrate neuromuscular junction.

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A trans-synaptic dialogue between the presynaptic axon machinery and its postsynaptic receptive apparatus is essential for the proper function of the nervous system. However, little is known regarding the molecules that mediate synaptogenesis. Wnt factors play critical roles in synapse formation, but their role at postsynaptic differentiation is currently unknown. Previous data suggest that Wnt signalling secreted by motoneurons enhances postsynaptic differentiation at the vertebrate neuromuscular junction (NMJ). In this work we have analyzed the possible involvement of particular muscle Frizzled (Fz) receptors in this effect. Through RT-PCR we found that Fz-1, -2, -3, -7 and -9 transcripts are expressed in both myoblasts and myotubes obtained from the muscle cell line C2C12 whereas Fz-5 is only expressed in myoblasts. We have focused our research on Fz9 as it has been shown that the mRNA for this receptor is down-regulated in denervated skeletal muscles, which suggest that Fz9 is important for proper nerve/muscle communication. Using a specific antibody we found by Western blot that Fz9 is expressed in C2C12 cells throughout *in vitro* myogenesis where it is distributed in the plasma membrane, as assessed by immunocytochemistry. In addition, Fz9 is also expressed in rat embryonic skeletal muscle during the stages of NMJ assembly (E13 to E20). To accomplish gain- and loss-of-function experiments, we have cloned full-length, HA-tagged and shRNA sequences of Fz9 into expression vectors. Control experiments using the TOPflash reporter gene showed that Fz9 enhanced Wnt canonical pathway activation triggered by Wnt2 in HEK293 cells; this effect was reverted upon co-expression of the shRNA for Fz9. Interestingly, Fz9 strongly inhibited the ability of Wnt-1 to stimulate Wnt canonical pathway in C2C12 myoblasts. Moreover, overexpression of Fz9 in C2C12 myotubes inhibited the ability of agrin, an organizer of postsynaptic differentiation, to cluster acetylcholine receptors on their surface. Thus, we conclude that Fz9 may have a role in post-synaptic differentiation at the NMJ.

Palavras-Chave: Wnt, Frizzled, NMJ, acetylcholine receptor

Apoio Financeiro: Anillo PBCT ACT-02 Grant, CHILE.

B.01.022

REGULAÇÃO DA EXPRESSÃO DO RECEPTOR A1 DE ADENOSINA EM CULTURAS DE RETINA DE EMBRIÃO DE GALINHA

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Objetivos: Adenosina (ado), um neuromodulador do SNC, atua via 4 subtipos de receptores: A1 e A3, acoplados negativamente à adenilil ciclase, e A2a e A2b, acoplados positivamente à adenilil ciclase. Trabalhos anteriores do laboratório mostraram que ado e os receptores A1 e A2 estão presentes nas retinas intactas de embriões de galinha. Os objetivos deste trabalho foram investigar a presença de receptores A1 em culturas mistas de retinas e o envolvimento dos receptores A2a na regulação da sua expressão.

Métodos: Utilizando a técnica de “binding” foram feitos experimentos para investigar a presença do receptor A1 nas culturas utilizando [³H]DPCPX, um antagonista radioativo do receptor. Para determinar a ligação específica ao A1, foi utilizado o seu agonista seletivo, CHA. Além disso, o RNAm do receptor foi detectado através de experimentos de RT-PCR.

Resultados: O receptor A1 apresentou uma baixa ligação ao antagonista [³H]DPCPX em culturas mistas. Entretanto, quando as culturas eram incubadas cronicamente com 8BrAMPc ou SpAMPc, análogos permeáveis de AMPc, a ligação aumentou em $1.010,9 \pm 90,6\%$ e $887,5 \pm 187,5\%$ do controle ($n=3$, $p < 0,001$), respectivamente. Além disso, o agonista A2a, DPMA, também aumentou a ligação em $393,5 \pm 60,2\%$ do controle ($n=4$, $p < 0,001$), um efeito totalmente bloqueado por H89, inibidor da proteína cinase dependente de AMPc (PKA), e pelo ZM 241385, antagonista de receptor A2a ($n=3$). DPMA também aumentou em $211,3 \pm 27,4\%$ do controle ($n=5$, $p < 0,001$) a quantidade de RNAm para o receptor A1. Culturas incubadas com ado deaminase ou inosina, produto do metabolismo da ado pela ado deaminase, apresentaram um aumento na ligação de $626,4 \pm 68,8\%$ do controle ($n=5$, $p < 0,001$) e $579,2 \pm 119,6\%$ do controle ($n=2$), respectivamente.

Conclusões: Os resultados indicam que AMPc e a ativação do receptor A2a regulam a expressão do receptor A1 nas culturas mistas de retina de embrião de galinha.

Palavras-Chave: Adenosina, receptor A1, receptor A2a, retina.

Apoio Financeiro: Capes, CNPq, Faperj, PRONEX-MCT.

B.01.023

Biophysical study of the complex formed by the cellular prion protein and the co-chaperone hop/STI1: in search for the structural basis of its signaling properties.

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Objectives

The physiological function of the normal cellular prion protein (PrP^C), the protein associated with prion diseases, is not fully resolved. Lately, it has been shown that it modulates diverse aspects of cellular processes. In particular, the recruitment of neuronal PrP^C by the co-chaperone hop/STI1, induces trophic, proliferative and neuroprotective signals. However, the molecular basis for this signaling remains unclear. In order to gain insight into the structural features of this interaction, we present an *in vitro* biophysical analysis of hop/STI1 and the PrP:hop/STI1 complex.

Methods

Heterogously expressed recombinant proteins were purified and used as working models (*r*PrP and *r*hop/STI1, namely). Synthetic peptides containing the binding sites of these proteins were used when necessary. Complex formation was assayed by fluorescence anisotropy of fluorescein-labeled *r*PrP. Secondary and tertiary structural modifications were studied by Circular Dichroism (CD) and fluorescence spectroscopy (FS), respectively. Small Angle X-ray Scattering (SAXS) data were processed for structural parameters and low-resolution structural models.

Results

Equimolar (1:1) complexation of *r*PrP and *r*hop/STI1 was observed, with a dissociation constant (K_d) in the order of 100 nM. Both CD and FS techniques indicated that binding triggers a significant structural rearrangement, in particular, an important stretching of *r*PrP helical structures. Furthermore, analysis of SAXS data also suggests a compaction of the *r*hop/STI1 tertiary structure when binding *r*PrP. Importantly, SAXS-derived molecular envelope models were obtained for both *r*PrP and the *r*PrP:*r*hop/STI1 complex.

Conclusions

The complexation-triggered structural modifications observed for both proteins are likely of biological significance since they may represent the structural basis for the signaling properties of their interaction. Their physiological relevance remains to be tested. The molecular envelope models obtained represent a novel and informative view on the intra and inter-molecular organization of both hop/STI1 and the PrP:hop/STI1 complex, since they stand as their first empirical representation.

Palavras-Chave: prion protein, hop/STI-1, SAXS, structural model

Apoio Financeiro: CNPq, CAPES, FAPERJ, PRONEX.

B.01.024

Identification of new putative PrP^C ligands through phage display screening

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Objectives: Cellular prion protein (PrP^C) is a glycosylphosphatidylinositol-anchored protein, the physiological functions of which are still under discussion (1). Various PrP^C ligands were described, including hop/STI-1 (2,3). The interaction between these two proteins triggers various signaling pathways such as AMPc/PKA, ERK and PI3K pathways. The AMPc/PKA pathway is related to neuroprotection and the ERK pathway to neuritogenesis and proliferation (4,5). Mechanisms of signal transduction triggered by engagement of PrP^C are still unclear. The aim of this work is to identify new PrP^C ligands involved in a signaling platform.

Methods and Results: In order to identify new PrP^C ligands, presumably involved in a signaling platform with the hop/STI-1 protein, we have used a phage-display approach. A random heptapeptide phage display library (Complexity ~2.8 x 10⁹ transformants) was used to isolate phages which bind to PrP^C after five biopanning rounds. The specificity of the binding of PrP^C to the selected clones was tested by ELISA. The isolated clones were sequenced and the sequence was analysed using the BLAST *algorithm* (psi-blast) to search for homologies to known membrane proteins in the non-redundant protein sequences database (NCBI). One of the clones showed homology to a region in the extracellular domain of Metabotropic Glutamatergic Receptor 1 (mGluR1). This homology region is located next to a putative hop/STI-1 binding site (Magdesian et al, unpublished data).

Conclusion: The results show that the mGluR1 may be a novel PrP^C ligand, but the interaction among hop/STI-1, PrP^C and mGluR1 has yet to be validated by biochemical methods.

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Palavras-Chave: hop/STI-1, prion cellular, phage-display and signaling

Apoio Financeiro: CAPES, CNPq, FAPERJ and FAPESP.

MODULAÇÃO DO SISTEMA GABAÉRGICO ENDÓGENO POR ADENOSINA NA RETINA DE GALINHA

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OBJETIVO: A adenosina é um neuromodulador amplamente distribuído no sistema nervoso central, inclusive na retina. Assim, objetivamos verificar a existência de efeitos modulatórios da adenosina sobre o sistema GABAérgico endógeno na retina de galinha. **MÉTODOS:** Retinas de animais pós-eclosão foram tratadas semi-*in vivo* com drogas agonistas e/ou antagonistas dos receptores de adenosina. As retinas foram fixadas, crioprotégidas e cortadas em criostato para serem, então, processadas imunohistoquimicamente para o GABA. Os corpos celulares de células amácrinas imunorreativos para GABA (IR-GABA) foram quantificados. Os valores apresentados correspondem à média $\pm$ desvio padrão da porcentagem de IR-GABA com relação ao controle (%IR-GABA), seguidos do *p*-valor.

RESULTADOS: O agonista não-seletivo NECA (100 μ M) não modificou a %IR-GABA (115,3 $\pm$ 10,9%, *p*>0,05). Porém, o agonista seletivo do receptor A₁ (A₁R), CHA (10 μ M), promoveu uma diminuição na %IR-GABA (35,6 $\pm$ 9,8%, *p*<0,01) cujo efeito foi totalmente bloqueado pela pré-incubação com o antagonista seletivo do A₁R, DPCPX (100 μ M) (108,4 $\pm$ 13,2%, *p*>0,05). Já o bloqueio dos receptores A_{2A} (A_{2A}R) pelo antagonista seletivo ZM 241,385 (10 μ M) causou uma diminuição na %IR-GABA (37,1 $\pm$ 6,0%, *p*<0,001) e esse efeito foi parcialmente revertido pela incubação posterior com NECA (88,9 $\pm$ 3,8%, *p*>0,05). **CONCLUSÃO:** Os resultados sugerem que a ativação de A₁R promova uma modulação positiva do sistema GABAérgico endógeno já que há uma diminuição na %IR-GABA, indicando um aumento da liberação desse neurotransmissor. Ao contrário, o antagonista do A_{2A}R diminuiu a %IR-GABA, levantando a possibilidade que a ativação do A_{2A}R por adenosina esteja modulando negativamente o sistema GABAérgico.

Palavras-Chave: GABA/ adenosina/ retina

Apoio Financeiro: CNPq, CAPES, FAPERJ, PRONEX, PROPP-UFF

Involvement of cannabinoid CB1 signaling in the antidepressant-like effects of eletroacupuncture in rats submitted to the forced swimming test.

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Previous work from our laboratory showed that electroacupuncture provided simultaneously at ST36 and SP6 promoted antidepressant-like effects in rats submitted to forced swimming and learned helplessness paradigm. Here we aimed at evaluating the influences of CB1 receptor signaling over antidepressant-like effects of electroacupuncture. Adult male Wistar rats (250g) were assigned to the following groups: simultaneous electroacupuncture (EA) stimulation of acupoints ST36 and SP6 ($N=8$); Sham ($N=8$), simultaneous stimulation of two nearby non-acupoints; Naïve ($N=8$), not subjected to needle stimulation; Sed ($N=8$): animals were only submitted to the brief ether exposure that is required for the needle stimulation procedure; SR-EA ($N=5$), animals treated with SR141716A (a CB1 antagonist) 5mg/kg, i.p., 30 min prior to acupoints stimulation; and SR ($N=4$), animals similarly treated only with SR141716A. The stimulation of acupoints and of non-acupoints consisted of a 2Hz, 50 μ A stimulus during 20min in each session. A total of 7 sessions, in alternated days were performed. After 24h of the last acupuncture treatment, the animals were submitted to a 15 min pretest of forced swimming. In the next day, the rats were submitted to the open field test (to avoid possible bias resulting from locomotion effects over the forced-swimming test), and immediately after animals were evaluated with the forced swimming test. As expected, ANOVA followed by Tukey *post hoc* revealed that EA diminished the time in immobility position as compared to Naïve and Sed groups [$F_{(5,35)}=6.85$ $p<0.01$]. The most relevant result is that SR141716A treatment blocks the effects of EA (given that SR-EA did not differ from Naïve and Sed) but does not alter the behavioral outcome of animals submitted to Sham electroacupuncture. These behavioral results were not due to an impairment of locomotor activity given that the animals of the different groups did not differ in terms of the number of squares crossed in the open field test [$F_{(5,35)}=1.18$ $p=0.15$]. Our data indicate that inhibition of CB1 signaling blocks the antidepressant-like effects associated with the stimulation of acupoints ST36 and SP6 in rats submitted to forced swimming test. These results suggest that the endocannabinoid system play a crucial role in the antidepressant-like effects of electroacupuncture.

Palavras-Chave: eletroacupuncture, endocannabinoid, depression

Apoio Financeiro: FAPESP, CNPq, CAPES

ETHANOL CHRONIC EXPOSURE ALTERS DERIVATED-PURINE NUCLEOTIDE HYDROLYSIS IN ZEBRAFISH (*Danio rerio*) BRAIN

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Ethanol is a drug widely consumed and exerts many actions in CNS, such as alters motor coordination, sensorial perception, and cognition. Ethanol modifies routes of signal transduction mediated by hormones and neurotransmitters. Zebrafish is an experimental model for the research of human diseases, including alcoholism and its dependence. However, there is no evidence about the effects of chronic ethanol exposure in the purinergic system, where ATP acts as neurotransmitter. After released, ATP is cleaved by ecto-nucleotidases, such as NTPDases and ecto-5'-nucleotidase, which has been previously characterized in zebrafish brain. Therefore, we verified the chronic effect of ethanol on adenine and guanine nucleotide hydrolysis in zebrafish brain. For *in vivo* experiments, animals have been exposed to 0.5% ethanol during 7 and 14 days (n=6). After exposure, brain membranes were prepared according a sequential centrifugation method and the enzymatic assays performed. The Pi released was quantified and specific activity was expressed as mean $\pm$ S.D. (nanomole of Pi per minute per mg of protein). The activity of control group was for ATP (504,72 $\pm$ 89,81), ADP (198,18 $\pm$ 26,84), AMP (34,50 $\pm$ 8,82), GTP (140,92 $\pm$ 25,11), GDP (33,78 $\pm$ 9,14), and GMP (32,67 $\pm$ 5,02). There were no significant changes on ATP and GTP hydrolysis after treatments. However, the data have shown a decrease of ADP (37% and 37%) and GDP (41% and 41%) hydrolysis after 7 and 14 days, respectively. After *in vivo* exposure to ethanol during 7 and 14 days, it was observed a significant decrease on AMP (40% and 24%) and GMP (50% and 36%) hydrolysis, respectively. Our results demonstrated an inhibition on NTPDase and 5'-nucleotidase activity in zebrafish brain. The nucleotide hydrolysis cascade could be a possible target of chronic toxicity promoted by ethanol, exerting a modulation of adenine and guanine nucleotides levels, as well nucleosides adenosine and guanosine for their specific receptors. Thus, the regulation of purinergic system by this drug could exert a key role for neurological mechanisms mediated by purines.

Palavras-Chave: Ethanol, Zebrafish, Ecto-nucleotidase, Purine

Apoio Financeiro: CAPES, FAPERGS, CNPq, FINEP "Rede Instituto Brasileiro de Neurociência (IBN-Net)" #01.06.0842-00.

EFEITO DO PRÉ-TRATAMENTO COM LÍTIO OU VALPROATO NA ATIVIDADE DAS MAPks, ERK E JNK, EM MODELO ANIMAL DE MANIA

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Objetivos: Avaliar os efeitos dos estabilizadores de humor, lítio e valproato nas vias de sinalização intracelulares no cérebro de ratos submetidos ao modelo animal de prevenção de mania.

Métodos: Foram utilizados ratos Wistar machos (60 dias), que foram tratados com lítio (47 mg/kg i.p) ou valproato (200 mg/kg i.p) e salina por 14 dias. No 8º dia de tratamento os animais tratados com valproato, lítio e salina foram divididos em dois grupos: um grupo recebeu solução salina e outro recebeu anfetamina (2mg/kg, i.p.). Os ratos foram mortos, o cérebro removido e o hipocampo dissecado. As proteínas foram separadas por SDS-PAGE e imunodetectadas por *western blotting*. Os complexos imunes foram quantificados com o software Scion Image. Os dados foram comparados com One Way ANOVA considerando estatisticamente significativo $p < 0,05$. Dados estão expressos como média da fosforilação em relação ao grupo controle (salina) $\pm$ desvio padrão ($n = 3$).

Resultados: Observamos que os tratamentos dos animais com estabilizadores de humor, lítio ou valproato, associados ou não com anfetamina não alteram a ativação das proteínas ERK1/2 (Control= $68,4 \pm 17,6$ e $68,5 \pm 24,3$; AMPH= $48,3 \pm 7,11$ e $46,9 \pm 31,2$, ERK 1 e 2 respectivamente) e JNK1/2 (Control= $18,4 \pm 1,8$ e $19,7 \pm 7,4$; AMPH= $17,7 \pm 45$ e $124 \pm 43\%$, JNK 1 e 2 respectivamente).

Conclusão: Os dados demonstram que os estabilizadores de humor ou a anfetamina não tem efeito sobre a atividade das proteínas ERK ou JNK no modelo de mania aplicado em ratos. Provavelmente estas vias não estão envolvidas nas alterações neuroquímicas induzidas pelos estabilizadores de humor, ou pela anfetamina no hipocampo destes animais.

Palavras-Chave: ERK, JNK, modelo de mania, lítio, valproato, anfetamina.

Apoio Financeiro: Unesc

TREATMENT WITH L-ARGININE DURING DEVELOPMENT DOSE-DEPENDENTLY FACILITATES CORTICAL SPREADING DEPRESSION IN RATS

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Objectives: The amino acid arginine (ARG) is considered essential early in life, and is the precursor of the nitric oxide synthesis. Recent studies strongly suggest that nitric oxide-mediated signaling is involved in the phenomenon of cortical spreading depression (CSD). It has been demonstrated that administration of L-arginine (300 mg/kg) during development can facilitate CSD propagation in adult rats (Frazão, Master Thesis, Recife, 2004). In this study, two additional doses of L-arginine have been used in order to further characterize the dose-response features of this CSD-effect.

Methods: Wistar rat pups received, from the 7th to the 28th day of life, by gavage, either ARG (150, 300 or 450 mg/day) or distilled water (H₂O). An additional “ingenuous” group (ING, which did not receive any treatment) was also studied. At 30-40 days of life, CSD recording was performed, for 4 hours. CSD was elicited every 20 min by KCl (2% solution), applied for 1 min to a point of the frontal cortex region and recorded at 2 points of the parietal cortex.

Results and Conclusions: Compared to the controls (H₂O- and ING-) groups, ARG-treated rats displayed significantly higher CSD velocities ($P < 0.05$), in a dose-dependent fashion. The mean $\pm$ sd CSD-velocities (in mm/min) were 3.78 ± 0.19 and 3.77 ± 0.14 for the H₂O- and ING-groups, respectively, and 4.03 ± 0.12 , 4.36 ± 0.17 , 4.41 ± 0.23 for the 150, 300 and 450 mg/kg/d ARG-groups. The CSD-velocity of the lower ARG-dose group (150 mg/kg/d) significantly differed from the other two ARG-groups, which presented similar CSD-velocities. The results reinforce previous data on a facilitating effect of L-arginine on CSD propagation, and shows that this effect is dose-dependent, suggesting that the optimal dose for this effect in the developing rat is around 300mg/kg/d. It can be suggested that L-arginine supplementation can modify brain CSD susceptibility by modulating nitric oxide synthesis. Further investigations shall clarify which ARG-dose could possibly protect or even damage brain cells, in an attempt to better understand the nitric oxide role in neurodegenerative diseases. **Palavras-Chave:** L-arginine, Cortical spreading depression, Development, Rat, Nervous system, Nitric oxide

Apoio Financeiro: CNPq, CAPES, UFPE, FINEP/IBN-Net

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Is arginylated calreticulin implicated in cell adhesion?

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Protein arginylation is a posttranslational modification mediated by arginyl-tRNA protein transferase (Ate 1) that transfers arginine from tRNA onto proteins that expose Glu, Asp or Cys at the N-terminal position. Whilst arginylation was discovered more than 40 years ago, the identity of proteins arginylated in vivo and its biological functions are largely unknown. We demonstrated the posttranslational incorporation of arginine into calreticulin (CRT) in vitro and in living cells. The arginylated form of CRT (R-CRT) was observed in the cytosol, in contrast to the non arginylated CRT which is in the endoplasmatic reticulum.

It has been previously demonstrated that CRT affects cell adhesion, and plays a role in the control of cell adhesiveness via regulation of vinculin expression. This may be mediated by direct interaction between CRT and the KxGFFKR of a integrins. Therefore, to functionally affect integrin cluster in focal contacts, CRT should be in the cytoplasm. The first direct evidence of cytosolic CRT is the arginylated form R-CRT.

By immunocytochemistry, using an antibody against R-CRT we observed an increased expression of both R-CRT and vinculin in cells subjected to focal adhesion disassembly and reassembly. In addition, by confocal microscopy we observed the colocalization between integrins and R-CRT in hippocampal neurons and fibroblasts. Further studies performed in cells lacking of arginyl-tRNA protein transferase (ate1 -/-) show a differential attachment to laminin and polylysine when compared to cells ate1 +/+ (provided by Dr. Kashina). When both cell types were transfected with CRT the ate1 -/- cells did not improve their adhesiveness to substrata as ate1 +/+ cells.

This evidence supports the idea that R-CRT is implicated in cell adhesion and spreading; thus posttranslational arginylation of CRT seems to regulate its intracellular localization and cytosolic function.

Palavras-Chave: arginylation, calreticulin, adhesion

Apoio Financeiro: Supported by SECyT-UNC, CONICET, ANPCyT-PICT.

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Antidepressant-like effect of lectin from *Canavalia brasiliensis* (ConBr) administered centrally in mice involves modulation of glutamatergic system

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Lectins are carbohydrate-binding proteins that may recognize glycoconjugates on cell surfaces regulating cell function. The brain displays a variety of lectins, however, their roles are not well known. ConBr is a lectin isolated from *Canavalia brasiliensis* seeds that demonstrated an antidepressant-like effect in the forced swimming test (FST) in mice by a mechanism dependent of monoaminergic system activation (Pharmacol. Biochem. Behavior, 85:160, 2006). In the present work the involvement of glutamatergic system and L-arginine-nitric oxide (NO)/cGMP pathway in the antidepressant-like effect of ConBr in the FST in mice was investigated. The results are expressed as mean $\pm$ S.E.M. and showed that ConBr (10 μ g/site, i.c.v.) caused a decrease of 40% $\pm$ 3.2 ($p < 0.01$) in the immobility time in the FST. The pre-treatment of mice with NMDA (0.1 pmol/site, i.c.v.) or D-serine (30 μ g/site, i.c.v.; glycine site agonist of the NMDA receptor) reversed this antidepressant-like effect of ConBr. Additionally, the co-administration of sub-effective doses of MK-801 (0.001 mg/kg, i.c.v.; NMDA receptor antagonist) and ConBr (0.1 μ g/site) caused a decrease of 35% $\pm$ 3.5 ($p < 0.01$) in the immobility time in the FST. In order to investigate the involvement of the L-arginine-NO/cGMP pathway in the anti-immobility effect of ConBr, a pre-treatment of mice with L-arginine (750 mg/kg, i.p., NO precursor) or sildenafil (5 mg/kg, i.p.; PDE5 inhibitor) was performed. The results showed that both drugs reversed the antidepressant-like effect of ConBr. Moreover, the pretreatment with the guanylyl cyclase inhibitor (1H-[1,2,4]oxadiazole[4,3-a]quinoxalin-1-one) (ODQ; 30 pmol/site, i.c.v.) produced a synergistic antidepressant-like effect with sub-effective doses of ConBr (reduction in the immobility time of 35% $\pm$ 6.3; $p < 0.01$). The treatments did not change the locomotor activity in the open-field test. Furthermore, *in vitro* studies suggest low toxicity for ConBr in hippocampal slices. Taken together, the results indicate that the reduction in the immobility time elicited by ConBr in the FST, seems to be dependent on NMDA receptor inhibition and a reduction in NO and cGMP production. **Palavras-Chave:** Lectins; ConBr; antidepressant-like effect; glutamatergic system

Apoio Financeiro: Supported by CNPq, FAPESC and FINEP (IBN-Net #01.06.0842-00)

INIBIDORES DA SÍNTESE DE PROTEÍNAS INDUZEM PRODUÇÃO DE ÓXIDO NÍTRICO E FOSFORILAÇÃO DAS CINASES AKT E ERK 1/2

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Objetivos: Trabalho anterior de nosso laboratório mostrou que a inibição da síntese protéica com cicloheximida (CHX) promove aumento da disponibilidade intracelular de L-arginina (L-ARG) e o aumento da síntese de NO através da enzima óxido nítrico sintase. NO atua como neuroprotetor em culturas purificadas de neurônios de retina e este efeito depende das proteínas cinases PKA, ERK1/2, CaMK e PI3K. O propósito deste estudo foi determinar o mecanismo pelo qual CHX e anisomicina (ANIS), outro inibidor de síntese protéica, produzem aumento da produção de NO e ativação da AKT e ERK em culturas de retina de embrião de pinto.

Métodos: Culturas de células de retina de embrião de pinto de 8 dias (E8) foram preparadas e após 72 horas foram estavadas com solução salina Hank's por 24 horas. Em seguida foi adicionado CHX ou ANIS em concentrações e tempos diferentes e as culturas processadas para a medida da fosforilação da AKT^{Ser-473} e ERK-1/2 pela técnica de western blot. O NO foi quantificado pela reação de Griess. Em culturas purificadas de neurônios (85x10⁴ células/placa) mantidas até E8C3 foi determinada a captação de ⁴⁵Ca²⁺ após 3 minutos de estímulo com SNAP(100 µM), um doador de NO, ou L-ARG (1mM). Em seguida, a radioatividade foi quantificada por cintilação líquida.

Resultados: Tanto CHX quanto ANIS produziram maior síntese de NO (0.844±0.034 µM e 1.056±0.054 µM em relação ao controle (0.526±0.043 µM, n=3) e induziram a fosforilação da AKT e ERK-1/2 de maneira tempo e dose-dependente. Esses resultados foram mimetizados pela L-ARG, substrato da nNOS, e bloqueados por L-NAME, inibidor da enzima nNOS, e por EGTA, um quelante extracelular de cálcio (Ca²⁺). Por outro lado, o NO aumentou o influxo ⁴⁵Ca²⁺ de 159.8±42.6 CPM/placa no controle para 602.3±95.4 CPM/placa (n=3).

Conclusões: Nossos dados sugerem que o NO endógeno produzido pelo acúmulo de L-ARG intracelular após inibição da síntese protéica com CHX ou ANIS e a ativação da NOS-neuronal promove a fosforilação da AKT ou ERK-1/2, sinais importantes na regulação da sobrevivência celular. O aumento produzido no influxo de cálcio pelo L-ARG pode ser fundamental para a ativação das cinases neuroprotetoras em culturas de retina.

Palavras-Chave: Cicloheximida, Anisomicina, Neurônios de retina, SNAP, Neuroproteção, AKT/PKB.

Apoio Financeiro: CAPES, CNPq, FAPERJ, PRONEX, PROPP-UFF

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Modulation of ATP-induced cell proliferation in the development avian retina: Role of P2Y1 receptor desensitization.

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ATP induces retinal cell proliferation through activation of P2Y1 receptors, phospholipase C, PKC and MAP kinases. Here, we show that the response of chick retinal cells to this nucleotide decreases during development *in vitro* and that activation of MAP kinases depends on ATP-induced desensitization. Cultures of chick retinal cells stimulated for short periods of time (2h) with 100µM ATP showed no increase in DNA synthesis (control: 1077±151; ATP:1151±66 cpm/culture. n=3). However, longer incubations in the presence of 100 µM ATP (8 and 24h) induced significant increases (109±18% and 227±15%, above basal levels respectively, n=3) in [³H]-thymidine incorporation in the cultured cells. Immunocytochemistry for the P2Y1 nucleotide receptor, using cultured retinal cells (E7C2 and E7C9) or intact tissues from 6- or 9-day-old embryos, revealed that this protein was localized in glial cells. BrdU immunocytochemistry and confocal microscopy revealed that only glial cells presented BrdU incorporation in the cultures. Incubation of cultured retinal cells with 100 µM ATP for 1h induced a significant increase (150±20% under basal levels. n=3) in the formation of [³H]-phosphoinositides. However, when cells were pre-incubated with 100 µM ATP for 24h and then stimulated with the nucleotide, no increase in [³H]-phosphoinositides was observed, an effect that was blocked by the P2Y receptor antagonist PPADS. Western blot assays revealed that ATP-induced phosphorylation of ERKs was completely inhibited by 300 µM monodansyl-cadaverine (MDC) or 10 µM chlorpromazine (CPZ), two inhibitors of clathrin-mediated endocytosis. Moreover, although cell death was detected with prolonged incubations of the cells with MDC, but not with CPZ, both compounds inhibited ATP-induced cell proliferation in a dose-dependent manner. These data together suggest that ATP increases proliferation of developing glial precursors in culture, a phenomenon that is concomitant with agonist-dependent P2 receptor desensitization. Our data also suggest that activation of endocytosis is also necessary for ATP-induced proliferation of retinal cells in culture. **Palavras-Chave:** Retina, Chick, P2 receptor, desensitization, Proliferation.

Apoio Financeiro: CNPq, Faperj, Propp-UFF

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Glutamato Modula A CREB Na Retina de Galinha Em Desenvolvimento

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Objetivos: A retina é um tecido neural especializado na fototransdução e, como outras estruturas do sistema nervoso central, apresenta células com fenótipos de diversos neurotransmissores, como o glutamato (Glu). A ativação de receptores de Glu está associada à produção de óxido nítrico (NO) e aciona cascatas intracelulares que culminam com a fosforilação de ERK e do fator de transcrição CREB. O resíduo serina 133 de CREB é crítico para sua ativação, e pode ser fosforilado por diversas cinases. Neste trabalho estudamos a expressão de CREB e o papel de NO e Glu na fosforilação de CREB e ERK na retina de embrião de galinha em desenvolvimento.

Métodos: Retinas de embriões de galinha eram incubadas por cinco minutos em salina com drogas. Em seguida o tecido era homogeneizado em tampão de amostra para Western blot e, após a determinação de proteínas pelo método de Bradford, as amostras eram processadas para imunodeteção de CREB, pCREB, ERK e pERK.

Resultados: CREB foi expressa na retina de galinha desde o período embrionário até a eclosão. A fosforilação basal de CREB aumentou bruscamente em E14 (catorze dias embrionários), que coincide com o período de sinaptogênese deste tecido. Glu (500 μ M) estimulou 109,7 $\pm$ 30,4% a fosforilação de CREB em retinas E8, enquanto o doador de NO SNAP (100 μ M) estimulou a fosforilação de CREB (134,4 $\pm$ 74%) e ERK (103,5 $\pm$ 42,5%) nas retinas E8, com envolvimento da via do GMPc. O aumento da pCREB induzido por Glu foi também observado nas retinas E11. Entretanto, o bloqueio dos receptores de Glu do tipo NMDA em E14 foi capaz de diminuir os níveis basais de pERK (39,9 $\pm$ 20,2% do controle), e de pCREB (53,6 $\pm$ 5,9% do controle).

Conclusões: Os resultados mostram que a sinalização por Glu é capaz de modular a atividade de CREB ao longo do desenvolvimento da retina, sugerindo sua participação na expressão gênica deste tecido. Além disso, os níveis de Glu endógeno parecem estimular a ERK e a CREB via receptores NMDA no período de sinaptogênese da retina.

Palavras-Chave: Glutamato, CREB, retina, desenvolvimento

Apoio Financeiro: FAPERJ, PRONEX, CAPES, CNPQ

B.01.035

Kinetic characterization of adenosine deaminase activity in zebrafish (*Danio rerio*) brain

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Objectives: Adenosine deaminase (ADA) activity is responsible for cleaving adenosine to inosine. The aim of this study was to describe the biochemical properties of adenosine deamination in soluble and membrane fractions of zebrafish (*Danio rerio*) brain.

Methods: Zebrafish were euthanized and whole brains were initially homogenized in 20 volumes (v/w) of chilled phosphate buffer saline, with 2 mM EDTA, 2 mM EGTA, pH 7.4. The soluble and membrane fractions were obtained by differential centrifugation (1). The proteins from both fractions were measured (2) and the adenosine deaminase activity was determined spectrophotometrically by measuring the ammonia produced over a fixed time using a Berthelot reaction (3). All assays were carried out in triplicate and means $\pm$ SEM of at least four independent experiments were presented. Data were analyzed by Student's t-test or one-way analysis of variance (ANOVA), followed by Tukey test as post-hoc considering $P < 0.05$ as significant.

Results: The optimum pH for ADA activity was in the range of 6.0–7.0 in soluble fraction and reached 5.0 in brain membranes. A decrease of 31.3% on adenosine deamination in membranes was observed in the presence of 5 mM Zn^{2+} , which was prevented by 5 mM EDTA. The apparent K_m values for adenosine deamination were 0.22 ± 0.03 and 0.19 ± 0.04 mM for soluble and membrane fractions, respectively. The apparent V_{max} value for soluble ADA activity was 12.3 ± 0.73 nmol $NH_3 \cdot min^{-1} \cdot mg^{-1}$ of protein whereas V_{max} value in brain membranes was 17.5 ± 0.51 nmol $NH_3 \cdot min^{-1} \cdot mg^{-1}$ of protein. Adenosine and 2'-deoxyadenosine were deaminated in higher rates when compared to guanine nucleosides in both fractions. Furthermore, a significant inhibition on adenosine deamination in both soluble and membrane fractions was observed in the presence of 0.1 mM of erythro-9-(2-hydroxy-3-nonyl)adenine.

Conclusions: These data may be important to clarify the mechanisms of adenosine cleavage in zebrafish, which could lead to better understanding about the role of the purinergic system in this species.

References: 1- [J. Neurochem.](#) 61:1685, 1993; 2- [Anal. Biochem.](#) 72:218, 1976; 3- [J. Biol. Chem.](#) 263:5266, 1988. **Palavras-Chave:** adenosine deaminase; brain; zebrafish

Apoio Financeiro: CAPES, CNPq, FAPERGS, FINEP (IBN-Net # 01.06.0842-00)

B.01.036

Adenosine modulates ERK activity in cultures of avian retinal glial cells: Involvement of A₁ receptors, PKC and Src kinase

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Objectives: Glial cells play fundamental roles in CNS such as regulation of synaptic transmission and neurotransmitter uptake and metabolism. Adenosine (Ado) is an important neuroactive substance regulating cell proliferation and differentiation, also participating in cellular repair processes and neuroprotection from glutamate-induced excitotoxicity. Ado receptors are present in glial cells where they regulate adenylyl cyclase activity. The MAP-kinase pathway appears to have an important role in several neural and glial functions. In this work we studied the involvement of A₁ ado receptors in the regulation of ERK activity in glial cells.

Methods: Cultures of glial cells were obtained from 11 day-old chick embryo retinas (E11) and incubated in MEM for 21 days. Cultures were then incubated with selective ado receptor agonists and antagonists and processed for determination of phosphorylated ERK (pERK) by western blotting or immunocytochemistry.

Results: Incubation of cultures with ado deaminase (0.5 U/ml) or DPCPX (10 μ M), an A₁ receptor antagonist, reduced the basal pERK levels to 52.1 ± 5.3 % (n=3) or 45.1 ± 9.2 % (n=3), respectively, indicating that endogenous ado regulates ERK phosphorylation through the activation of A₁ receptors. Incubation with CHA (1 μ M), a selective A₁ receptor agonist, promoted an increase of 119.4 ± 16.6 % (n=19) in ERK phosphorylation, an effect completely blocked by DPCPX (10 μ M) (5.8 ± 16.5 %, n=4). PD 98059 (25 μ M), a MEK inhibitor, blocked both basal and CHA-stimulated levels to 53.7 ± 16.2 % below basal levels (n=3), and the same effect was observed with PP1 (10 μ M), a Src inhibitor, or Chelerythrine chloride (100 nM), a PKC inhibitor (28.2 ± 40.5 % and 31.7 ± 16.5 % below basal levels respectively, n=3). Immunocytochemical data revealed that the increase of pERK levels observed by activation of A₁ receptors is mainly located in the cytosol.

Conclusion: The results clearly demonstrate that A₁ ado receptors modulate ERK activity in retinal glial cells.

Palavras-Chave: Adenosine, ERKs, Glial Cells

Apoio Financeiro: FAPERJ, CNPq, PRONEX – MCT and CAPES

B.01.037

Glutamate modulates cyclic-AMP responsive element binding protein (CREB) phosphorylation in cultured Müller glia cells through an AMPA receptor and NO-dependent mechanism.

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Glutamate signaling in the mature retinal tissue is very important for accurate sensory decoding by retinal neurons and orchestrates the fine-tune output from the retina to higher-order centers at the cerebral cortex. It is also known that Nitric Oxide (NO) system is present and functional since early periods of retinal development. Mixed primary cultures of eight-day-year-old embryonic retinal neurons were used to assess glutamate and NO modulation of ERK2 (extracellular regulated kinase 2) and CREB (cyclic-AMP responsive element binding) transcription factor phosphorylation. Western blot analysis show that 250μM glutamate induces a rapid ERK2 (262,8±68,2, n=5) and CREB (336,7±89,7, n=6) phosphorylation in cultured developing retinal neurons, a process reliant of AMPA receptors and NO signaling, but not NMDA receptors since is blocked by 200μM of the AMPA/kainate antagonist DNQX (109,4±83,3, n=3 for ERK2 and 152,3±106,4, n=3 for CREB) as well as inhibiting Nitric Oxide Synthases with 100μM L-NAME (16±66,1, n=3 for ERK2 and -5,4±23,6, n=3 for CREB), but not by 10μM of the NMDA channel blocker MK-801 (212,6±117,9, n=3 for ERK2 and 312,8±154,7, n=3 for CREB). The effect of NO on ERK2 and CREB involves Src kinases and the classical NO/sGC/cGMP/PKG pathway as is inhibited by 10μM of the Srcs inhibitor PP1 (-16,7±39,4, n=3 for ER2 and -9,9±38,2, n=3 for CREB), by 5μM of the soluble Guanylate Ciclase (sGC) blocker ODQ (16,8±42,4, n=3 for ERK and 10,6±19,7, n=3 for CREB) and by 1μM of the Protein Kinase G inhibitor KT5823 (18,3±56,3, n=3 for ERK2 and -21±40,1, n=4 for CREB), respectively. Moreover, NO-induced CREB phosphorylation is dependent on prior integration of multiple kinases signaling pathways, such as, ERK2, PKC, PI3K and CaM kinases. Immunocytochemistry data suggest that increased CREB phosphorylation in response to glutamate occurs in glial cell nuclei. We also have supporting evidence that neuronal-produced NO directly reaches the glial cell and stimulates CREB phosphorylation. Hence, the results indicate that the interplay between neuronal-glial communication and glutamate/NO/CREB linkage may be important during retinal tissue development.

Palavras-Chave: CREB, glutamate, nitric oxide, retina

Apoio Financeiro: FAPERJ, CAPES, CNPq, Pronex

TREATMENT WITH TIANEPTINE DURING DEVELOPMENT INCREASES CORTICAL SPREADING DEPRESSION PROPAGATION IN YOUNG RATS

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Previous work has demonstrated that cortical topic treatment with tianeptine, a reuptake serotonin enhancer, facilitates cortical spreading depression (CSD) propagation in adult rats (II Simpósio do Instituto Internacional de Neurociências de Natal, 2007). In this study, systemic administration of tianeptine has been used, instead of topic application, in order to confirm its influence on CSD by using a clinically more important way of pharmacotherapy. Wistar rats were injected subcutaneously from postnatal day 2 to 24 with tianeptine (T; 5 or 10 mg/kg/d) or equivalent volume of saline solution (S). CSD was recorded immediately after weaning (25-30 days of life). The phenomenon was elicited every 20 min by KCl (2% solution) applied for 1 min to a point of the frontal region and its propagation was recorded at 2 points of the parietal cortex. Systemic administration of tianeptine significantly increased ($p<0.05$) CSD velocities when compared to the S group (mean $\pm$ SD of the 4h of recordings, in mm/min: 3.98 ± 0.13 , 4.61 ± 0.38 and 3.79 ± 0.19 for the groups T5, T10 and S, respectively). These results suggest that the serotonergic system is putatively involved in CSD propagation mechanisms, reinforcing previous data with other serotonergic drugs (Exp. Neurol. 200:275, 2006). By considering that important neurological diseases like epilepsy and migraine are related to serotonergic processes, as well as to CSD, this study could contribute for future strategies of therapy against those diseases. Additionally, the possibility is raised, that tianeptine-induced reduction in the brain serotonin availability can be used as a tool to better understand brain function, concerning the serotonergic system. **Palavras-Chave:** Tianeptine, Spreading depression, Serotonin, Development, Nervous system, Rat

Apoio Financeiro: FACEPE, CNPq, UFPE

B.01.039

DARPP-32 and NCS- 1 expression in PC12 cells are not altered by antipsychotics

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Schizophrenia is one of the most complex and devastating mental illness which is characterized by positive and negative symptoms. Although the cause of the disease is still unknown, it is well known that the dopamine D2 receptors are the main target of antipsychotics. Literature has shown that DARPP-32 has a central role in the dopaminergic signaling pathway and its expression is decreased in dorsolateral prefrontal cortex (DLPFC) of patients with schizophrenia and bipolar disorder. In this same region of schizophrenia and bipolar disorder patients, it was found an upregulation of neuronal calcium sensor-1 (NCS-1) which inhibits D2 receptor phosphorylation and internalization. Our previous studies showed that there is no alteration in DARPP-32 and NCS-1 levels in brain of rats treated chronically with typical and atypical antipsychotics. However, levels of both proteins in brain of untreated rats are not changed as schizophrenia and bipolar disorder patients. Recently we observed that PC12 cells stably overexpressing NCS-1 (PC12 clone cells) have downregulation in DARPP-32 expression. In this way, we aimed to investigate the role of typical or atypical antipsychotics on the expression of DARPP-32 and NCS-1 in PC12 wild type (PC12 wt cells) and PC12 clone cells. For that, we treated both cells with three different concentrations of typical antipsychotic haloperidol and two atypical antipsychotics, risperidone and clozapine, for 7 and 14 days. We analyzed DARPP-32 and NCS-1 expression by western blot and we have not found any changes in DARPP- 32 and NCS-1 expression in both PC12 cells. Therefore, since the mechanism of action of antipsychotics seems to be not correlated to changes in DARPP-32 and NCS-1 expression, we are allowed to suggest that downregulation of DARPP-32 and upregulation of NCS-1 in DLPFC of schizophrenia and bipolar disorder subjects might be correlated with the diseases and not with drugs treatment. **Palavras-Chave:** Antipsychotics, DARPP 32, NCS1, PC12 cells, Schizophrenia

Apoio Financeiro: CNPq, FAPEMIG, John Simon Guggenheim Foundation

B.01.040

Quantitative morphological study of the P2X₂ receptor, nitric oxide synthase (NOS), choline acetyltransferase (ChAT) and calretinin in neurons of the small intestine of obese female mice (*ob/ob*).

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Objectives: Obesity is a chronic heterogeneous disorder characterized by the excess of corporal fat. Alterations in the intestinal motility have been observed in obese individuals and can be related with the myenteric neurons. In this work, we analyzed the effect of the obesity in P2X₂ receptor-ir (immunoreactivity), NOS-ir, ChAT-ir and Calretinin-ir (CalR) neurons, for this was carried by colocalisation and morphometric studies in ileum female mice.

Methods: The ileum of the obese group (OBG) (C57BL/6J *ob/ob*) and control group (CG) (+/+) were used and the areas of the segments were obtained. The tissues were submitted to the double immunostaining technique the P2X₂ receptor with NOS (mark for inhibitory neurons), ChAT (mark for excitatory neurons) or CalR (mark for sensory neurons).

Results: The qualitative analysis demonstrated the P2X₂ receptor expression in the cytoplasm and in the nuclear and plasmatic membranes of the myenteric neurons in OBG and CG. Also, it was observed NOS, ChAT and CalR in the nerve cell bodies in both groups. Colocalizations results have shown in the CG for P2X₂ receptor with NOS-ir, ChAT-ir and CalR-ir neurons: 23.6±4%, 24.3±2% and 23.7±4%, and in OBG were 19.5±4%, 24.6±4% and 21.6±1%, respectively. Inversely, the colocalization of NOS-ir, ChAT-ir or CalR-ir neurons with the P2X₂ receptor were in turn of 100% in both groups. The neuronal density (neuron/mm²) has shown a decreased in NOS-ir and ChAT-ir neurons of the OBG (p<0.05). The values were: in CG 220±2 for P2X₂-ir, 85±3 for NOS-ir, 103±5 for ChAT-ir and 87±3 for CalR-ir neurons; in OBG was observed 336±105 for the P2X₂-ir, 49±1 for NOS-ir, 48±8 for ChAT-ir and 89±2 for CalR-ir. Morphometric studies have demonstrated an increase in the cell body profile area (µm²) (p<0.05) of NOS-ir neurons (CG 234±63 and OBG 312.3±67); ChAT-ir neuron (CG 210±24 and OBG 253±14) and CalR-ir neurons (CG 203±41 and OBG 315±47). There were not changes in P2X₂-ir neurons (CG 325.4±23 and OBG 336±67).

Conclusion: It is possible that the obesity can affect the density and morphometric data of the P2X₂-ir, NOS-ir, ChAT-ir and CalRir neurons and this may cause intestinal dysfunctions.

Palavras-Chave: myenteric neurons, P2X₂-receptor, obesity, small intestine

Apoio Financeiro: FAPESP/CAPES

A ZONA INCERTA DO SAGUI (*Callithrix jacchus*): CARACTERIZAÇÃO NEUROQUÍMICA COM POLIPEPTÍDEO INTESTINAL VASOATIVO, PROTEÍNA ACÍDICA FIBRILAR GLIAL E DELIMITAÇÃO CITOARQUITETÔNICA COM NISSL E NEUN.

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Objetivos: A zona incerta (ZI) é um agrupamento neuronal localizado na região do tálamo ventral, medial ao núcleo geniculado lateral dorsal, e dorsal ao núcleo subtalâmico. A este conjunto de neurônios são atribuídas diferentes funções, principalmente ativação cortical, ingestão hídrica, comportamento sexual e movimentos sacádicos dos olhos. Nosso objetivo foi caracterizar uma possível subdivisão da ZI no sagui através da delimitação citoarquitetônica com Nissl e NEUN, um marcador nuclear de neurônios, além de caracterização neuroquímica para polipeptídeo intestinal vasoativo (VIP) e proteína acídica fibrilar glial (GFAP).

Métodos e Resultados: Os animais foram anestesiados e perfundidos transcardialmente. Os encéfalos foram submetidos à microtomia por congelamento obtendo-se secções coronais de 30µm, processadas imunohistoquimicamente, através do protocolo ABC, para a revelação de GFAP, VIP e NEUN. Algumas secções foram coradas por Nissl. Foram observadas imunorreatividades contra VIP, e marcação quase que completamente negativa para GFAP. O VIP delimitou uma porção na ZI que corrobora com a delimitação com Nissl e NEUN, admitindo-se uma subdivisão em duas regiões, ventrolateral e dorsomedial.

Conclusão: Diferente da subdivisão da ZI em outras espécies em porção dorsal e ventral, neste trabalho encontramos as subdivisões ventrolateral e dorsomedial. O VIP delimita a porção ventrolateral. O GFAP delimitou a ZI de forma a revelar nos três níveis anterior, intermediário e posterior a sua extensão, bem como para o núcleo subtalâmico, não encontrado em estudos anteriores.

Palavras-Chave: Zona incerta, NEUN, VIP, sagui, imunohistoquímica

Apoio Financeiro: CNPq, CAPES, PROPESQ-UFRN, FAPESP

B.01.042

Participação de Rac1 na morte neuronal induzida por glutamato na retina de ratos.

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Objetivo: Apesar do alto influxo de cálcio ser considerado o evento central da morte neuronal desencadeada pela excitotoxicidade por glutamato, a cascata de sinalização envolvida nesse processo ainda não está completamente elucidada. Uma das possibilidades seria via GTPases Rho, recentemente relacionadas à morte de neurônios cerebelares e à degeneração de fotorreceptores. Nosso objetivo foi investigar a possível participação das GTPase RhoA e Rac1, na retina de ratos após tratamento excitotóxico com glutamato e agonista.

Métodos: Injeções intraoculares de NMDA (100 nmol) foram feitas em ratos *Wistar* (250-350g) e os animais sacrificados após 3, 6, 12 e 24 hs. A indução de morte foi analisada pelo método de TUNEL. Explantes de retina foram cultivados em DMEM (soro fetal bovino 10%, glicose 0,8%, HEPES 25mM, B-27 2% e penicilina/estreptomicina 1%) e tratados com glutamato (Glu, 5mM Sigma), MK-801 (10mM) e NSC (100mM) por 24 hs. A eficiência da excitotoxicidade foi verificada por ensaio de lactato desidrogenase (LDH). As retinas foram fixadas, seccionadas, processadas para imunofluorescência para Rac1 e RhoA (Santa Cruz) e analisadas em microscópio confocal (Nikon).

Resultados: Após injeções intraoculares de NMDA, um aumento de células TUNEL-positivas e Rac1-positivas nas camadas nuclear interna (INL) e ganglionar (GCL) foram detectadas, principalmente a partir das 6 hs. Por outro lado, RhoA permaneceu inalterada. Em explantes, a excitotoxicidade por glutamato provocou aumento significativo da % de LDH (média± desvio padrão, 72±6%) em relação ao controle (22±2%) (Teste de Tukey, p<0,001) após 24 hs. A adição do antagonista MK-801, assim como do inibidor específico de Rac1 reduziu parcialmente esse efeito (41±1% e 51±6%) e provocou uma diminuição das células Rac1-positivas, aumentadas após tratamento com Glu. Nenhuma diferença significativa foi observada nos grupos Glu+NSC, Glu+MK e Glu+MK+NSC (51±6%, 41±1% e 50±4%).

Conclusão: Rac1 pode ser uma possível proteína downstream da via de sinalização de morte ativada por receptores NMDA. No entanto, a participação de outras GTPases Rho e subunidades de receptores de Glu na morte induzida por glutamato não está descartada.

Palavras-Chave: Glutamato, GTPases Rho, Retina

Apoio Financeiro: Apoio financeiro: FAPESP e CNPq.

B.01.043

Modulation of DARPP-32 expression by NCS-1 through Akt pathway

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Schizophrenia is a chronic, severe and disabling psychiatric disorder which causes remains unknown. There are evidences that levels of Akt and *dopamine and cyclic adenosine 3':5'-monophosphate regulated phosphoprotein of relative molecular mass 32,000* (DARPP-32) are decreased in prefrontal cortex (PFC) of schizophrenic patients. Also, it was shown that PFC of schizophrenics has increased levels of neuronal calcium sensor-1 (NCS-1). It is well known that brain volume of schizophrenic subjects is reduced and that serine/threonine kinase Akt is involved in intracellular signaling of apoptosis. Dopamine receptor D2 is the main target of antipsychotics and DARPP-32 has a central role in dopamine intracellular signaling pathway through the inhibition of protein phosphatase-1 (PP-1). Many functions of NCS-1 were described such as survival factor and as an important key of dopamine signaling, inhibiting dopamine receptor D2 internalization. Recently, we have shown that PC12 cells stably overexpressing NCS-1 (Clone) have downregulation of cAMP/PKA signaling pathway and reduced levels of DARPP-32. Several studies have demonstrated that D2 modulates Akt pathway through beta-arrestin 2 and PP2A. Also, it was demonstrated that DARPP-32 expression is regulated by phosphorylation of Akt when tyrosine kinase B (TrKB) was activated. Given that, we hypothesized if NCS-1 modulates DARPP-32 expression through Akt signaling pathway. Immunoblotting analysis of wild type (WT) and Clone PC12 cells showed that there was no difference in Akt expression and phosphorylation levels at serine 473. However, we observed a decrease in Akt phosphorylation at threonine 308 in Clone cells. These results indicate that DARPP-32 is modulated by NCS-1 through p-Akt(Thr308) and that PC12 cells are a suitable model to dissect the important signaling pathways modulated by DARPP-32 and NCS-1.

Palavras-Chave: schizophrenia, AKT, DARPP-32, NCS-1

Apoio Financeiro: CNPq, Programa Institutos do Milênio/CNPq/FINEP e John Simon Guggenheim Foundation

B.01.044

Análise da expressão do RNAm da Tirosina Hidroxilase, do NeuropeptídeoY, da Arginina Vasopressina e Oxitocina no Núcleo Paraventricular do Hipotálamo de ratos SHR machos e fêmeas, durante o envelhecimento.

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Objetivos: O núcleo paraventricular do hipotálamo (PVN) é importante para várias funções do organismo, como o controle cardiovascular. Nele, encontram-se diversos neurotransmissores, como o NPY, Arginina Vasopressina (AVP), Oxitocina (OT) e Tirosina Hidroxilase (TH), que atuam no controle da PA. É conhecido o desenvolvimento da hipertensão de acordo com a idade e nas fêmeas é diferenciado comparando-as aos machos, sugerindo a influência do dimorfismo sexual na hipertensão. O objetivo deste trabalho foi analisar a expressão do RNAm da TH, NPY, AVP e OT em ratos SHR machos e fêmeas, durante o envelhecimento.

Métodos e Resultados: Utilizaram-se ratos SHR, machos e fêmeas com 3 meses, 1 ano e 2 anos (N= 6) de idade. Registrou-se a medida de pressão arterial indireta, através de pletismógrafo. Os animais foram decapitados, os encéfalos congelados e seccionados na região do diencéfalo, (área do PVN) e submetidos à técnica de hibridização *in situ*, expostos ao filme radiográfico e quantificados por análise de imagem. Utilizou-se ANOVA two-way para análise estatística. Apresentam-se os resultados como média $\pm$ erro padrão. Os sistemas de neurotransmissão analisados sob influência do dimorfismo sexual na expressão do RNAm, são: OT: comparamos macho ($0,643 \pm 0,038$) e fêmea ($0,390 \pm 0,072$) de 3 meses, ($p < 0,05$), macho ($0,679 \pm 0,089$) e fêmea ($0,486 \pm 0,088$) de 2 anos ($p < 0,05$); para o NPY macho ($0,073 \pm 0,013$) e fêmea ($0,030 \pm 0,003$) 3 meses, ($p < 0,05$). Sistemas sob influência etária na expressão do RNAm: NPY, ao compararmos fêmeas 3 meses ($0,030 \pm 0,003$) e de 1 ano ($0,076 \pm 0,012$) com as de 2 anos ($0,083 \pm 0,010$), ($p < 0,05$); AVP, ao compararmos fêmeas 3 meses ($0,038 \pm 0,001$) com as de 1 ano ($0,056 \pm 0,005$) e 2 anos ($0,058 \pm 0,004$), ($p < 0,05$). Para TH, não houve influência significativa.

Conclusão: Podemos observar através dos resultados que alguns sistemas envolvidos na regulação cardiovascular sofrem a influência do dimorfismo sexual e da idade o que pode auxiliar na compreensão das alterações de PA entre machos e fêmeas, bem como durante o envelhecimento.

Palavras-Chave: Neurotransmissores, hipertensão, dimorfismo sexual.

Apoio Financeiro: fapesp, CAPES, CNPq

B.01.045

Effects of nicotine on the expression of adenosine A2a receptor mRNA in cultured neurons from medulla oblongata of normotensive and hypertensive rats

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Introduction

The nucleus of the solitary tract (NTS) plays an important role in cardiovascular control. Within the NTS there are several neurotransmitters and neuromodulatory substances, such as adenosine, which acts on purinoreceptors A1, A2a and A3. Stimulation of NTS adenosine A2a receptor (A2aR) may induce a decrease in blood pressure by different mechanisms. Nicotine, that acts in several areas of central nervous system including the NTS, it contributes for the development of hypertension in subjects with genetic predisposition to this disease. The goal of this study was to analyze the modulatory effects of nicotine on the expression of A2aR mRNA in neurons cultured neurons from medulla oblongata of normotensive (WKY) and spontaneously hypertensive rats (SHR).

Methods and Results

Cell cultures were made using the medulla oblongata of one-day old WKY and SHR (n=30). Viable cells were plated and subjected to dose-response and time-course experiments. Cells were treated with 1, 10 and 100 μ M of nicotine for 24 hours in dose-response experiments. For time-course experiments cells were treated with 10 μ M of nicotine for 4, 12, 24 and 48 hours. Total RNA was extracted and Real time RT-PCR was carried out for quantitative analysis of A2aR mRNA. All data were evaluated by two-way analysis of variance. Results were expressed significantly as fold-change $\pm$ standard deviation. Cells from the SHR express more A2aR mRNA (1.75 ± 0.03) as compared to WKY (1.00 ± 0.01) at the control situation. We also observed a decrease in A2aR mRNA expression after nicotine treatment in SHR (at a maximum of 0.68 ± 0.05 , 10 μ M) as compared to SHR own control. WKY showed an increased after 1 μ M of nicotine (1.44 ± 0.04) followed by a decrease at 10 μ M (0.59 ± 0.04) and 100 μ M (0.86 ± 0.03), as compared to its own control without nicotine. The time-course experiments revealed an increased A2aR mRNA at 4 hours after treatment (1.43 ± 0.06) in the WKY, followed by a time-related decrease (maximum of 0.60 ± 0.14 , 48h). On the other hand, A2aR mRNA decreased at 4 and 12 hours, and increased at 24 and 48 hours in SHR after nicotine treatment.

Conclusion

Nicotine might be able to modulate A2aR mRNA expression in both strains, however each strain responds differently to nicotine treatment. It was also observed that A2a mRNA is more expressed in SHR cells when compared to WKY.

Palavras-Chave: Neurotransmission - NTS - A2a receptor - Hypertension

Apoio Financeiro: CAPES, CNPQ e FAPESP

PRESENÇA DE COLINA ACETILTRANSFERASE E NADPH-diaforase NO COMPLEXO PARABRAQUIAL DO SAGUI (*Callithrix jacchus*).

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Objetivos: O complexo parabraquial (PB) é um grupo de neurônios que circunda o pedúnculo cerebelar superior. O PB é dividido em duas principais sub-regiões, a lateral e a medial. Funcionalmente este complexo é considerado o relé de integração das informações sensorial-viscerais. Diversos trabalhos em roedores demonstram uma co-localização de NADPH-diaforase com colina acetiltransferase (ChAT) em células do PB. Estes achados sugerem que a maioria dos axônios e terminais positivos para NADPH-diaforase no tálamo emanam da região parabraquial. Portanto, temos como objetivo identificar células imunorreativas a ChAT, bem como células marcadas com NADPH no complexo PB do sagüi, verificando se há similaridade na distribuição dos terminais em primatas.

Métodos e Resultados: Os animais foram previamente anestesiados e submetidos à perfusão transcardíaca. Os encéfalos passaram por uma microtomia de congelamento obtendo secções de 30µm. Os cortes foram processados por imunohistoquímica contra ChAT e histoquimicamente para revelar a atividade de NADPH-diaforase. Posteriormente as secções foram montadas em lâminas gelatinizadas e os resultados observados em microscópio óptico. Foi possível visualizar células marcadas com NADPH-diaforase e ChAT nas duas sub-regiões do PB. Estas células estão localizadas na região mais medial do PB, no ápice do pedúnculo cerebelar superior.

Conclusão: A marcação de NADPH e ChAT em células da mesma região do complexo PB do sagüi, aponta para uma possível co-localização destas substâncias nesta região, similar ao que é observado em roedores.

Palavras-Chave: Complexo Parabraquial, NADPH-diaforase, Colina acetiltransferase, Sagüi, Imunohistoquímica, Histoquímica

Apoio Financeiro: CNPq, CAPES, PROPESQ-UFRN, FAPESP

B.01.047

Pharmacological activity of tobacco nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and N-nitrosornicotine

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Background: The addictive effect of tobacco has been attributed to nicotine as agonist of nicotinic acetylcholine receptors (nAChR). However, substances whose affinities for some nAChR subtypes are higher than nicotine's affinity were found in tobacco, including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosornicotine (NNN). Actually, many effects attributed to nicotine might be related to these compounds (Life Sciences 80:2274, 2007).

Objectives: The pharmacological activity of NNN and NNK over nAChR subtype $\alpha 3$, $\beta 4$ shall be determined and possible competitive and non-competitive mechanism of inhibition of these compounds shall be studied.

Materials and Methods: Electrophysiological studies were made by using the patch-clamp technique in whole-cell mode with HEK cells transfected with rat $\alpha 3$, $\beta 4$ nAChR subunits. NNK and NNN activity is studied in the presence of nicotine.

Results and Discussion: The plot of nicotine's dose-response curve provided a hill slope of 1.28 ± 0.8 and an EC_{50} of $34 \pm 15 \mu M$. Partial agonists or competitive antagonists are expected to reduce this EC_{50} in a dose-response curve using variable concentrations of nicotine and a constant concentration of one of the nitrosamines, while non-competitive antagonists are expected to reduce maximum response in this situation. Total agonists are expected to not change the properties of nicotine's dose-response curve. It is possible to separate partial agonists and competitive antagonists due to the fact that only partial agonists are able to cause a whole-cell current when applied alone.

Palavras-Chave: nicotinic acetylcholine receptors, N-nitrosornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

Apoio Financeiro: FAPESP

PI3K/PKB and MEK/ERK signaling pathways modulate NCS-1 expression in lymphocytes

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Introduction: Schizophrenia is a severe psychiatric disorder that affects 1% of the world wide. Several studies have shown a decrease in the brain volume and an altered apoptotic signaling pathway in the pre-frontal cortex (PFC) of schizophrenic (SCZ) patients. PKB and ERK are important proteins involved in the cellular survival signaling. It has been demonstrated that lymphocytes of SCZ patients have a decrease in the levels of PKB and in the GSK-3b phosphorylation. Recently, it was shown that a neuronal calcium sensor-1 protein (NCS-1) is a survival factor for neuroendocrine cells and has increased expression levels in the PFC of schizophrenics. However, we have shown that NCS-1 expression in lymphocytes of SCZ patients are reduced compared to controls. Therefore, we sought to investigate whether the survival cellular pathway is involved in the modulation of NCS-1 expression in lymphocytes at first in control subjects. Our next step will consist of repeating this study concerning SCZ patients' cells.

Methodology: With PKB and MEK (PD0956 and UO126) inhibitors we have investigated the participation of MEK/ERK, PI3K/PKB intracellular signaling pathways in the NCS-1 expression from CD4+ T lymphocytes, stimulated in vitro, using flow cytometry.

Results: Data showed a downregulation of NCS-1 expression in lymphocytes in the presence of both MEK and PKB inhibitors, when cells were stimulated. The activation of cells was accompanied by CD69 increased expression.

Conclusion: PI3K/PKB or MEK/ERK modulate and have additive effects in the NCS-1 expression in CD4+ T lymphocytes. Then, these cells could be a good model for NCS-1 studies.

Palavras-Chave: NCS-1, PKB, lymphocytes, schizophrenia, bipolar disorder

Apoio Financeiro: CNPq, FAPEMIG

B.01.049

Relato Clínico e Bioquímico do Primeiro Caso de Deficiência de Tirosina Hidroxilase na Região Norte do Brasil

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Objetivo: Descrever os protocolos de investigação clínico-laboratorial do primeiro caso de deficiência da enzima Tirosina Hidroxilase (TH) da Região Norte do Brasil.

Métodos: Para a realização deste trabalho, inicialmente o paciente passou por uma avaliação clínica; em seguida foram colhidos dados da história familiar, a exemplo de outros casos semelhantes na família, antecedentes gestacionais, bem como, histórico de infecções, convulsões e problemas nutricionais. Como protocolo laboratorial, foi realizada a triagem bioquímica na urina; quantificação de metabólitos por cromatografia líquida de alto desempenho na urina e no sangue (HPLC) e a dosagem de neurotransmissores na urina e líquido cefalorraquidiano (LCR); além da análise molecular para mutações no gene da (TH).

Resultados: A triagem bioquímica na urina revelou resultados normais, no entanto a análise da urina por HPLC constatou aminoacidúria generalizada (excreção elevada de ácido aspártico, serina, histidina, glutamina, glicina, treonina, alanina, arginina, tirosina, valina, fenilalanina, leucina e lisina). A análise no pasma por HPLC não demonstrou alterações. A dosagem na urina de ácido homovalínico (HVA), ácido manilvamílico e noradrenalina apresentaram-se muito baixos e a de 5-hidróxiindol acético (5-HIAA) foi normal. A dosagem de HVA, 3-metoxi-4-hidroxifenilglicol e HIAA no LCR apresentou valores abaixo da normalidade. Finalmente a pesquisa molecular demonstrou a presença das mutações R233H e A241T no éxon 6 do gene da TH.

Conclusões: Os resultados das dosagens de HVA, MPGH, 5-HIAA, VMA e noradrenalina apontaram para um defeito na síntese de TH. A partir desses dados foi realizada a identificação das mutações R233H/A241T no gene que codifica a enzima tirosina hidroxilase. A investigação para deficiência de tirosina hidroxilase seguiu um protocolo baseado na análise clínica e bioquímica o que culminou no diagnóstico correto, proporcionado a utilização de uma terapêutica satisfatória, que melhorou a qualidade de vida do paciente em questão.

Referências: 1-Annals of Neurology **56:65, 2003, 2-Eur J Neurosci.** 580:92, 2008.

Palavras-Chave: deficiencia de tirosina hidroxilase, neurotransmissor, distonia responsiva a dopa

Apoio Financeiro: PIBIC/CNPq, SECTAM, PROEx/UFPa, CAPES.

B.01.050

FoxO1 levels modulated by Aripiprazole in rat brains

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Introduction:

Schizophrenia is a complex genetic disorder characterized by profound disturbances of cognition, emotion and social functioning. The pathophysiology of schizophrenia is still unclear; however, this disease is believed to involve genetic abnormalities in developmental processes leading to abnormal synaptic plasticity. Pharmacological evidence indicates that drugs used in the management of psychosis modulate PI3K/Akt pathway. FoxO1 (forkhead transcription factor member in the O class), which have been shown to be involved in proliferation, cell survival and apoptosis, is a downstream target of Akt. Indeed, as a key signaling intermediate downstream of both dopaminergic and serotonergic receptors, Akt is instrumental in behaviors.

Objectives:

The purpose of our study was to investigate if chronic treatment with typical or atypical antipsychotics in rats induced alterations in FoxO1 expression in prefrontal cortex and hippocampus.

Methods: Male Wistar adult rats were treated with typical (Haloperidol - 1.5 mg/kg) and atypical (Clozapine - 25 mg/kg, Olanzapine - 2.5, 5 or 10 mg/kg or Aripiprazole - 2, 10 or 20 mg/kg) antipsychotics in a 1.0 ml/kg volume for 28 days. Prefrontal cortex and hippocampus extracts were prepared with lysis buffer and samples were submitted to electrophoresis and transferred to a nitrocellulose membrane. Membranes were incubated with antibody anti-FoxO1 (Cell Signaling - L27) and anti-actin (Chemicon - MAB1501R) and examined using chemiluminescent detection. The results were analyzed using the ImageJ software. Differences among experimental groups in experiments evaluating protein expression were determined by one-way ANOVA.

Results: We observed that levels of FoxO1 were dose-dependent decreased in hippocampus of rats treated with Aripiprazole. However, there were no alterations in FoxO1 levels in hippocampus treated with Haloperidol, Clozapine and Olanzapine. Also, levels of FoxO1 were not altered in rat PFC treated with both typical and atypical antipsychotics.

Conclusion: Our results suggest that atypical antipsychotics Aripiprazole may modulate FoxO1 expression.

Palavras-Chave: Schizophrenia, FOXO1, Antipsychotics

Apoio Financeiro: CNPq Universal grant, Programa Institutos do Milênio/CNPq/FINEP and John Simon Guggenheim Foundation

B.02.001

NEURÔNIO AFERENTE QUE RESPONDE AO ESTÍMULO MECÂNICO NO CORDÃO NERVOSO VENTRAL DA MINHOCÁ

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Objetivo: Identificar um neurônio aferente que responde ao estímulo mecânico aplicado em seu epitélio complementando o papel de dois neurônios, M-1 e M-2, identificados no circuito gerador motor do comportamento da retração corporal da minhoca, *Amyntas hawayanus* (Chang, et al, Braz. J. Med. Biol. Res., 31:1285, 1998).

Método: O estímulo mecânico aplicado pelo estilete manual foi usado neste teste porque o estímulo mecânico gerado pelo estimulador eletrônico contem os componentes acústico e eletromagnético, sensíveis também à minhoca. A intensidade do estímulo manual foi medida em mdyne, $\text{grama.cm/s}^2 \times 10^{-6}$, por dois transdutores, TME-ISBM-D e TME-ISBM-D1 (Chang e Ferraz, XXI FeSBE, 49.004, 2006). Este estímulo foi aplicado no epitélio da preparação epitélio-músculo-nervo-cordão, EMNC, e sua resposta foi registrada do neurônio no cordão pelo microeletrodo com Amarelo de Lúifer-CH. O neurônio com a latência mais curta é considerado como mais provável de ser aferente.

Resultado: Uma intensidade limiar de 26.4 ± 9.8 mdyne deste estímulo evocou uma espiga graduada de 2.1 ± 0.5 mV no neurônio-H (Bartoszeck, et al, XV FeSBE, 04.054, 2000) após uma latência de 10.0 ± 3.4 ms (N = 20). A amplitude desta espiga aumentou exponencialmente até 7.4 ± 2.1 mV na intensidade máxima de 75.5 ± 20.1 mdyne. A latência desta resposta diminuiu exponencialmente até 1.7 ± 0.4 ms nesta intensidade máxima.

Conclusão: O neurônio-H fica confirmado como situado no lado aferente do circuito do comportamento de retração corporal, pela proporcionalidade de sua resposta com a intensidade do estímulo e com uma curta latência, enquanto os neurônios M-1 e M-2 são situados no lado eferente deste circuito.

Palavras-Chave: Palavra chave: Neurônio aferente. Estímulo mecânico manual. Amplitude e Latência. Comportamento de retração corporal.

B.02.002

Relationship between the sensory modality and the electrophysiological properties of petrosal ganglion neurons.

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Several studies tried to find a relationship between the electrical properties and the sensory modality of primary afferents neurons, but decisive conclusions were not reached due to the complexity of the sensory ganglia studied. The petrosal ganglion (PG) is a suitable model to test the hypothesis that the electrical properties are related to the sensory modality, because the PG contains the soma of primary afferent neurons with two (chemo or mechano) sensory modalities.

Therefore, we studied the electrophysiological properties of chemo- and mechanosensory PG neurons from adult male New-Zealand White rabbits anaesthetized with ketamine:xylazine (75:7.5 mg/kg). Mechano- and chemosensory neurons were identified if they respond to a mechanical (application of hank's direct to the neuron) or chemical (neurotransmitters) stimuli respectively.

Immunofluorescence studies were made to establish the location of different ionic channels in the PG neurons.

In the whole superfused in vitro PG, we found that neurons can be divided in two groups according to the shape of the action potential (AP): one group of neurons with a “hump” or inflection in the repolarization phase of the AP and a long lasting hyperpolarization, and another group with AP without this inflection and short lasting hyperpolarization.

Patch Clamp recording from PGs primary cultures showed that all the chemosensory neurons had humped AP, while all mechanosensory neurons showed AP without the hump. The hump is associated with the activation a Ca^{2+} current, which activates a Ca^{2+} -dependent K^+ current involved in the generation of the long lasting hyperpolarization.

PG neurons express more than one type of Ca channel and both maxik and SK Ca^{2+} -dependent K^+ .

Present results suggest that the difference in the form of the AP its related with the sensory modality of the PG neurons. **Palavras-Chave:** Petrosal ganglia, sensory neurons, action potential, ionic channels

Apoio Financeiro: FONDECYT 1070584 and 1040638

B.02.003

Nitric oxide mediates inhibition of NMDA receptor currents by pro-NGF in postsynaptic densities transplanted into *Xenopus* oocytes

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Neurotrophins are essential regulators of synaptic function in the adult CNS. We have recently shown that the pan-neurotrophin receptor p75^{NTR} is an integral part of postsynaptic densities (PSDs) at excitatory synapses of the rat forebrain (Sandoval *et al.* 2007). p75^{NTR} inhibits NMDA receptor- (NMDA-R) mediated currents, however, the underlying mechanisms are unknown. To get insight into possible signalling pathways, we micro-transplanted rat forebrain postsynaptic densities (PSDs) into *Xenopus* oocytes following the method described in (Sandoval *et al.* 2007). NMDA receptor currents were elicited in a dose-dependent manner with a K_a of 59.02±8.46 µM. One-minute incubations of oocytes with 150 ng/mL pro-NGF, the ligand we used to selectively activate p75^{NTR} in PSDs, inhibited NMDA-R currents by 44±11%, an effect that depended on p75^{NTR} because it was blocked by the use of a loss-of-function antibody. We hypothesized that nitric oxide (NO), that has been reported to inhibit NMDA-R currents by S-nitrosylation, or alternatively protein phosphatases, might mediate the inhibition. Application of 1µM N^ω-Nitro-L-Arginine (NNA), but not of the phosphatase inhibitor 1 mM NaF reversed the inhibition caused by pro-NGF. Our data suggest that nitric oxide synthase might be involved in p75^{NTR} downstream signalling at excitatory synapses and might therefore modulate synaptic plasticity. **Palavras-Chave:** Postsynaptic densities, p75NTR, NMDA receptor, nitric oxide

Apoio Financeiro: Programa Bicentenario para Ciencia y Tecnologia (Anillo ACT 09-2006)

B.02.004

Caracterización funcional de receptores nicotínicos en neuronas amacrinas de retina de rata.

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En la retina de vertebrados, las neuronas amacrinas modulan las interacciones laterales entre las células bipolares y ganglionares. Los sistemas colinérgicos presentes en estas neuronas participan de esta modulación a través, en parte, de la activación de receptores nicotínicos neuronales (AChRs). Sin embargo la caracterización de los AChRs en las neuronas amacrinas y sus posibles funciones durante el desarrollo de la retina no han sido establecidas.

Objetivos: Identificar funcionalmente subtipos de AChR neuronales en células amacrinas en cultivo y determinar sus efectos sobre el crecimiento de neuritas durante la activación o bloqueo de dichos receptores.

Métodos: Realizamos registros de corrientes macroscópicas en neuronas amacrinas a distintos tiempos de su desarrollo *in vitro*. Identificamos las neuronas por su morfología y por técnicas de microscopia de fase e inmunocitoquímicas utilizando el anticuerpo monoclonal HPC1. Utilizamos microscopia confocal y la sonda fluorescente Fluo-3/AM para evidenciar influjo de Ca^{2+} .

Resultados: Por medio de marcación con α -bungarotoxina (α -BTX) demostramos la presencia del receptor nicotínico $\alpha 7$ en la superficie de células amacrinas. A tiempos tempranos del desarrollo no observamos corrientes activadas por agonistas en las células amacrinas. Una vez que las neuronas alcanzaron un avanzado proceso de diferenciación, observamos dos tipos de respuestas: una lenta, evidenciada por corrientes con constante de decaimiento (t_d) del orden de 70 ms y otra rápida con t_d del orden de 10 ms. Esta última podría corresponder al receptor $\alpha 7$. Debido a que éste es altamente permeable a iones Ca^{2+} , utilizamos Fluo-3/AM para evidenciar su actividad. La activación con nicotina o ACh reveló el ingreso de Ca^{2+} a células amacrinas. El bloqueo con α -BTX inhibió la señal, indicando la presencia de receptores $\alpha 7$ funcionales. El agregado de nicotina a los cultivos neuronales indujo un aumento significativo en el largo de neuritas con respecto a los cultivos control.

Conclusión: Los resultados muestran que las neuronas amacrinas presentan al menos dos subtipos de receptores nicotínicos funcionales sugiriendo un rol de estos receptores durante el establecimiento de la sinaptogénesis en la retina. **Palabras-Chave:** Receptor nicotínico, Patch clamp, Neuronas amacrinas

Apoio Financeiro: UNS, CONICET y ANCyT

B.02.005

La interfase entre el dominio unidor de agonista y el poro iónico de receptores Cys-Loop, gobierna la cinética de apertura y desensibilización del canal.

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Los receptores Cys-loop son canales iónicos activados por neurotransmisores que intervienen en sinapsis químicas. La respuesta sináptica es gobernada por la cinética de apertura, cierre y desensibilización del canal. Los mecanismos moleculares de estos procesos no se conocen todavía.

Objetivo:

Dilucidar la contribución de la interfase entre el dominio extracelular, unidor del agonista, y el dominio transmembranal, a la activación y desensibilización de receptores Cys-Loop.

Métodos:

Se construyeron subunidades quiméricas conteniendo secuencias de alfa-7 y serotonina (5-HT_{3A}), y se evaluó la funcionalidad mediante la técnica de patch-clamp.

Resultados:

Medimos corrientes unitarias y macroscópicas de receptores alfa-7 y 5-HT_{3A}. Las duraciones de apertura de los canales de alfa-7 son breves porque el receptor pasa rápidamente a un estado desensibilizado. Por el contrario, la desensibilización del receptor de 5-HT_{3A} es lenta, y la duración del canal abierto es prolongada. La quimera compuesta por el dominio extracelular de alfa-7 y la región transmembranal de 5-HT_{3A} (alfa7-5HT_{3A}) muestra un comportamiento intermedio. Con el fin de determinar si este comportamiento es debido a que la interfase es estructuralmente heterogénea, generamos, a partir de alfa7-5HT_{3A}, quimeras conteniendo distintas secuencias en regiones de la interfase. Encontramos que cuando la interfase tiene toda la secuencia de 5-HT_{3A}, la quimera posee un tiempo de estado abierto y una velocidad de desensibilización similares a los del receptor 5-HT_{3A} salvaje. Por el contrario, si la interfase tiene secuencia alfa-7, la cinética se aproxima a la del receptor alfa-7. Sustituciones parciales revelaron la existencia de una interdependencia funcional entre las diferentes regiones de la interfase.

Conclusión:

La interacción entre los distintos loops de la interfase no sólo permite el acoplamiento de la unión del agonista con la apertura del canal sino también gobierna la cinética de cierre y desensibilización del receptor, controlando de esta manera la duración de la respuesta sináptica y el período refractario.

Palabras-Chave: Receptor Nicotínico, Receptores Cys-Loop, Patch-clamp, Quimera

Apoio Financeiro: UNS, CONICET, ANCyT

B.02.006

MODULATION OF ADENOSINE UPTAKE AND RELEASE BY INHIBITORS OF ERK 1/2 IN CULTURES OF AVIAN RETINAL CELLS

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Objectives: Adenosine (Ado) is an important modulator of neuronal survival and differentiation in the CNS. Our previous work showed that Ado transporters are present in cultures of chick retinal cells and that activation of Ado A1 receptors promotes an increase in ERK phosphorylation in glial cultures. Our aim in this work was to study the participation of the ERK pathway in Ado uptake and release by different types of retinal cultures.

Methods: The uptake was measured after incubation with (³H)adenosine for 15 minutes and cell lysis to determine intracellular radioactivity. Basal and glutamate-stimulated radioactivity released from cells was determined by liquid scintillation spectroscopy.

Results: Ado uptake involves 2 components, one saturable and one linear, in mixed or glial cultures, but the saturable component is much smaller in glial cultures. In glial cultures, ERK inhibition with PD98059 (25 μ M) reduced the uptake in $37.1 \pm 4.3\%$ (n=4). In mixed cultures, PD98059 (25 μ M) or UO126 (10 μ M), two MEK inhibitors with different chemical structures, reduced the uptake in $39.1 \pm 4.5\%$ (n=5) and $48.6 \pm 2.9\%$ (n=3), respectively. UO124 (10 μ M), an inactive UO126 analog, was much less effective in inhibiting Ado uptake ($17.9 \pm 3.4\%$ vs control, n=3). The analysis of inhibition curves with MEK inhibitors showed that the blockade is observed mainly on the saturable component at both types of culture. Glutamate (Glu) induced the release of purines from mixed cultures ($136.9 \pm 31.0\%$ increase vs control, n=3) and this effect was not significantly inhibited by PD98059 ($79.6 \pm 18.8\%$ increase vs Glu, n=3, p < 0.05).

Conclusions: MEK inhibition dramatically reduces adenosine uptake in mixed neuronal/glial or purified cultures of retinal glial cells, apparently not affecting purine release induced by Glu. Since ERK activity is regulated by stimulation of A1 receptors in the retina, the results suggest that adenosine regulates its own transporter activity via activation of the ERK pathway. **Palavras-Chave:** Adenosine, ERKs, Transporters

Apoio Financeiro: CAPES, CNPq, FAPERJ and PRONEX-MCT

B.02.007

In vitro selection of RNA aptamers as specific inhibitors of P2Y₂ purinergic receptors

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The development of the nervous system is one of the most important morphogenetic events occurring in the embryo. This process is accompanied by cell proliferation and differentiation as well as by tissue organization into a specific architecture. Although the specific molecular pathways that drive these events remain unresolved, it is widely believed that proliferation and differentiation programs of the neural progenitors require the interaction of extrinsic and intrinsic signals. While the function of growth factors in controlling neuronal differentiation is well documented, there also is increasing persuasive evidence for a role of neurotransmitters and their respective receptors in this process. One such neurotransmitter is ATP, whose biological actions are mediated by both ionotropic purinergic receptors (P2X) and G-protein-coupled metabotropic purinergic receptors (P2Y). Based on purinergic receptor pharmacology we have determined the participation of P2Y₂ receptors in the formation of embryonic bodies as prerequisites for phenotype determination of P19 murine embryonal carcinoma progenitor cells. Final neuronal maturation of P19 cells in the presence or absence of purinergic receptors agonists and antagonists implicated the involvement of P2Y₂ receptor subtypes in the determination of the final neuronal phenotype, such as expression of NMDA-glutamate and cholinergic receptors (Resende et al. Neuroscience, 146: 1169, 2007). In order to further evaluate the function of this receptor and in view of the absence of specific inhibitor for this receptor subtype, we have used the SELEX technique (Systematic Evolution of Ligands by Exponential Enrichment) as a RNA-based combinatorial library approach for developing specific inhibitors of P2Y₂ receptors. A protocol for reiterative in vitro selection has been established which allows the enrichment and identification of RNA aptamers with high affinity for human P2Y₂ receptors. **Palavras-Chave:** purinergic receptor P2Y₂, SELEX, P19 embryonal carcinoma cells, neuronal differentiation

Apoio Financeiro: Supported by FAPESP, CNPq and CAPES

Eag1 Potassium Channel Immunohistochemistry in the Nigrostriatal Pathway of 6-Hydroxydopamine Lesioned Rats

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Objectives: Dopamine (DA) mediated effects can include a direct action on potassium (K⁺) channels. The *ether-a-go-go* (Eag) gene is a K⁺ channel derived from a *Drosophila melanogaster* hyperexcitable mutant strain that is almost exclusively expressed in the CNS. The aim of this study was to provide an analysis of Eag1 expression pattern in the rat basal ganglia with or without dopaminergic lesion induced by the neurotoxin 6-hydroxydopamine (6-OHDA).

Methods: Male *Wistar* rats (250 g, n=6-8/group) received unilateral injections of saline or 6-OHDA (3µl, 8µg/µl) into the medial forebrain bundle. Apomorphine (APO)-induced rotational behavior (0.5 mg/kg, s.c.) was evaluated 21 and 28 days after surgery. Rats were sacrificed by decapitation 5 days later, the brains removed, fixed and paraffin embedded. Adjacent coronal sections (5 µm) were stained for tyrosine hydroxylase (TH) and Eag1 immunohistochemistry. Optical and cell density were analyzed in dorsal striatum (ST), accumbens nucleus (Acb), globus pallidus (GP) and substantia nigra compacta (SNc). Results were analyzed by ANOVA followed by the Duncan post-hoc.

Results: Eag1 immunoreactivity was found throughout the striatal nuclei, particularly in ST, Acb, GP and SNc. Complete DA cells loss (evaluated by TH immunoreactivity) was observed in rats presenting intense rotational behavior after APO in the ST, Acb ($F_{(2,18)}=10.046$ and 32550.59 ; $P<0.001$) and SNc ($F_{(2,17)}=1981.22$; $P<0.001$). Similar effects were observed in rats with partial lesions of DA cells (ST: $F_{(2,18)}=32550.59$; $P<0.001$; SNc: $F_{(2,17)}=1981.22$; $P<0.001$). Eag1- and TH- immune-positive cells were co-localized in the SNc but not in the raphe nuclei. Partial and complete DA cell lesions induced reduction (25% and 75%, respectively) of Eag1 labeled cells in the ipsilateral SNc ($F_{(2,17)}=103.64$; $P<0.001$).

Conclusion: The results showed that Eag1 channels are expressed in nuclei of the striatal pathway. Lesions of DA-positive cells were associated with a decrease in Eag1-labeled cells in SNc, which was dependent on the intensity of the lesion. These results indicate that Eag1 channels could be involved in the physiology and pathophysiology of the striatal pathway.

Palavras-Chave: dopamine; 6-hydroxydopamine; substantia nigra; striatum; accumbens nucleus; globus pallidus; potassium channel; Eag1

Apoio Financeiro: CNPq, FAPESP, DAAD/CAPES and FAEPa.

B.02.009

Análise da atividade (Na^+/K^+)-ATPásica durante o desenvolvimento pós-natal da retina de ratos: Efeito do fator de crescimento semelhante à insulina.

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Objetivos: Avaliar os níveis de atividade (Na^+/K^+)-ATPásica no tecido retiniano, durante o desenvolvimento pós-natal de ratos pigmentados. Determinar o papel do fator de crescimento semelhante à insulina (IGF-1) na modulação da atividade desta enzima.

Métodos: Retinas de ratos Lister Hooded de diferentes idades pós-natais (P1, P7 e P14) foram dissecadas em solução salina e homogeneizadas em solução tampão. Após centrifugação (1.500g), o sobrenadante foi utilizado para medir a atividade (Na^+/K^+)-ATPásica, determinada pela diferença entre o Pi liberado na ausência e na presença de 1mM ouabaina. Nos experimentos com IGF, após dissecação, as retinas foram incubadas por 1 hora, em meio de cultura completo, à 37°C, com 10 ng/mL ou 100 ng/mL desta citocina. Após este período procedeu-se a homogeneização.

Resultados: A atividade (Na^+/K^+)-ATPásica em retinas P1 foi de: $62,02 \pm 8,49$ nmoles $\text{Pi} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$. Quando analisamos retinas P7 observamos aumento de aproximadamente 100% na atividade enzimática ($119,53 \pm 3,24$ nmoles $\text{Pi} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$) e em retinas P14, a atividade obtida foi ainda maior, ($178,48 \pm 11,55$ nmoles $\text{Pi} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$) correspondendo à aproximadamente 200% em relação a P1. Quando retinas de animais P7 foram incubadas com 10 ng/mL de IGF-1 houve um aumento da atividade em cerca de 86% em relação ao controle. Um aumento menor, mas significativo, de 51% foi observado quando da incubação com 100 ng/mL de IGF-1.

Conclusões: Nossos resultados sugerem que o aumento da atividade (Na^+/K^+)-ATPase observado até o 14º dia, período em que usualmente ocorre a abertura dos olhos, pode estar correlacionado com a diferenciação do circuito retiniano. Os resultados obtidos com IGF-1 indicam que este fator possa desempenhar um importante papel na regulação da atividade desta enzima ao longo do desenvolvimento pós-natal.

Palavras-Chave: (Na^+/K^+)-ATPase, desenvolvimento, retina, IGF-1

Apoio Financeiro: CNPq e FAPERJ

B.02.010

Nicotinic Acetylcholine Receptor is Internalized via a Rac-dependent, Dynamin-Independent Endocytic Pathway

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Objectives. Endocytosis of the nicotinic acetylcholine receptor (AChR) is a proposed mechanism of neuromodulation at neuromuscular junctions and in the central nervous system. Here we characterize muscle-type AChR internalization in mammalian cells.

Methods. To label AChR we used fluorescence derivatives of the competitive antagonist α -bungarotoxin (α BTX) or monoclonal antibodies against the α subunit. Cells were imaged using a combination of wide-field, confocal, and TIRF microscopies.

Results. Binding of α BTX or antibody-mediated crosslinking induces the internalization of cell-surface AChR to late endosomes when expressed heterologously in CHO cells or endogenously in C2C12 myocytes. Internalization occurs via sequestration of AChR- α BTX complexes in narrow, tubular, surface-connected compartments, as shown by differential surface-accessibility of fluorescently-tagged α BTX-AChR complexes to small and large molecules, and real-time TIRF imaging. Internalization occurs in the absence of clathrin, caveolin or dynamin, but requires actin-polymerization. α BTX-binding triggers c-Src phosphorylation, and subsequently activates the Rho GTPase Rac1. Consequently, inhibition of c-Src kinase activity, Rac1 activity or actin polymerization inhibits internalization via this unusual endocytic mechanism.

Conclusions. AChR is internalized via a novel, Rac-dependent, dynamin-independent endocytic pathway. This pathway may regulate AChR levels at ligand-gated synapses.

Palavras-Chave: AChR endocytosis bungarotoxin

Apoio Financeiro: CONICET ANPCyT

B.02.011

A distinct site of action at the nicotinic acetylcholine receptor disclosed by the anticonvulsive drug Lamotrigine.

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Recent studies from our laboratory showed that Lamotrigine (LTG), in the presence of acetylcholine, behaves as a typical muscle nicotinic acetylcholine receptor (AChR) open-channel blocker (1).

Objectives: In the present work we studied whether LTG alone had any effect over the AChR.

Methods: single-channel and whole-cell patch-clamp recordings; fluorescence microscopy; ligand binding assays; fluorimetric measurements.

Results: LTG was found to activate the AChR. Single-channel patch-clamp recordings showed that the application of LTG (0.05-100 μ M) alone was able to open AChR channels by itself. [¹²⁵I] α -bungarotoxin binding studies further indicated that LTG does not bind to the canonical ACh binding site. Moreover, fluorescence experiments using the probe crystal violet demonstrated that LTG induces the transition from the resting state to the desensitized state of the AChR in the presence of α -bungarotoxin, that is, when the agonist site is blocked. Allosterically-potentiating ligands or an open-channel blocker exhibited a behavior different from that of LTG.

Conclusion: LTG activates the AChR through a site that is different from that of the agonist/competitive antagonists and that of allosterically-potentiating ligands.

References: 1- Neuroreport 18: 45, 2007.

Palavras-Chave: AChR, Lamotrigine, Agonist, APL

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Expression Patterns of Kinin-B2, Muscarinic Acetylcholine and Purinergic P2X Receptors during Mouse Encephalic Development

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A previous study of our laboratory has demonstrated the crucial role of kinin-B2 receptors in neuronal differentiation using P19 embryonic carcinoma cells as *in vitro* model (Martins et al. J. Biol. Chem. 280:19576, 2005). This was observed because the gene and protein expression of kinin-B2 expression increased together with bradykinin secretion into the culture medium after induction of neuronal differentiation. Moreover, selective B2 receptor inhibition with HOE-140 compromised terminal differentiation of the P19 cells to neurons, decreasing the expression and functionality of the muscarinic acetylcholine receptors and indicating a probable cross-talking among these receptors during the neuronal differentiation process. Furthermore, work of our group has shown the participation and the differential expression of several types of metabotropic (muscarinic, kinin-B2 and purinergic P2Y) and ionotropic (nicotinic and purinergic P2X) receptors in neuronal differentiation *in vitro* (Resende *et al.* Exp. Cell Research 314:1429, 2008; Resende *et al.* submitted). We now study the mouse encephalic development as a complementary model in order to verify whether the results obtained for *in vitro* differentiation reflect processes encountered during neuronal development. We have used *in situ* hybridization and RT-PCR techniques for studying gene expression during mouse brain development. We report the differential expression of M2-M3 muscarinic, P2X2-P2X6 purinergic and kinin-B2 receptors in critical periods of the developing embryo and in the adult mouse. The *in situ* hybridization experiments provide us with a spatial and temporal picture of this process.

Palavras-Chave: bradykinin receptors, muscarinic cholinergic receptors, purinergic receptors, *in situ* hybridization, brain development

Apoio Financeiro: Acknowledgments: This work is supported by FAPESP, CAPES and CNPq.

B.02.013

Interneurons and medium spiny neurons in the nucleus accumbens of the rat express distinct patterns of AMPA-type receptor subunits

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Objectives:

The nucleus accumbens (Acb) is a key structure of the reward system and crucially involved in different aspects of adaptive and emotional behaviors. The great majority of neurons in the Acb are medium spiny projection neurons (MSNs) that receive converging dopaminergic and glutamatergic inputs and are modulated by different types of interneurons. There is little information which AMPA-type receptor subunits are expressed by MSNs and interneurons of the Acb. For this purpose we investigated the distribution of all AMPA-type receptor subunits in the Acb and their co-localization with the neuronal phosphoprotein DARPP-32, which is expressed in all MSNs expressing dopamine D1 or D2 receptors, as well as with markers selectively expressed in interneurons such as parvalbumin and Chat.

Methods:

Adult Wistar rats (n= 40) were perfused with 4% paraformaldehyde and brains were cut on a freezing microtome. Coronal sections through the Acb were either incubated with antibodies to the AMPA-type receptor subunits GluR1, GluR2, GluR2/3, and GluR4 alone, or together with antibodies to DARPP-32, parvalbumin, Chat and the neuronal marker NeuN. Standard immunoperoxidase or fluorescence techniques were used for visualization.

Results:

The GluR2/3 and GluR2 subunits showed by far the strongest expression and were expressed in all DARPP-32+ neurons. Double-staining with NeuN revealed that the GluR2 subunit was expressed in almost all neurons of the Acb, except in cholinergic Chat+ interneurons. In contrast, the GluR1 and GluR4 subunits were only weakly expressed in the Acb and often co-localized with parvalbumin. All markers investigated showed a similar distribution in the core and shell of the Acb.

Conclusions:

Results indicate that 1.) all MSNs receive converging glutamatergic and dopaminergic inputs; 2.) the GluR2 subunit might play a prominent role in mediating dopamine – glutamate interactions; and 3.) MSNs and interneurons in the Acb express distinct patterns of AMPA-type subunits, with the GluR1 and GluR4 subunits exclusively expressed in interneurons. **Palavras-Chave:** Dopamine, Glutamate, GABA, reward, ventral striatum

Apoio Financeiro: FAPESP, CAPES

B.02.014

High and Low rearing rats selected in the open field differ in the binding of [³H]Flunitrazepam to the hippocampus

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Objective: High and low rearing rats differ in the susceptibility to clonic convulsions induced by methyl-6,7-dimethoxy-4-ethyl-b-carboline-3-carboxamide (DMCM), a benzodiazepine inverse agonist. The aim of this study was to verify if Low (LR) and High (HR) rearing subgroups differ in the binding of [³H]Flunitrazepam ([³H]FNT), a benzodiazepine agonist, to the hippocampus.

Materials and Methods: Naive adults (3 months old), male Wistar rats were submitted to a 3-min session in the open field test. Ambulation and the total number of rearings were scored. Those rats showing ≤ 19 rearings were selected as the LR (14.3 ± 0.63 , $n=29$; mean $\pm$ S.E.M) responders and those showed ≥ 28 rearings were selected as the HR (34.8 ± 0.85 , $n=27$; mean $\pm$ S.E.M.) rats. Twenty days after the open field session, the rats were sacrificed by decapitation, the hippocampus was dissected, weighed and kept frozen (-20°C) until the preparation of the homogenates. The homogenates (2.5% W/V) were prepared in 0.32 M sucrose. Saturation curves of [³H]FNT binding were carried out using 8 concentrations of the ligand (0.4-13.5 nM). Non-specific binding was determined in the presence of DMCM (10 μM concentration in the assay). The data was statistically analysed using the unpaired Student's t test with a significance level set at $p \leq 0.05$, two-tailed.

Results: A statistically significant difference between LR and HR subgroups in the affinity constant (K_d) was observed (LR: 3.9 ± 0.3 nM; $n=8$; mean $\pm$ S.E.M.; HR: 5.8 ± 0.3 nM; $n=7$; $t=4.0$, $df=13$, $p=0.001$). There was also a significant difference between the subgroups in the density (B_{max}) of [³H]FNT binding in the hippocampus (LR: 750.0 ± 45.3 fmol/mg protein; $n=8$; mean $\pm$ S.E.M.; HR: 929.4 ± 54.1 fmol/mg protein; $n=7$; $t=2.5$, $df=13$, $p=0.028$).

Conclusions: Our data suggest that the innate inter-individual differences in exploratory behavior is influenced by GABA neurotransmission through the GABA_A/BZD site receptor and that this difference may be due to the existence of differences in the expression of GABA_A/BZD site receptor subtypes in the hippocampus of HR and LR subgroups of rats.

Palavras-Chave: rearing; open-field test, GABA_A, BZD

Apoio Financeiro: FAPESP, AFIP

RAPID EYE MOVEMENT SLEEP DEPRIVATION (REMSD) INDUCED CHANGES IN Na^+/K^+ -ADENOSINETRIPHOSPHATASE (Na^+/K^+ -ATPase) ACTIVITY IN THE RAT BRAIN REGIONS
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Objective: The REMSD produces alteration in brain excitability (Physiol. Behav. 7: 103-106, 1971). The Na^+/K^+ -ATPase has an important role in the neuronal excitability (Amer. J. Physiol. 279: C541-C566, 2000). The objective of this work was to determine if the REMSD induces alteration in the K^+ -p-NPPase activity, the reaction of dephosphorylation of phosphorylated Na^+/K^+ -ATPase, in discrete regions of the brain.

Results: Adult, male, Wistar rats were deprived of REM sleep for 96 h by the flower-pot technique. A home-cage and a large platform group control were also used. The enzyme activity was determined in homogenates (Brain Research, 1058: 178-182, 2005). The results showed an increase in the K^+ -p-NPPase activity in the Striatum; Hippocampus and Brainstem. Statistical analysis: One-way ANOVA, followed by Bonferroni's multiple comparison Test, $p < 0,05$, two tailed.

Na^+/K^+ -ATPase (nmol p-nitrophenol/mg protein/min) means $\pm$ S.E.M.

Brain Region	GROUPS		
	Control	Large Platform	REMSD
Frontal cortex	82,2 $\pm$ 6,3, (7)	81,8 $\pm$ 3,2 (8)	85,4 $\pm$ 6,5 (8)
Striatum	67,7 $\pm$ 6,3, (6)	90,5 $\pm$ 6,9 * (8)	99,5 $\pm$ 4,1* (8)
Hippocampus	83,6 $\pm$ 8,7 (8)	106,9 $\pm$ 7,2 (8)	116,2 $\pm$ 3,7* (8)
Limbic cortex	64,2 $\pm$ 4,0 (7)	62,9 $\pm$ 4,5 (7)	62,2 $\pm$ 3,9 (8)
Brainstem	112,3 $\pm$ 2,8 (5)	109,0 $\pm$ 4,1 (8)	134,4 $\pm$ 6,1* (8)

* Differ from control group, $p < 0,05$. () = number of rats.

• Differ from Large Platform, $p < 0,01$.

Conclusion: The pattern of changes in K^+ -p-NPPase may be involved in the differences induced by REMSD in the brain excitability.

Palavras-Chave: SLEEP DEPRIVATION, Na^+/K^+ -ATPase

Apoio Financeiro: AFIP

B.02.016

Type-2 corticotropin releasing hormone receptors are localized in glutamatergic subcortical afferences to the ventral tegmental area

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Recently, it has been shown that cocaine experience determines a corticotrophin releasing hormone (CRH)-mediated sensitization of glutamate release in the ventral tegmental area (VTA) (Wang et al, J. Neurosci. 25:5389, 2005). This CRH-mediated sensitization of VTA glutamate release is due to the activation of type-2 CRH receptors (Wang et al, Psychopharmacology 193:283, 2007). The present study was undertaken to determine whether type-2 CRH receptors are localized in VTA glutamatergic terminals. To this end, we prepared VTA synaptosomes with low postsynaptic density material and performed double immunofluorescence. Antibodies against vesicular glutamate transporters type 1 and 2 (vGluT1 and vGluT2) were used to identify cortical and subcortical VTA glutamatergic terminals, respectively. Specific antibodies against type-2 CRH receptors allowed studying their presynaptic localization. As expected, the results show that vGluT1 and vGluT2 antibodies labeled different VTA synaptosomes, and qualitative analysis indicates that the majority of VTA synaptosomes are vGluT2-positive. Double immunofluorescence shows that type-2 CRH receptors are co-localized in vGluT2 containing VTA synaptosomes but not with vGluT1 containing synaptosomes. These results indicate that type-2 CRH receptors are presynaptically localized in VTA glutamatergic afferences of subcortical origin. It is tempting to suggest that modifications in the number or in the signaling properties of type-2 CRH receptors localized in subcortical VTA glutamatergic terminals could explain the sensitization of glutamate release observed after chronic cocaine.

Palavras-Chave: Receptor, VTA, Glutamatergic afferences

Apoio Financeiro: Funded by MSI grant N° P06/008-F and FONDECYT 1070340

B.02.017

Subgroups of rats with high and low threshold to clonic convulsion induced by methyl 6,7-dimethoxy-4-ethyl- β -carboline-3-carboxylate (DMCM), a benzodiazepine inverse agonist, differ in [^{3} H]-L-Glutamate binding in brain regions

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Objectives: Glutamate is considered the main excitatory neurotransmitter in the brain. This study sought to verify if the difference in the susceptibility to clonic convulsion induced by DMCM is related to the glutamate receptor in rats.

Methods: Adult, male Wistar rats received two intraperitoneal injections (one-week interval between them) of a clonic convulsant dose (CD_{50}) of DMCM (0,63 mg/kg). Following this procedure two subgroups were formed: rats susceptible to convulsion (SC, N=8), which presented clonic convulsion in both the administrations, and rats non susceptible to convulsion (NSC, N=8), which did not manifest any sign of motor disturbance in both administrations of the drug. Twenty-five days after the second injection of DMCM the rats were sacrificed, the brain regions were homogenized and [^{3} H]-L-Glutamate binding assays to the brain structures were carried out in the presence of 50 nM (Pharmacol. Biochem. Behav. 67:169, 2000). The incubation was terminated by a rapid vacuum filtration and the radioactivity trapped in the filters was determined by liquid scintillation.

Results: The results, expressed in mean $\pm$ SEM of fmol of [^{3} H]-L-glutamate specific bound/mg protein, were: hippocampus (SC: $1353 \pm 97,8$, NSC: $1862 \pm 161,5$; $p=0.01$, unpaired Student's t test); frontal cortex (SC: 1305.0 ± 107.40 ; NSC: 1741.0 ± 152.60 ; $p=0,03$); limbic cortex (SC: 1755.0 ± 88.70 ; NSC: 2375.0 ± 180.90 ; $p=0,008$), and striatum (SC: 1322.0 ± 114.90 ; NSC: 1299.0 ± 146.20 ; $p>0,05$).

Conclusion: The results obtained in this study suggest that the threshold to clonic convulsion induced by DMCM is related to glutamatergic receptors in hippocampus, limbic cortex and frontal cortex, but not in striatum.

Palavras-Chave: DMCM, binding, glutamate, rats, benzodiazepine inverse agonist

Apoio Financeiro: AFIP and Capes

B.02.018

Neurochemical identity governs cholinergic phenotype across hippocampal basket cell networks.

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Objectives: To test the hypothesis that cholinergic modulation of hippocampal basket cells (BCs) depends on their neurochemical phenotype. **Methods:** Combining whole-cell recordings, single cell PCR (scPCR), morphological identification, and visual targeting of GFP in parvalbumin (PV) or GAD65-expressing interneurons, we determined mAChR (M1R-M5R) mRNA expression profiles in electrophysiologically, neurochemically and morphologically identified BCs. **Results:** Bath application of muscarine (10 μ M) showed that non-fast spiking GAD65-BCs underwent a transient hyperpolarization while fast-spiking PV-BCs underwent depolarization. In response to depolarizing current steps, mAChR activation increased firing frequency in both GAD65- and PV-BCs, but a larger mAChR-induced afterdepolarization (mADP) was elicited in GAD65-BCs (7.2 ± 2.5 mV, n=11) than in PV-BCs (0.9 ± 0.7 mV, n=9, p=0.0007). To determine whether cholinergic neuromodulation tunes GAD65- and PV-BCs differently, we systematically varied the frequency (1-100Hz) and number of action potentials (APs; 1-100 at 20Hz) elicited in both BC subtypes. In GAD65-BCs, the mAChR-induced ADP (mADP) was largest at 20Hz and was generated with as few as 10APs (5.7 ± 2.1 mV, n=6, p=0.02). In contrast, the mADP of PV-BCs exhibited no frequency or AP preference. scPCR revealed CCK mRNA present in all mADP-containing GAD65-BCs. In contrast, in all PV-BCs, PV but not CCK mRNA was present. An analysis of mAChR mRNA indicated that M1R mRNA was present in all both PV-BCs and CCK-BCs. Telenzepine, an M1R antagonist, prevented the mADP in both CCK and PV-BCs, confirming the importance of M1Rs. However, mAChR-induced increase in firing frequency in CCK-BCs was not prevented by telenzepine (27 ± 3 to 39 ± 4 , p=0.008, n=10), indicating non-M1Rs present. Indeed, M3R mRNA was expressed in higher relative abundance in CCK-BCs than in PV-GFP cells. **Conclusions:** PV and CCK BCs undergo mAChR modulation differently. M1Rs emerge as critically involved in both BCs responses, yet M3Rs also appear important in CCK-BC modulation. Therefore, the specific cholinergic specializations of hippocampal BCs relate directly to their neurochemical identities.

Palavras-Chave: Interneurons, Acetylcholine, Neuromodulation, Afterdepolarization

Apoio Financeiro: This work was supported by an NICHD Intramural research award to CMcB

B.02.019

Beyond endocytosis: LRP-1 function in Müller glial cell migration.

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Purpose: Müller cells (MC) are known to undergo functional and morphological changes and to produce matrix metalloproteinases (MMPs) during retinal proliferative diseases. Alpha 2- macroglobulin (α_2 -M) receptor (LRP-1) is a large endocytic receptor expressed in most cell types. In previous studies we have demonstrated that MC express LRP-1 and its ligand, α_2 -M, is able to induce MMP-2 activity. However, the biochemical mechanism by which α_2 -M/LRP-1 system may control this activity is unclear, which could involve both endocytic and intracellular pathways activation. Herein we investigate the putative mechanisms of α_2 -M/LRP-1 system on the MMPs regulation and cell migration in a Müller cell line.

Methods: The human MIO-M1 was cultured in the absence or presence of 60 nM α_2 -M for different time periods. The effect of α_2 -M on MMPs activity was evaluated in MC supernatants by zymography. The expression level of MT1-MMP was analyzed by RT-PCR, Western blot (WB) and immunofluorescence (IF) analysis. MMP-2 and TIMP-2 expression were also examined by RT-PCR. Cell migration was evaluated on collagen or laminin-coated surface using time-lapse video microscopy. The α_2 -M induced signalling pathways were evaluated by WB. Cell binding and internalization of α_2 -M were evaluated by flow cytometry using anti LRP-1:RPE and Alexa fluor 488-conjugated α_2 -M.

Results: By zymography we observed that α_2 -M increased MMP-2 activity whereas MMP-9 activity was not modified. α_2 -M also increased MT1-MMP and TIMP-2 mRNA and MT1-MMP protein, which was associated with the MMP-2 activity. Under α_2 -M induction MT1-MMP was localized at the cell surface of MC. This MMPs regulation was also accompanied by an increase of cell motility on coated surfaces. Finally, using pharmacological inhibitors of signaling pathways and endocytosis we observed that MMPs regulation and MIO-M1 cell migration were dependent on the intracellular signal activation induced by the α_2 -M/LRP-1 system.

Conclusion: The present study demonstrates that the α_2 -M/LRP-1 system regulates the proteolytic activity of MMP-2 in MIO-M1 cells by inducing the MT1-MMP and TIMP-2 expression, suggesting that MAPK and PKB pathways are involved.

Palavras-Chave: Müller cells, LRP-1, α_2 -M, endocytosis, migration, intracellular signaling pathways

Apoio Financeiro: FONCyT, CONICET and SECyT-UNC.

B.02.020

VIAS DEPENDENTES DE CÁLCIO NOS EFEITOS DO CLORETO DE METILMERCÚRIO EM CULTURAS DE CÉLULAS GRANULARES CEREBELARES

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Objetivos: Investigar o papel do Ca^{2+} na toxicidade induzida por cloreto de metilmercúrio ($\text{CH}_3\text{-Hg}$) em culturas cerebelares primárias de cerebelo de ratos.

Métodos: Toxicidade aguda associada à exposição ao $\text{CH}_3\text{-Hg}$ foi avaliada em culturas primárias cerebelares de ratos neonatos Wistar (P5-P7), através do ensaio de redução do MTT (J Immunol Methods 65:55, 1983). A viabilidade celular foi determinada como percentual da atividade dos poços-controle. As culturas foram tratadas no 8º dia por 12 h, 24 h, 3 e 5 dias, nas concentrações de 10 nM, 100 nM, 1-10 μM . Vias dependentes de Ca^{2+} foram investigadas com exposição concomitante a nifedipina (100 nM, 1 e 10 μM) e vermelho de rutênio (1, 3 e 5 μM), por 24 horas.

Resultados: Comparadas ao controle, o efeito do $\text{CH}_3\text{-Hg}$ resultou em marcante perda neuronal dependente de dose e de tempo de exposição. Por exemplo, com exposição por 12 h houve perda celular significativa somente a partir de 1 μM ($10 \pm 4,0\%$) até um máximo com a utilização de 10 μM ($57,6 \pm 4,1\%$). Exposição por 24 h promoveu perda celular significativa desde a dose de 100 nM ($6,1 \pm 1,7\%$) até um máximo de perda celular ($63,2 \pm 2,1\%$) com a dose de 10 μM . Exposição por 3 dias resultou em perdas celulares em todas as concentrações testadas, desde 10 nM ($9,6 \pm 2,5\%$) até 10 μM ($96 \pm 0,8\%$), assim como na exposição por 5 dias, com perda celular de 9 ($\pm 3,6\%$) a 96,5 ($\pm 0,1\%$) para exposição às concentrações mínima (10 nM) e máxima (10 μM). Concorrendo para estes efeitos, parece haver influência de cálcio extracelular, pois a utilização de nifedipina (100 nM) promoveu redução da perda celular associada a exposição ao $\text{CH}_3\text{-Hg}$ (4 μM por 24 h) de $26 \pm 2,5\%$ para $13,6 \pm 1,4\%$. Não foram observadas alterações nos valores de perda celular com a utilização de vermelho de rutênio.

Conclusão: Os efeitos da exposição ao $\text{CH}_3\text{-Hg}$ sobre células granulares cerebelares em cultura mostraram-se dependentes de dose e de tempo de exposição, sugerindo alterações na homeostasia do cálcio, principalmente de canais dependentes de voltagem do tipo-L.

Palavras-Chave: células granulares, cerebelo, mercúrio, cálcio.

Apoio Financeiro: CAPES, SEDECT (PA).

B.02.021

PACAP Aumenta a Eficiência de Captação de D-Aspartato em Explantes Retinianos

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D e L-Aspartato (D e L-ASP) são aminoácidos excitatórios (AAE) envolvidos na sinalização neuronal. Na retina de galinha, o ASP foi descrito como um agonista seletivo dos receptores NMDA. Embora o ASP esteja presente na retina de galinha e participe da sua sinalização neuroquímica pouco se sabe sobre os processos envolvidos nessa sinalização. O PACAP é um neuropeptídeo que foi descrito pela capacidade de promover o acúmulo de AMPc e atua através de receptores próprios (PAC1) Nesse trabalho estudamos a captação de D-ASP e possíveis mecanismos regulatórios desta captação. Experimentos realizados com explantes de retinas de galinhas no 13º dia embrionário (E13) e animais pós-eclosão demonstraram que a captação de D-ASP é 5 vezes maior em retinas embrionárias quando comparadas ao tecido diferenciado. A captação de D-ASP é dependente de Na⁺ e abolida em baixas temperaturas. Experimentos realizadas com culturas de células sugerem que os neurônios são os principais tipos celulares envolvidos na captação de D-ASP. Tratamento de retinas de E13 por 15 horas com o neuropeptídeo PACAP 38 (10 hM) levaram a um aumento de 50% na capacidade de captação de D-ASP e esse efeito do peptídeo foi bloqueado na presença de PACAP 6-38 (100 hM), um antagonista dos receptores de PACAP. Nossos dados sugerem que PACAP é um importante agente regulatório da homeostasia de AAE no sistema nervoso central. Portanto, importante em manter a higidez do tecido nervoso protegendo-o da excitotoxicidade **Palavras-Chave:** D-Aspartato, PACAP, Retina, Captação

Apoio Financeiro: CNPq, FAPERJ e PRONEX

MODULATION OF MITOCHONDRIAL PERMEABILITY TRANSITION PORE BY METALS CATIONS

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Objectives: Mitochondrial Ca^{2+} overload results in the dramatic increase of the permeability of mitochondrial (Mit) inner membrane due to the activation of permeability transition pore (PTP). The latter leads to Mit swelling, developing of low energy state, inhibition of oxidative phosphorylation. This results in the Ca^{2+} efflux from Mit following by cell damage and death. However the mechanism of Mit swelling and modulation of this process by different ions and substances are unknown. The aim of this study was to find out the kinetic characteristics of the Ca^{2+} - and Cd^{2+} -induced Mit swelling and possibilities to modulate this process.

Methods: The experiments were performed on isolated Mit obtained by differential centrifugation. Mit swelling was measured using spectrophotometric method.

Results: We showed that increase of extra-Mit concentration of Ca^{2+} and Cd^{2+} (5-100 and 1-50 mM respectively) caused decrease in the initial slow phase (lag time) of the Ca^{2+} - and Cd^{2+} -induced Mit swelling; did not induce significant changes in the amplitude of Ca^{2+} -stimulated induced Mit swelling but evoked decrease Cd^{2+} -induced stimulated Mit swelling. The rate of Mit swelling significantly increased upon the rise of extra-Mit Ca^{2+} concentration. The dependence of the rate of Mit swelling on $[\text{Ca}^{2+}]_e$ was power ($\text{EC}_{50} = 19,85 \mu\text{M}$, $V_{\max} = 0,609 \text{ A}_{540}/\text{min}\cdot\text{mg protein}$). The rate of Cd^{2+} -induced Mit swelling was also significantly increased upon the rise of extra-Mit Cd^{2+} concentration, but this dependence was sigmoidal ($\text{EC}_{50} = 7,28 \mu\text{M}$, $V_{\max} = 1,084 \text{ A}_{540}/\text{min}\cdot\text{mg protein}$).

We also found pronounced inhibitory effect of immunosuppressant cyclosporin A (10 μM) on Ca^{2+} -induced Mit swelling and its inability to influence Cd^{2+} -stimulated Mit swelling.

Conclusion: The observed differences between the kinetic of Ca^{2+} - and Cd^{2+} -induced Mit swelling and opposite effect of cyclosporin A on these processes allowed us to suggest that distinctive mechanisms are involved in the interaction of Ca^{2+} and Cd^{2+} with inner Mit membrane during the PTP activation.

Palavras-Chave: mitochondria, permeability transition pore, cell death

B.02.023

Expression of the mannose receptor (MR) in olfactory ensheathing glia *in vitro* and *in vivo*

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The mannose receptor (MR) is a transmembrane glycoprotein, included in the family of pattern recognition receptors (PRRs), of basic importance in the organism's defense. MR is a putative link between innate and adaptive immune responses. MR is expressed in microglia, astrocytes, and Schwann cells, but not in adult or developing oligodendrocytes. Furthermore, there is no information on MR in olfactory ensheathing glia (OEG), a unique glial type that crosses the boundary between peripheral and central nervous system (CNS) and may have a significant role in the regrowth of CNS axons.

We show that *in situ* OEG (olfactory fibers layer – OFL – of the olfactory bulb – OB) of perfusion-fixed adult rats binds a highly mannosylated neoglycoprotein – mannosyl/bovine albumin/-fluorescein isothiocyanate (Man/BSA-FITC) – in a very specific manner. Incubation with Man/BSA-FITC and flow cytometric analysis show that 71% of OFL recently isolated cells, also immunoreactive for 2'3' cyclic nucleotide 3'-phosphodiesterase (CNPase, marker of oligodendrocytes, mature Schwann cells and OEG), internalize Man/BSA-FITC. OEG in OFL or from OB isolated cell cultures reacts with a polyclonal antibody against the c-terminal domain of the macrophage MR (anti-cMR). In OB, there is partial colocalization of cRM and CNPase, particularly in perinuclear OEG areas.

Protein extracts of total or superficial OB (mostly OFL) separated by electrophoresis and immunoblotted with polyclonal anti-cRM or K1K2 (against MR whole molecule) show a single 180kDa band. Our results suggest that OEG expresses a MR-like protein, similar to that of macrophages, in a potentially functional state in infectious/ inflammatory states and/or in tissue homeostasis. This expressions is of possible relevance in studies of regeneration of the nervous system.

Financial support: CNPq, FAPERJ, SR2/UFRJ

AUSÊNCIA DE EFEITOS NEUROPROTETORES DO 17 β -ESTRADIOL SOBRE OS EFEITOS NEUROTÓXICOS DO CH₃-Hg EM CULTURAS PRIMÁRIAS DE HIPOCAMPO

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Objetivos: Investigar efeitos do pré-tratamento com 17 β -estradiol (E2) sobre os efeitos neurotóxicos induzidos por CH₃-Hg em culturas primárias de hipocampo de ratos neonatos (P4).

Métodos: A toxicidade aguda associada à exposição ao CH₃-Hg foi avaliada em culturas primárias de hipocampo de ratos neonatos Wistar, através do ensaio de redução do MTT (J Immunol Methods, 65:55, 1983). A viabilidade celular foi determinada como percentual da atividade dos poços-controle. As culturas mistas foram tratadas no 8º dia por 24 h, 3 e 5 dias, nas concentrações de 1-30 uM. Foi realizado pré-tratamento com E2 (10 e 100 nM) no 7º dia de cultura e, a partir de então, associado ao CH₃-Hg.

Resultados: Comparadas ao controle, o efeito do CH₃-Hg resultou em perda de viabilidade celular dependente de dose e de tempo de exposição. Culturas mistas expostas a CH₃-Hg (1-30 uM) por 24 horas sofreram redução significativa na viabilidade celular apenas a partir de 5 uM (7,8 $\pm$ 3,3%) até a máxima concentração de 30 uM (84,3 $\pm$ 4,3%), enquanto que a exposição por três dias mostrou perda celular desde a concentração de 1 uM (9,1 $\pm$ 1,8%), até 30 uM (99,0 $\pm$ 0,2%). Após 5 dias de exposição, também houve redução significativa em todas as concentrações testadas, desde a concentração mínima (9,2 $\pm$ 2,16%) até a máxima concentração testada (98,5 $\pm$ 0,04%). Análises iniciais do efeito da administração de E2 (10 e 100 nM, 3 dias) não mostraram alteração significativa no efeito neurotóxico do mercúrio.

Conclusões: Confirmando os achados da literatura, os efeitos da exposição ao CH₃-Hg em culturas primárias de hipocampo de ratos neonatos mostraram-se dependentes de dose e de tempo de exposição. Resultados preliminares apontam para ausência de efeitos neuroprotetores do estradiol neste modelo *in vitro* de exposição ao mercúrio orgânico. **Palavras-Chave:** Culturas primárias, hipocampo, mercúrio, estrógeno.

Apoio Financeiro: CAPES, SEDECT (PA).

B.02.025

CAPTAÇÃO E LIBERAÇÃO DE ASCORBATO EM CULTURAS MISTAS DE RETINA: MODULAÇÃO POR RECEPTORES IONOTRÓPICOS DE GLUTAMATO E DEPENDÊNCIA DO SVCT-2

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Objetivos: No SNC, Ascorbato (Asc) é captado principalmente através do SVCT-2, que transporta seletivamente Asc de maneira dependente de Na⁺. Nesse trabalho estudamos os processos de captação de Asc e a modulação de sua liberação por glutamato em culturas de retina.

Métodos: Culturas mistas de retina de embrião de pinto foram utilizadas para captação ou liberação de [¹⁴C]Asc. Na captação, culturas foram pré-incubadas por 10 minutos com diferentes drogas, incubadas com 0,3µCi/ml de [¹⁴C]Asc por 40 minutos e lisadas para determinação da radioatividade. Na liberação, após a captação de [¹⁴C]Asc, culturas foram incubadas por períodos sucessivos de 10 minutos com diferentes tratamentos, as frações liberadas recolhidas e a radioatividade determinada. Para western blot, após eletroforese as proteínas foram transferidas para membrana PVDF, incubadas com anticorpo contra SVCT-2 e reveladas com ECL. A imunofluorescência foi realizada com culturas fixadas com paraformaldeído 1% por 10 minutos e incubadas com anticorpo contra SVCT-2 e secundário alexa 488.

Resultados: Foi observada a presença do SVCT-2 na cultura por western blot e imunofluorescência. A captação de Asc entrou em equilíbrio em cerca de uma hora, apresentando K_M de 18,9±4,3 e V_{MÁX} de 200,4±26,6. A captação foi inibida na ausência de Na⁺ (79,3±2,5% de inibição, n=3) ou por Sulfipirazona 1mM (87,7±1,4%, n=3). Glutamato foi capaz de promover a liberação de Asc, o que também foi observado com kainato 50 µM (54,4±3,9% de aumento, n=7) ou NMDA 50 µM (43,1±7,2%, n=5), que foram bloqueados por seus respectivos antagonistas DNQX (50 µM) (3,3±6,2%, n=5) e MK-801 (10µM) (2,7±9,4%, n=5). A liberação basal ou estimulada por glutamato foi inibida na ausência de Na⁺ (-10,9±4,5%, n=3) ou por Sulfipirazona 1mM (-23,1±1,8%, n=3), indicando a participação de transportador.

Conclusão: Células de retina em cultura captam e liberam Asc através do SVCT-2. A liberação de Asc pode ser estimulada pela ativação de receptores ionotrópicos de Glutamato. A presença de um sistema específico para a captação de Asc e sua modulação por receptores de glutamato sugere um papel importante para essa substância na retina. **Palavras-Chave:** SVCT-2, Ascorbato e Glutamato

Apoio Financeiro: CNPq, CAPES, FAPERJ, PRONEX/MC

B.02.026

Mechanism of Inhibition of The Rat $\alpha 3\beta 4$ Subtype of Neuronal Nicotinic Acetylcholine Receptor by Tacrine

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Background. The neuronal nicotinic acetylcholine receptors (nAChRs) are integral membrane proteins and belongs to the family of ligand-gated cation channels composed by α and β subunits. These receptors play a key role at the signal transmission between neurons in the central and peripheral nervous system. The $\alpha 3\beta 4$ subtype, for example, is a major one of the neuronal nAChR in autonomic ganglia; $\alpha 3$ -containing nAChRs are present at particularly high density in the superior cervical ganglia, pineal, and adrenal glands. They are also present in the *substantia nigra*, striatum, hippocampus, *locus ceruleus*, habenulo-interpeduncular tract, and cerebellum.

Objective. In this study, we investigated the mechanism by which tacrine (9-amino-1,2,3,4-tetrahydroacridine), an agent used clinically to treat Alzheimer's disease, inhibits the rat $\alpha 3\beta 4$ nAChR expressed in KX $\alpha 3\beta 4$ R2 cells (*Mol. Pharmacol.* 54:322, 1998.).

Methods. To determine the inhibition mechanism relevant for channel opening, currents obtained in fast kinetic whole-cell recording experiments, following stimulation of cells by nicotine in the presence of tacrine, were compared to those ones obtained by nicotine alone.

Results. The nicotine dissociation constant for the site controlling receptor activation, K_d , is $(22,9 \pm 0,2)$ microM, and the channel opening equilibrium constant, f^1 , is $4,3 \pm 1,3$. Experiments were performed to investigate whether tacrine is able to activate the $\alpha 3\beta 4$ nAChR. No ion currents were observed upon superfusion with this drug, demonstrating that this drug by itself is not agonist of $\alpha 3\beta 4$ nAChR. Supported by a mathematical model for receptor kinetics (*Biochemistry* 40:8419, 2001), the inhibition factor was plotted as function of concentration of inhibitor. Analysis of data indicated a competitive inhibition mechanism for tacrine, with an apparent inhibiton constant, K_i , of 0,8 microM.

Conclusion. Taken together, these data allow the prediction of a model for $\alpha 3\beta 4$ nAChR inhibition, and we can obtain additional information about the mode of action of tacrine.

Palavras-Chave: Tacrine, Neuronal Nicotinic Acetylcholine Receptor, Fast Kinetic Whole-Cell Recording.

Apoio Financeiro: FAPESP and CNPq.

B.02.027

Effects exerted by diacylglycerols on the surface expression of the nicotinic receptor in CHO cells

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Results from our laboratory indicated that sphingomyelin (SM) and ceramides have a key role in the trafficking of nicotinic receptor (AChR) to the cellular membrane (1,2). We have postulated that SM formation as well as diacylglycerols (DAG), a coproduct in the biosynthesis of SM, are significantly important in receptor trafficking.

Objetives: in the present work we studied the possible modulatory effects of exogenous DAGs and phorbol ester on the trafficking of AChR in CHO-K1/A5 cells

Methods: ligand binding assays and fluorescence microscopy experiments.

Results: we determined using the specific ligand ¹²⁵I a-bungarotoxin, that long chain DAG (palmitoyl-oleoyl glycerol) exogenously supplied in concentrations of 200 and 400 mM by 3 h, significantly increases the surface expression of AChR to 115±2 and 130±8% respectively. The higher amount of the receptor detected in treated cells respect to control ones occurs without change in its affinity for the ligand (20 nM). The analysis of receptor distribution in cells treated with 300 mM of DAG reveals an increase on the total amount of the AChR mainly detected as consequence of the increase at the cellular membrane. In addition, short chain DAG (dioctanoyl glycerol) induces a higher increase (180%) of the AChR detected at the plasma membrane after 30 min of treatment. Experiments conducted in the presence of a phorbol ester, allowed us to determine that the DAG analog was more effective in increasing the AChR expression at the plasma membrane than long chain diacylglycerol, affecting also the affinity of the receptor for a-bungarotoxin.

Conclusion: DAG positively modulates AChR trafficking to the plasmalemma.

References: 1- J. Neurochem. 2007; 101, 1072; 2- Biochim. Biophys. Acta, 1778:917, 2008

Palavras-Chave: nicotinic receptor, diacylglycerol, receptor traffic

Apoio Financeiro: PGI No. 24/B135 from Universidad Nacional del Sur, Argentina, to F.B., PGI No. 24/BZ34 from Universidad Nacional del Sur to M.P., PICT 5-20155 to F.B. and PIP 6327 to F.B.

B.02.028

Participação de Canais TRP na nocicepção produzida por cristais de urato monossódico (MSU) em ratos

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Objetivos: A gota é uma doença inflamatória que parece ser provocada pela precipitação de cristais de MSU em articulações. A crise de artrite aguda é caracterizada por dor, edema, eritema e rigidez na articulação, e os mecanismos responsáveis pela dor são pouco conhecidos. Assim, o objetivo deste trabalho foi investigar a nocicepção produzida pela administração de cristais de MSU na articulação de ratos e o papel dos receptores TRPA1 e TRPV1 neste efeito.

Métodos: Ratos Wistar adultos machos (200-300g) (N=4-10) receberam injeção intra-articular de cristais de MSU (1,25 mg/sítio). Os parâmetros avaliados foram alodínia mecânica (avaliado pelo método acima-abaxo), hiperalgesia térmica (avaliada pelo teste plantar), edema (aumento no diâmetro da articulação) e nocicepção espontânea (escore de pressão sobre a pata 0-4). A participação de receptores TRPA1 e TRPV1 foi avaliada pela co-administração de cânfora (antagonista dos receptores TRPA1; 150 nmol/sítio) e SB 366791 (antagonista do receptor TRPV1; 1 nmol/sítio) juntamente com MSU.

Resultados: Os resultados demonstraram que cristais de MSU administrados na articulação de ratos produziram alodínia mecânica (por até 4 hs após a administração) com redução de limiar de 28 ± 7 vezes, hiperalgesia térmica com efeito máximo ($E_{\max}$) = $78 \pm 14\%$, edema com $E_{\max}$ = $86 \pm 7\%$ e nocicepção espontânea com $E_{\max}$ = $79 \pm 11\%$. Os parâmetros analisados foram reduzidos pela co-administração da cânfora que causou reversão parcial, tanto da alodínia mecânica (inibição máxima ($I_{\max}$) = $84 \pm 10\%$) quanto da hiperalgesia térmica ($I_{\max}$ = $72 \pm 16\%$) e do escore de pressão ($I_{\max}$ = $61 \pm 14\%$), não sendo eficaz no efeito edematogênico. Entretanto, o tratamento com SB 366791 foi capaz de reduzir o edema ($I_{\max}$ = $81 \pm 12\%$), além do prevenir a alodínia ($I_{\max}$ = $42 \pm 21\%$), a hiperalgesia ($I_{\max}$ = $64 \pm 16\%$) e a nocicepção espontânea ($I_{\max}$ = $81 \pm 12\%$).

Conclusão: A estimulação dos receptores TRPV1 e TRPA1, que são importantes canais codificadores de estímulos nocivos externos e internos em nociceptores, são essenciais para o desenvolvimento dos comportamentos nociceptivos induzidos por cristais de MSU.

Palavras-Chave: gota, cristais de urato monossódico, dor, edema.

Apoio Financeiro: Auxílio Financeiro: CNPq, CCS/CCNE/UFSM.

GABA TRANSPORT MEDIATED BY GAT1 IN CULTURE OF CELLS OF EMBRYONIC RETINA: MODULATION BY SECOND MESSENGERS.

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Objectives: g-aminobutyric acid (GABA), is the major inhibitory neurotransmitter in the central nervous system (CNS) including the retina. In the retina of chicken was demonstrated two mechanisms of GABA release, a vesicular Ca^{+2} -dependent and another Na^{+} -dependent mediated by GAT1 (1). To study the intracellular mechanisms which mediate GABA transport can be important to understand neuropathology such as epilepsy, emotional diseases and others (2). Therefore, in the present work, it was evaluated the GABA uptake mediated by GAT1 and its modulation by second messengers utilizing retina cells cultures as experimental model.

Methods: Retinas from 8-day-old chick embryos leghorn (E8) were dissected from other structures of the eye and dissociated with trypsin (0.05%). The cultures were maintained at DMEM 10% FBS, 5U/ml penicillin and 5µg streptomycin in humidified atmosphere with 5% CO_2 at 37°C. The uptake assay (E8C7) was performed in Hank's solution with or without Ca^{+2} containing 0,5µCi [^3H]-GABA during 10min. The quantification was expressed at pmoles/dish and the data were statistically evaluated using one-way ANOVA and the Teste-t Pos-test.

Results: For evaluate the system specificity it was used NNC-711 50µM, a GAT1 inhibitor, and in the presence this inhibitor it was observed a decrease of 80% in the uptake (None: 0,329 ±0,030; NNC: 0,068 ±0,002; $P<0,001$). We also showed that in the absence of Ca^{+2} the [^3H]-GABA uptake is decreased about 20% (None: 0,474 ±0,020; Ca^{+2} : 0,391 ±0,020; $P=0,003$) and the pre-treatment with EGTA 2mM, a Ca^{+2} scavenger, resulted at inhibition of approximately 30% (None: 0,474 ±0,020; EGTA: 0,345 ±0,004; $P<0,001$). The ion Ca^{+2} is an important signaling pathway, then we investigated the role of calmodulin, a protein Ca^{+2} -binding in the [^3H]-GABA uptake. Thus, we used W7 50µM, an inhibitor to this protein, and we observed an inhibition of 40% in the [^3H]-GABA uptake (None: 0,437 ±0,027; W7: 0,279 ±0,026; $P<0,001$).

Conclusions: The Ca^{+2} signaling pathway can be important in the [^3H]-GABA uptake regulation mediated by the GAT1 transporter.

References: 1- Braz. J. Med. Biol. Res. 25:379, 1992; 2- Neurochem. Internat. 49:769, 2006.

Palavras-Chave: GABA, retina, transporter, uptake and calmodulin.

Apoio Financeiro: Capes, PIBIC/CNPq e FINEP.

B.02.030

EFEITO PROTETOR DA MINOCICLINA SOBRE A NEUROTOXICIDADE INDUZIDA POR ROTENONA

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Objetivo: A doença de Parkinson (DP) é uma desordem neurodegenerativa de etiologia desconhecida, na maioria dos casos. Neste trabalho foram investigados os efeitos da administração de minociclina, um inibidor de ativação microglial, na neurotoxicidade induzida por rotenona (ROT) em culturas primárias mistas derivadas de mesencéfalo ventral de ratos neonatos (P2-P4).

Métodos: A toxicidade aguda associada à exposição à rotenona foi avaliada nestas culturas através do ensaio de redução do MTT (J Immunol Methods, 65:55, 1983). A viabilidade celular foi determinada como percentual da atividade dos poços-controle. As culturas foram expostas à ROT no 7º dia, por 1, 3, 5 e 7 dias nas concentrações de 10, 20 e 30 nM. Em alguns casos, as culturas foram pré-tratadas por 30 minutos com minociclina (20 ou 30 mM).

Resultados: Adição de ROT às culturas (10-30 nM) produziu perda celular dependente de dose e de tempo de exposição, como detectado pelo teste de viabilidade celular. Assim, por exemplo, culturas expostas à rotenona por 1 dia apresentaram redução na viabilidade celular de $11,7 \pm 0,4\%$, $10,8 \pm 2,4\%$ e $14,6 \pm 3,6\%$ para as concentrações de 10, 20 e 30 nM, respectivamente, em relação ao controle. Para o mais longo período de exposição (7 dias) houve perda celular de $30,6 \pm 0,2\%$ e $35,1 \pm 1,4\%$, para as concentrações de 20 e 30 nM, respectivamente. Em experimentos iniciais, o pré-tratamento com minociclina em culturas expostas à rotenona (30 nM, 3 dias) resultou em bloqueio do efeito neurotóxico previamente observado, com valores de viabilidade celular similares aos observados nos poços-controle ($103 \pm 4,02\%$ e $100 \pm 3,81$, para as concentrações de 20 e 30 mM de minociclina, respectivamente).

Conclusão: A minociclina, um inibidor de ativação microglial, mostrou-se capaz de bloquear os efeitos neurotóxicos induzidos por rotenona, o que indica a ativação microglial como um importante mecanismo envolvido nos eventos celulares observados neste modelo in vitro de doença de Parkinson.

Palavras-Chave: mesencéfalo, doença de Parkinson, microglia, rotenona

Apoio Financeiro: SEDECT (PA), PIBIC/UFPA

B.02.031

Modulation of 5-HT receptor signaling by CRF peptide

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The neuropeptide corticotropin releasing factor (CRF) has been shown to be the principal mediator of stress-related activity in the neuroendocrine system. Many studies have already demonstrated that CRF has substantial effects on brain serotonergic system.

Objectives: Investigate at a molecular level the mechanisms behind the cross-talk between the CRF and serotonin (5-HT) G protein-coupled receptors (GPCRs).

Methods: For evaluation of 5-HT receptor signaling, we measured inositol phosphate formation in a heterologous cell culture system as well as in mouse pre-frontal cortical slices. We also used biochemical techniques such as co-immunoprecipitation and FACs to assess receptors interaction and distribution as well as live confocal microscopy to study receptors trafficking.

Results: Here we show that CRF stimulation of cells expressing both human CRF1alpha and human 5-HT_{2A} or 5-HT_{2C} receptors can affect 5-HT signaling and trafficking. When pre-treated with CRF peptide, HEK cells showed a 40% increase in inositol 1,4,5-trisphosphate (IP3) formation upon 5-HT stimulation. These results were confirmed in mouse pre frontal cortical slices. The IP3 formation increase is not seen when cells expressing 5-HT_{2A} and Beta₂AR receptors are pre-treated with Beta₂AR agonist, suggesting that the results are not due to overexpression of the receptors. More importantly, cells expressing only CRF1alpha receptor do not show any IP3 formation when stimulated with CRF peptide. Interestingly, cells expressing both receptors treated with monensin to prevent receptor recycling failed to show an increase after CRF peptide treatment. In addition, both serotonin receptors exhibited co-localization with CRF1alpha receptors in HEK cells. Interestingly, when those cells were stimulated with CRF, both receptors were localized in the same intracellular vesicles. On the other hand, when stimulated by 5-HT, only 5-HT_{2A} receptor was localized in intracellular vesicles. We also show that 5-HT_{2A} and CRF1alpha receptors can associate by co-immunoprecipitation techniques.

Conclusion: Taken together, these results suggest that CRF1alpha receptors may modulate 5-HT receptors signaling by altering trafficking of these receptors.

Palavras-Chave: GPCR, trafficking, signalling, mood disorders

Apoio Financeiro: CIHR

NEUROCHEMICAL ACTIVITY OF PbTx10, A NOVEL FRACTION FROM THE VENOM OF THE SPIDER *Parawixia bistriata*.

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Objective:

This work investigated the neurochemical activity of PbTx 10, isolated from the venom of the spider *P. bistriata* on neurotransmitter uptake and release, using Wistar rat cerebral cortex.

Material and Methods

Specimens of *P. bistriata*, collected in Ribeirão Preto, SP, were sacrificed by freezing at -20°C. Venom glands and reservoirs were carefully removed and crushed in Milli-Q water, centrifuged and the supernatant was filtered (>3000Da). Filtered extract was then fractionated by HPLC (Preparative C-18 column), and the active fraction was lyophilized, weighted and dissolved in order to perform bioassays. Synaptosomes from cerebral cortices of male Wistar rats were obtained according to Coutinho-Netto *et al.*, [1]. Uptake and release assays initiated by adding L-[³H]-Glu, [³H]-Gly or [³H]-GABA to synaptosomal suspension and incubation for 5 and 15 min (uptake and release) at 37°C for L-[³H]-Glu and [³H]-Gly and 25°C for L-[³H]-GABA. Reactions stopped by centrifugation (3000 x g, for 3 min at 4°C). In uptake assays, supernatant were discarded and pellets were washed, homogenized and centrifuged. In release assays, supernatants were collected. At the end, aliquots of final supernatants were transferred to scintillation vials containing 5 ml of the biodegradable scintillation cocktail ScintiVerse, and their radioactivity was quantified in a scintillation counter (Beckman LS-6800) with a counting efficiency of 50% for ³H.

Results

We observed a maximum increase of 50% in [³H]-L-Glu uptake [F(7,23)=16.34; p<0.0001] and 40% in [³H]-Gly uptake [F(7,23)=6.134; p=0.0013], whereas [³H]-GABA uptake did not change. Also PbTx 10 caused an increase in [³H]-GABA release by 17% [F(7,23)=2.251 p<0.0001], but did not alter [³H]-L-Glu or [³H]-Gly release.

Conclusions

The interaction of PbTx10 with L-Glu and Gly transport systems may indicate its pharmacological potential in the investigation of neurodegenerative processes in which these transmitters are crucially involved.

References: [1] J Neurochem;35:558-565, 1980.

Palavras-Chave: Parawixia bistriata, uptake, L-glutamate, GABA, glycine, synaptosomes

Apoio Financeiro: CAPES, FAPESP

CLASS V MYOSIN WORKING AGAINST THE CLOCK: THE PARTICIPATION OF MYOSIN V IN A β PEPTIDE ENDOCYTOSIS AND ACCUMULATION.

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Objectives: The aim of this work is to verify the Amyloid-beta peptide (A β) internalization and its accumulation in neuronal cells as well as the association of intraneuronal A β with the actin-based molecular motors myosin V. Class V myosin, one of 19 known classes of the myosins superfamily, is a two-head, actin based molecular motor implicated mainly in organelle transport. Interaction with actin filament and generation of force upon ATP hydrolysis support its movements. Vesicles are abundant in the cells and motor-mediated transport is required for proper distribution. Myosin V is absolutely essential for transport cargos in actin-rich cortical regions, dendritic spines and axon terminal (1,2). Alzheimer's disease is the commonest neurodegenerative disorder in ageing human populations. The fundamental amyloid hypothesis suggests that the accumulation and deposition of the β -amyloid peptide (A β) in the brain precedes and induces the neuronal abnormalities that underlie dementia driving AD pathogenesis (3). Amyloid plaques were thought to form from the gradual accumulation and aggregation of secreted A β in the extracellular space, but recently, the accumulation of A β has been demonstrated to occur within neurons with AD pathogenesis as well as alterations in the axonal trafficking and morphological abnormalities that precede and lead to senile plaques. Moreover, intraneuronal A β accumulation has been reported to be critical in the synaptic dysfunction, cognitive dysfunction and the formation of plaques in AD (4).

Methods: In this work, we use cultured neurons from retinal chick embryos as a model of the central nervous system, immunohistochemistry assays and confocal microscopy.

Results: We show a time course process of endocytosis of the A β peptide. After 10 minutes exposure it exhibits a characteristic intracellular vesicle containing A β and confocal microscopy showed an endocytic A β accumulation in association with myosin V, suggesting a recruitment of myosin V dependent on time course treatment.

Conclusions: Once upon recently described that class V myosins plays multiple roles in the trafficking of secretory granules, these observations are consistent with one hypothesis for AD in which the disease process results from an imbalance between A β production and A β clearance and the importance of myosins on this process.

References: 1- Biol. Cell 99:411, 2007; 2- Traffic 9:290, 2008; 3- Science 297:353, 2002; 4- Neurobiol Aging 26:1235, 2005.

Palavras-Chave: Amyloid-beta; myosin; vesicle transport

Apoio Financeiro: FAPERJ, CNPq, CAPES and PRONEX.

POSSÍVEL PAPEL DOS TRANSPORTADORES DE GLUTAMATO DEPENDENTES DE Na⁺ NO EFLUXO DE GLUTATIONA EM CULTURAS DE RETINA DE PINTO.

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Objetivo: A glutathione (GSH) é um antioxidante essencial para manutenção da homeostasia celular da retina (1). Neste trabalho pretendemos avaliar os mecanismos de liberação de GSH em culturas de retina uma vez que seus níveis extracelulares são de suma importância para manutenção do estado redox da célula.

Métodos: Culturas de retina de embrião de galinha (7º dia embrionário) com dois, quatro, seis e oito dias de desenvolvimento (C2, C4, C6 e C8), foram incubadas em meio Hank com ou sem Na⁺ por 1 h. Culturas C2 foram tratadas por 1 h com L-glutamato (Glu) a 100 e 500µM; PDC a 10, 50 e 500 µM ou aspartato (Asp) a 500 µM. A quantificação de GSH extracelular (GSHe) foi realizada pelo método descrito por Anderson (1969) com algumas modificações. O nível de GSHe é expresso em nmol/mg de proteína, sendo o valor de proteína determinado pelo método de Lowry (1951).

Resultados: Observamos que a GSHe diminui de acordo com o desenvolvimento da cultura (8,82 ± 0.8 em C2; 7,25 ± 1.1 em C4; 4,97 ± 0.5 em C6; 3,23 ± 0.7 em C8). Verificamos também que a GSHe diminui 59,84% na ausência de Na⁺. O L-glutamato causou um aumento dependente de concentração nos níveis de GSHe (9,82 ± 0.2 no controle; 12,25 ± 0.7 a 100 µM; 14,62 ± 0.2 a 500 µM). O inibidor transportável e inespecífico dos transportadores de glutamato, PDC, também causou um aumento dependente de concentração na GSHe (17,16 ± 1.4 a 10 µM; 20,02 ± 0.9 a 50 µM; 22,27 ± 1.4 a 500 µM). Culturas tratadas com Asp também mostraram um aumento na quantidade de GSHe, similar ao provocado por Glu (9,84 ± 0.5 no controle; 14,40 ± 0.09 para Asp; 14.42 ± 0.6 para Glu).

Conclusões: Há um mecanismo de liberação de GSH dependente de Na⁺ na retina nos primeiros dias de desenvolvimento que é estimulado por Glu, PDC e Asp sugerindo um possível papel dos transportadores de glutamato na liberação de GSH em culturas de retina.

Referências: 1 - Exp Eye Res. 72:661, 2001.

Palavras-Chave: Glutathione, Glutamato, Retina, Transportadores

Apoio Financeiro: CAPES, PIBIC/CNPq, PADCT/CNPq, FINEP

PHARMACOLOGICAL CHARACTERIZATION OF GLUTAMATE TRANSPORT IN RETINAL CELLS CULTURES: IMPLICATIONS IN THE GLUTATHIONE METABOLISM.

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OBJECTIVE: Characterize, pharmacologically, the presence and activity of glutamate (GLU) transport mechanisms in retinal cells culture and the role of these transport mechanisms in the glutathione levels

MATERIAL AND METHODS: Retinal cells cultures were obtained from 7 days old chick embryos. Once dissociated, the cells were plated and maintained in DMEM with 10% fetal bovine serum (FBS). After 7 days, cultures showed a confluent monolayer containing both neurons and glial cells. Intracellular levels of reduced glutathione (GSH) were determined by the method described by Anderson (1969).

RESULTS: Our results demonstrated that the absence of Na⁺ led to a significant decrease in the ³H-GLU uptake (@52%), therefore, indicating the presence of two distinct mechanisms of glutamate transport in the retina: a Na⁺-dependent transport system and a Na⁺-independent transport system. These mechanisms of transport represented, respectively, 52±7,69% and 48±4,83% of the total glutamate uptake. We also observed that different concentration of cysteine (500µM) decreased Na⁺ independent ³H-GLU uptake (31±4,67%), suggesting that this system presented characteristics related to the x_{CG}⁻ system. The huge activity of x_{CG}⁻ system in retinal cells, led us to investigate a physiological role for this system, besides only amino acid transport. We demonstrated that the use of cysteine decreased intracellular GSH content (43±2,34%) in relation with control groups, being this effect reverted by the treatment with PDC (23±4,76), a Na⁺-independent ³H-GLU uptake blocker.

CONCLUSIONS: In retina, there are two different mechanisms of glutamate transport: a Na⁺-dependent transport system and a Na⁺-independent transport system and these mechanisms seemed to have a possible involvement in processes related to the synthesis and release of glutathione (GSH), an antioxidant molecule.

Palavras-Chave: glutamate transport, glutathione, cysteine, retina

Apoio Financeiro: PADCT/CNPq; FINEP; CAPES; FAPESP

B.03.001

Effect of Methamidophos pesticide on glutamate uptake.

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Organophosphates (OP) are commonly used for agricultural purposes and have long been known to have neurotoxic potential and be generally related to neurobehavioral findings (Sheets et al. *Bioorganic & Med. Chem. Letters* 8:2787, 1997). The main mechanism of action of OP is an inhibition of acetylcholinesterase (AChE) however other toxic mechanisms are associated such as oxidative stress and DNA damage (Bagchi et al. *Toxicology* 104:129, 1995). The failure of inhibited AChE to hydrolyze the neurotransmitter acetylcholine results in an over-stimulation of cholinergic receptors (Eddleston et al. *Q J Med* 99:513, 2006). Methamidophos (Meth) is a highly active, systemic, a potent AChE inhibitor and residual OP. In fact, there is now a large body of evidence supporting the concept that OPs do interact directly with other molecular targets or cellular functions in the CNS, such as membrane channels, receptors, and even neurotransmitters, but results are inconsistent (Meister. *Farm chemicals handbook* 95, 1995). The purpose of this project was to assess the effects of Meth in glutamate uptake and cellular viability in rat hippocampal slice. The experiments were performed using Wistar rats. Brain was removed, dissected and the slices (0.4 mm) were obtained by transversal cuts of hippocampus using a McIlwain chopper. The glutamate uptake was performed according to Frizzo et al. (*Cell Mol. Neurobiol.* 22:353, 2002) with few modifications. Cell viability in hippocampus slices was quantified by measuring the reduction of MTT (T. Mosmann. *J. Immunol. Methods* 16:55, 1983). In both experiments, we used different concentrations of Meth (0.5, 1, 10, 50 and 100 μ M). Our findings indicate that Meth increased the glutamate uptake at 1 and 100 μ M ($p < 0.05$). However, Meth in the concentrations used here did not modify the cellular viability in rat hippocampus slices. Based on results we can suggest that the Meth interference in the glutamatergic system by affecting glutamate uptake did not affect the MTT assay. Further studies are necessary to explain the exact mechanism of action of Meth in the central nervous system as well as to investigate its effects on the glutamatergic system.

Palavras-Chave: organophosphates, Methamidophos, glutamate uptake, cellular viability

Apoio Financeiro: CNPq, Fapergs, Capes

B.03.002

AÇÃO DA TOXINA TX3-4 ISOLADA DO VENENO DA ARANHA *Phoneutria nigriventer* EM TERMINAÇÕES NERVOSAS DA MEDULA ESPINHAL DE RATOS

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Na medula há muitos canais de cálcio sensíveis à voltagem (CCSVs) em neurônios nociceptivos que desenvolvem papel principal na transmissão sináptica nos mecanismos de dor. A distribuição de canais do tipo P/Q complementam à medida que co-localizam com o tipo N em neurônios contendo CCSVs. Este estudo nos leva a investigar a ação da toxina Tx3-4, presente no veneno da aranha *Phoneutria nigriventer*, na liberação de glutamato evocada por capsaicina e no aumento da $[Ca^{++}]_i$ induzido por capsaicina em terminações nervosas da medula espinhal (sinaptossomas) e também comparando sua ação com o efeito já conhecido do bloqueador de cálcio do tipo N, ω -Conotoxina-MVIIA (ziconotida). Foram realizados experimentos com sinaptossomas de medula espinhal de ratos Wistar machos (180-250g). O P2 pellet foi resuspenso em tampão Krebs-Ringer, aerado com 95% O₂ / 5% CO₂ e 500ml de suspensão foi aliqotada para cada tubo com 1500ml de tampão Krebs. Foram adicionados em cada solução de 2ml, NADP (1mM), glutamato desidrogenase (50 unidades) e Tx3-4 (35-140nM) ou Ziconotida (35-140nM). As toxinas foram adicionadas 100s antes da despolarização da membrana com capsaicina (100 μ M). Este ensaio gerou a liberação de NADPH por ação da glutamato desidrogenase em presença de glicose, com NADPH sendo medido fluorometricamente. Também foi realizada a medida da concentração de cálcio intracelular utilizando 5 μ M de fura-2 AM. A Tx3-4 (105-140nM) reduziu a liberação de glutamato após a despolarização da membrana com capsaicina, com inibição máxima de 98,49 $\pm$ 5,96% e um CI50 de 74,5(54-101)nM. A ziconotida diminuiu a liberação de glutamato com Imax (81,64 $\pm$ 10%). Na dosagem de cálcio interno, a Tx3-4 reduziu o aumento da entrada de cálcio evocado por capsaicina, com uma inibição de 69,56 $\pm$ 17,05% e um CI50 de 225(129-392)nM. Já a Ziconotida teve uma inibição máxima de 70,70 $\pm$ 9,79%. Desse modo, a toxina Tx3-4 poderá ser uma possível ferramenta para o tratamento da dor.

Palavras-Chave: *Phoneutria nigriventer*, Tx3-4, ziconotida, glutamato, canais de cálcio sensíveis à voltagem.

Apoio Financeiro: FAPEMIG, CNPq

B.03.003

SH3-domain-containing proteins regulate the dynamin-dependent kiss-and-run in chromaffin cells

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Two modes of exocytosis have been described in neuroendocrine cells: full fusion and kiss-and-run. During full-fusion, secretory vesicles collapse in the plasma membrane releasing their total content; then vesicle membranes are retrieved by an endocytotic mechanism that includes the participation of clathrin, dynamin and SH3-domain-containing proteins, such as amphiphysin, intersectin, endophilin and syndapin. These proteins bind to proline-rich domain (PRD) of dynamin. During kiss-and-run, secretory vesicles release their content partially, and are retrieved intact through a mechanism that is dependent on dynamin-1 and independent of clathrin. The role of the SH3-domain-containing proteins in this process is unclear. Therefore, we investigated the contribution of these proteins to the kiss-and-run mode in bovine chromaffin cells using amperometry. With this technique the time course of catecholamine release during a single exocytotic event can be measured in real time. Amperometric parameters such as spike area (Q) and half-width ($t_{1/2}$) indicate the amount of catecholamines released per event and the duration of the events respectively. Changes in Q values are related with a shift in the mode of exocytosis.

Intracellular injection of an anti-dynamin-1 antibody increased Q and $t_{1/2}$ of the exocytotic events induced by depolarization, confirming the presence of dynamin-dependent kiss-and-run events. Antibodies against endophilin or syndapin had no effects on the exocytosis. However, antibodies against amphiphysin or intersectin significantly decreased Q and $t_{1/2}$ indicating a prevalence of kiss-and-run events. A recombinant peptide (Dyn₈₂₉₋₈₄₂) encompassing a PRD motif of dynamin-1 inhibited the co-precipitation of amphiphysin and intersectin with dynamin. Moreover Dyn₈₂₉₋₈₄₂, but not its mutant version, dramatically decreased Q and $t_{1/2}$. The increase in kiss-and-run events promoted by Dyn₈₂₉₋₈₄₂ was reverted by co-injecting an anti-dynamin-1 antibody. Taken together, our results suggest that amphiphysin and intersectin have a role in regulating dynamin-1 availability for the kiss-and-run.

Palavras-Chave: kiss-and-run, exocytosis, endocytosis, dynamin, amphiphysin, intersectin, chromaffin cells

Apoio Financeiro: Fondecyt 1085266; Anillo de Ciencia y Tecnología (ACT46)

B.03.004

EXOCYTOSIS OF IMMEDIATELY RELEASABLE POOL IS COUPLED WITH P/Q Ca^{2+} THROUGH A SPECIFIC INTERACTION MECHANISM.

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Chromaffin cell exocytosis is triggered by a localized intracellular Ca^{2+} increase due to the activation of voltage dependent calcium channels. It was described that a small population of the ready releasable pool (RRP) called immediately releasable pool (IRP), can be released by short pulses, supposedly because of a close proximity to calcium channels. We used capacitance measurements on cultured chromaffin mouse cell, where IRP estimation was $31 \pm 3 \text{ fF}$ for control conditions, which represents the 25% of RRP.

Channels blockers and P/Q KO mice's let us to demonstrate that P/Q are the dominant calcium channels associated to the release of IRP. A possible hypothesis is that the coupling between P/Q channel and the IRP vesicles could be dependent of the synaptic protein interaction site, synprint, present in $\alpha 1$ subunit. This site interacts with key vesicular and exocytotic proteins. Chromaffin cells were transfected with pIRES-EGFP with inserted synprint cDNA. We expected that free exogenous synprint peptide will compete with the endogenous P/Q synprint, loosing IRP vesicles and those channels coupling. Expression of synprint peptide induced a significative reduction in the IRP exocytosis ($31 \pm 4 \text{ fF}$ vs $10 \pm 4 \text{ fF}$, $p < 0.05$). As expected, application of P/Q blocker ω -agatoxin-IVA on transfected cells did not induced additional reduction of IRP. Stronger stimulation that releases the RRP did not show a significative effect. This results support the idea that the IRP vesicles are physically coupled to the P/Q channels.

Different distributions of channels and RRP vesicles are modeled in order to reproduce the proportion of vesicles in close proximity to the channels that yield the presence of IRP: A) a uniform and random distribution channels and vesicles, B) a fix grid for the calcium channels and random vesicles distribution, and C) nonuniform distributions in which a fix percentage of RRP vesicles are forced to be placed at no more than 30nm distance from channels. In the first two conditions only 6% of RRP vesicles were associated to IRP. On the other hand, the model supports that an IRP of approximately 25% RRP can be explained by some type of specific interaction restriction.

Palavras-Chave: voltage dependent calcium channels

B.03.005

Identification and Characterization of Early Endocytosis Processes in Mice Chromaffin Cells

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Chromaffin cells release catecholamines through vesicle exocytosis, generating plasmamembrane excess and vesicle pools depletion. Endocytosis and vesicular cycling counteract these two effects. Using epifluorescence and confocal microscopy we studied endocytosis and vesicular cycling pathways involved in membrane homeostasis maintenance and vesicular pools refilling. To investigate endocytosed membrane fate, we designed a protocol that allows quantifying exocytosis, endocytosis and vesicular cycling in the same cell, using the membrane tracer FM 1-43. We particularly investigated endocytic mechanisms involved in fast cycling of releasable vesicles. Fast cycling is usually associated with kiss and run (Nature.394:581,1998), and in chromaffin cells it was proposed to be related to the fast endocytosis mechanism. A 50mM K⁺ stimulus triggered an excess retrieval process after exocytosis. It resulted to be PKC dependent, inhibited by Ba²⁺ and completed within ~30 s. These data suggest that a rapid endocytosis mechanism might be involved in our experiments (PNAS.92:8328,1995). Keeping FM1-43 in bath solution for additional 30 s after the end of stimulus, we noted the existence of a supplementary exocytosis process asynchronous with the stimulation that balanced the effect of excess retrieval, making total exocytosis identical to endocytosis. Vesicles fused during supplementary exocytosis might come from a pool of vesicles that had already been formed before stimulus application. Only ~50% of total endocytosed membrane is cycled to releasable vesicles and this cycling is produced >2 min. These results suggest that the fast endocytic process might be constituted in part by a retrieval mechanism that produces releasable vesicles in a short time.

The degree of excess retrieval varied with the type of stimuli (high K⁺, or nicotine) and seemed to correlate with the size of Ca²⁺ signal. Rapid endocytosis was proposed to be Ca²⁺ dependent and particularly associated to Ca²⁺ entrance through L type Ca²⁺ channels (BBRC.357:834,2007). We found some evidences suggesting that also Ca²⁺ released by endoplasmic reticulum would regulate this process. **Palavras-Chave:** Chromaffin; FM1-43; Endocytosis

Apoio Financeiro: ANCyT; CONICET; UBA

B.03.006

Synaptic alterations at the calyx of Held in S218L KI mice mutant of Cav 2.1 Ca²⁺ channel responsible of familial hemiplegic migraine type 1 disease in humans.

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Familial hemiplegic migraine type-1 (FHM1) is a Mendelian subtype of migraine that is caused by missense mutations in the CACNA1A gene. Cav2.1 (P/Q-type) calcium channels are located throughout the mammalian nervous system at presynaptic terminals, where they play a prominent role in controlling neurotransmitter release. We used knockin transgenic mice with the pathogenic FHM1 human mutation S218L (Tottone A et. al. 2005) to investigate presynaptic calcium currents and transmitter release. We used auditory brainstem slices containing the Medial Nucleus of the Trapezoid Body (MNTB), whose principal neurons receive a giant synapse (calyx of Held). We used wild type (WT) and mutant mice (MM). Using whole-cell configuration a little shift was found in the I-V curve to more negative potentials (WT: -10 mV; MM: -15 mV). Related to the spontaneous events, the frequency and amplitude was highest in the mutant mice in comparison with the wild type. The mean frequency was WT: 2.03 ± 0.55 and MM: 4.29 ± 1.07 . Related to the amplitude, not clear difference exists between both groups (WT: 45 ± 3 pA and MM: 45.82 ± 1.3 pA). In the other hand, some cells of the MM animals presented high frequency and low amplitude or vice versa (mean amplitude in the last group was 201.55 ± 9.19 pA). A jitter was produced in a group of MM under stimulation in the middle line with a bipolar electrode. These results could be indicating that the mutation could produce alterations in the synaptic transmission at the calyx of Held and provide important information to understand the basic features of the physiology of neurological disease such as migraine.

Palavras-Chave: migraine , P/Q calcium channel, Synaptic Transmission

Apoio Financeiro: BID 1728 OC.AR.PICT.2005 N°32113

B.03.007

Inibição da neurotransmissão em hipocampus de ratos pela α,β -epoxi-carvona

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Objetivos:

Estudar os possíveis efeitos do monoterpeneo α,β -epoxi-carvona no mecanismo de excitação sináptica na região CA1 do hipocampo de ratos.

Métodos e Resultados:

Foram utilizados ratos Wistar de 21 dias nos experimentos. Após rápida remoção do cérebro, fatias de 400 micrômetros foram feitas com o auxílio de um vibratômo, sendo as mesmas rapidamente transferidas para uma câmara de incubação contendo ACSF (*Artificial Cerebral Spinal Fluid*) borbulhado com uma mistura gasosa de 95% de O₂ e 5 % de CO₂ em temperatura ambiente. As fatias permaneceram incubando por uma hora e meia para permitir a recuperação dos danos sofridos durante o processo de fatiagem. Potenciais de campo excitatório pós sinápticos (EPSPs) foram evocados na região CA1, através de estímulos elétricos ortodrômicos aplicados no Colateral de Schaffer. A seleção das fatias, para uso nas medições eletrofisiológicas, foi feita através da transferência de cada uma destas para a câmara de registro, com perfusão constante de solução padrão de ACSF, contendo um veículo (tween, 0.01%). Tendo-se escolhido as fatias apropriadas e obtido uma linha de base estabilizada para os EPSPs, trocou-se o ACSF padrão por outro onde foi adicionada α,β -epoxi-carvona (1 mM). O protocolo de registro foi o de facilitação de pulso pareado (PPF, *Paired Pulse Facilitation*). Na presença da droga a amplitude do EPSP se reduziu em 70±6% (SEM, N=6) em relação ao controle, tomado na mesma fatia. A droga não alterou o PPF, associado à probabilidade de liberação de neurotransmissores, mostrando também uma pequena alteração nos potenciais de ação pré sinápticos (*Fiber Volley*). Em seguida, retornou-se a perfusão ao ACSF normal onde verificamos a recuperação da amplitude do EPSP.

Conclusão:

O α,β -epoxi-carvona afeta o mecanismo da neurotransmissão no hipocampo através do bloqueio dos receptores para glutamato existentes na membrana pós sináptica dos dendritos existentes nas células piramidais do CA1.

Palavras-Chave: α,β -epoxi-carvona, Potenciais de campo excitatório pós sinápticos

Apoio Financeiro: FAPEMIG, CNPq,

B.03.008

Extracellular carbonic anhydrase contributes to regulation of post synaptic calcium influx in hippocampal CA1 pyramidal neurons

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Objectives: Extracellular ionic shifts associated with spreading depression (SD) have been described in a variety of neuronal cells. The ionic displacements are accompanied by extracellular pH changes (pH_e) consisting of a rapid interstitial alkaline shift. These shifts influence the propagation of SD. In the hippocampal CA1 area the alkaline shifts are especially large but the mechanism of their regulation is not clear. It was shown that in the CA1 region stimulation of the Schaffer collaterals (SC) elicits an alkaline pHe shift that is limited by extracellular carbonic anhydrase (ECA). When ECA is inhibited by benzolamide (BZ) the alkalosis is enhanced and NMDA receptor (NMDAR) mediated post-synaptic currents are augmented. Accordingly, the dendritic Ca²⁺ influx elicited by synaptic excitation would be expected to increase if ECA was blocked.

Methods: We used concentric pH- and Ca²⁺-selective microelectrodes, Fluo-5F imaging of [Ca²⁺]_i transients and whole cell current clamp recording. Shifts in pHe, [Ca²⁺]_e, and [Ca²⁺]_i were elicited by stimulation of the SC fibers with three 200 ms current pulses (100 Hz).

Results: We showed that stimulation of the SC elicited an alkaline pHe transient, a decrease in extracellular Ca²⁺ concentration ([Ca²⁺]_e) and rise in intracellular Ca²⁺ concentration ([Ca²⁺]_i). Inhibition of ECA with BZ amplified and prolonged recovery of the pHe and [Ca²⁺]_e responses and dendritic [Ca²⁺]_i transients. BZ increased the amplitude of alkaline responses by 165±41% and decay half time by 165±19%; increased the peak fall in [Ca²⁺]_e by 53±10% and peak of dendritic [Ca²⁺]_i responses by 43±5%. The increase in amplitude caused by BZ did not occur in the presence of the NMDAR antagonist APV indicating that ECA can shape dendritic Ca²⁺ dynamics governed by NMDARs by virtue of its regulation of concomitant activity-dependent pHe shifts.

Conclusion: Inhibition of ECA causes amplification of alkaline pHe transients and an NMDARs-dependent augmentation of [Ca²⁺]_e, [Ca²⁺]_i and post-synaptic electrical responses. These data suggest that ECA acts to limit excitability and can thereby shape the amplitude and time course of dendritic Ca²⁺ responses.

Palavras-Chave: Depression; basic, receptors, signal transduction, extracellular carbonic anhydrase

B.03.009

FINE-TUNING REGULATION OF Ca^{2+} RECRUITMENT VIA P- AND L-TYPE VSCCs BY ADENOSINE $\text{A}_{2\text{A}}$ RECEPTORS IN TOXIN-INDUCED MYASTHENIC RATS

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Objectives: During short stimulation trains, P-type Ca^{2+} channels clustered at active zones control nerve-evoked ACh release. Rapid decline of P-type channel function as consequence of a Ca^{2+} -dependent inactivation might contribute to transmission tetanic failure. During intermittent high-frequency bursts, decline of transmission within a train may completely recover by the onset of the next train probably due to the recruitment of Ca^{2+} via quiescent high-capacity / slow-inactivating L-type channels located away from the active zones. The fine-tuning control of Ca^{2+} currents through P- or L-type channels is likely to be mediated by endogenous adenosine. Tonic activation of presynaptic $\text{A}_{2\text{A}}$ receptors operating Ca^{2+} influx via L-type channels contributes to overcome tetanic depression in adult rats (Oliveira *et al.*, 2004). In this study, we investigated whether manipulation of $\text{A}_{2\text{A}}$ receptors activation was also relevant to preserve neuromuscular transmission in a-bungarotoxin-induced myasthenia gravis (TIMG).

Methods: Rats with TIMG were generated as described by Plomp and colleagues (1992). [^3H]ACh release was evoked by phrenic nerve stimulation with either 5 Hz-trains (750 pulses) or 50 Hz-bursts (five bursts of 150 pulses, applied with a 20-s interburst interval). Fatigue tests were achieved by intermittent stimulation (17 pulses per second) at a frequency of 50 Hz during 3 min. The kinetics of exocytosis was tested by following unloading of the FM 4-64 fluorescent probe.

Results: The release of [^3H]ACh from rat phrenic nerve endings decreased upon increasing the stimulation frequency from 5-Hz ($83 \pm 4 \times 10^3$ dpm/g, $n=11$) to 50-Hz ($30 \pm 3 \times 10^3$ dpm/g, $n=5$). The P-type Ca^{2+} channel blocker, w-agatoxin IVA (100 nM) reduced ($40 \pm 10\%$, $n=6$) the release of [^3H]ACh evoked by 50-Hz trains, while nifedipine (1 mM, an L-type blocker) was inactive. Tetanic depression was overcome ($88 \pm 6 \times 10^3$ dpm/g, $n=12$) by stimulating the phrenic nerve with 50-Hz bursts; this was confirmed by fluorescence microscopy using FM 4-64. In these conditions, the w-agatoxin IVA (100 nM) failed to affect transmitter release, whereas nifedipine (1 mM) decreased [^3H]ACh release by $21 \pm 7\%$ ($n=4$). The inhibitory effect of w-agatoxin IVA (100 nM) was rehabilitated ($35 \pm 6\%$, $n=4$) in TIMG animals, but these animals were insensitive to nifedipine (1 mM). Inactivation of endogenous adenosine, with adenosine deaminase (ADA, 0.5 U/ml), or blockage of $\text{A}_{2\text{A}}$ receptor, with ZM 241385 (50 nM), decreased [^3H]ACh release induced by 50-Hz bursts by $54 \pm 8\%$ ($n=5$) and $46 \pm 7\%$ ($n=6$), respectively. This effect was attenuated respectively to $18 \pm 6\%$ and to $19 \pm 9\%$ ($n=4$) in TIMG animals. ZM 241385 (50 nM) potentiated fatigue of diaphragm contractions in control animals, but not in TIMG animals. Blockade of $\text{A}_{2\text{A}}$ receptors activation, with ADA (0.5 U/ml) or ZM 241385 (50 nM), rehabilitated w-agatoxin IVA (100 nM) inhibition and prevented the effect of nifedipine (1 mM).

Conclusions: Data suggest that adenosine $\text{A}_{2\text{A}}$ facilitatory tonus is attenuated in TIMG rats. Decline of the co-ordinated shift on Ca^{2+} channel dynamics, from the prevalent P-type to the “facilitatory” L-type channel, operated by $\text{A}_{2\text{A}}$ receptors activation might contribute to muscle weakness in *myasthenia gravis*.

Palavras-Chave: Myasthenia gravis; Neuromuscular junction; Adenosine; $\text{A}_{2\text{A}}$ receptors; P- and L-type calcium channels; Synaptic plasticity

Apoio Financeiro: This work was supported by FCT (FEDER funding).

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EFEITOS DO EXERCÍCIO FÍSICO SOBRE A EXPRESSÃO DE RECEPTORES DE GLUTAMATO NO ENCÉFALO DE RATOS.

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O exercício físico crônico beneficia o sistema nervoso central, promovendo a melhora da memória, aprendizado e cognição. Entretanto, poucos estudos analisam o efeito do exercício agudo. Este estudo visou observar os efeitos plásticos agudos do exercício no córtex, hipocampo, estriado e cerebelo, avaliados pela expressão de subunidades dos receptores de glutamato do tipo AMPA (GluR1-3).

Utilizaram-se ratos Wistar adultos, que foram divididos em 3 grupos experimentais (corrida 3 dias (COR3), 7 dias (COR7) e 15 dias (COR15)) e grupo controle (CONT). Os experimentais foram submetidos ao protocolo de exercício físico moderado em esteira (KT 3000 – IMBRAMED). Após o treino, os encéfalos foram submetidos às técnicas de imuno-histoquímica e “immunoblotting”. Para análise estatística utilizou-se o teste ANOVA e o teste de Tukey, quando apropriado. Adotou-se nível de significância de 5%.

A expressão das subunidades GluR1-3 apresentou mudanças distintas nas estruturas analisadas após o exercício físico, tanto na imuno-histoquímica quanto no “immunoblotting”. No cerebelo e no hipocampo houve uma queda significativa na expressão de GluR1 em COR3 (17% e 40%, respectivamente) em relação ao CONT, retornando a níveis basais no hipocampo em COR7. No cerebelo, houve um acréscimo significativo em COR7 (23%), voltando à expressão do CONT em COR15. No córtex cerebral observou-se uma crescente queda da expressão de GluR1, com máxima queda em COR7 (52%), retornando à expressão basal em COR15. O estriado não sofreu alterações na expressão de GluR1 ao longo dos primeiros 7 dias, tendo um aumento significativo em COR15 (90%).

A expressão de GluR2/3 não foi alterada em relação ao CONT, exceto no cerebelo, onde houve um decréscimo significativo em dois momentos distintos, COR3 (55%) e COR15 (25%), retornando à expressão basal em COR7.

Os nossos dados revelam que o exercício físico agudo foi capaz de promover alterações plásticas ao longo do treinamento de forma distinta nas estruturas e subunidades dos GluRs estudadas, sugerindo uma participação específica desses receptores em cada estrutura e tempo de treino.

Palavras-Chave: exercício físico, receptores de glutamato, AMPA, neuroplasticidade, sinapses

Apoio Financeiro: FAPESP e CNPq

B.04.002

AEROBIC PHYSICAL EXERCISE DURING DEVELOPMENT INCREASE PARVALBUMIN EXPRESSION IN THE HIPPOCAMPAL FORMATION OF ADULT RATS

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Although the effects of physical exercise in the central nervous system of adult animals have been well documented, little is known of its effects in the developing rats. In our laboratory, the expression of the calcium-binding protein parvalbumin (PV) has been used to analyze the hippocampal formation plasticity after physical exercise in adult animals. To investigate whether physical exercise would promote morphological changes in the hippocampal formation of rats during development, we performed an immunohistochemical study using PV after aerobic exercise program. Male Wistar rats at postnatal day 21 (P21) were divided into two groups: exercise group (n=4), control group (n=4). Animals of the exercise group were submitted to daily exercise in the treadmill between P21 and P60. Running time and speed gradually increased during the subsequent days until 18 m/min during 60 min. After the aerobic exercise program, animals of all groups were sacrificed and Western analysis was performed. PV density enhanced significantly in the hippocampal formation of rats submitted to aerobic treadmill exercise (~30%, 1.27 ± 0.1 , $p > 0.0019$) when compared to the control group (1.0 ± 0.001 ; Student's t-test). These findings showed that physical exercise during rat development promotes neuroplastic changes in hippocampal formation examined in the adult life. **Palavras-Chave:** Exercise, Hippocampal formation, Parvalbumin, Developing brain, pups.

Apoio Financeiro: FAPESP, CAPES

B.04.003

EFEITOS PLÁSTICOS AGUDOS DO EXERCÍCIO SOBRE A EXPRESSÃO DE NEUROFILAMENTOS E PROTEÍNAS SINÁPTICAS NO ENCÉFALO DE RATOS

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Objetivo: O exercício físico traz inúmeros benefícios para o sistema nervoso central (SNC), dentre eles a melhora da memória e cognição, o aumento da plasticidade e aprendizado, além de um efeito protetor em relação ao declínio mental decorrente do envelhecimento e de lesões do SNC. Este estudo visa observar os efeitos plásticos agudos do exercício em regiões do encéfalo de ratos relacionados a funções cognitivas e motoras, avaliados pela expressão de proteínas sinápticas como a sinapsina I (SYS) e a sinaptofisina (SYF), e de proteínas estruturais como os neurofilamentos (NF).

Métodos: Foram utilizados ratos machos da linhagem Wistar em um protocolo de corrida em esteira. O protocolo constou de dois dias de adaptação dos animais à esteira, exclusão dos animais que não correram na adaptação e divisão dos demais em um grupo controle (C), que permaneceu sem correr a partir deste momento, e três grupos experimentais de acordo com os tempos de corrida a que seriam submetidos: 3 dias (3D), 7 dias (7D) e 15 dias (15D). Os animais correram durante 40 minutos por dia a uma velocidade de 0,6km/h, exercício considerado leve/moderado. Os animais foram sacrificados ao final dos tempos de corrida e seus encéfalos rapidamente coletados para posterior análise por *Western-blotting*.

Resultados: Foram observadas alterações na expressão destas proteínas em todas as regiões avaliadas. No hipocampo, foi observado um aumento estatisticamente significativo entre C e 3D para SYS ($p<0,05$) e NF68 ($p<0,001$). No cerebelo houve aumento da expressão de SYS em 7D ($p<0,05$) e de NF68 em 3D ($p<0,005$), porém houve uma diminuição da expressão de SYF em 15D ($p<0,05$). Já para o corpo estriado e substância negra, foram observados aumentos do NF68 em 3D ($p<0,05$) e, apenas na substância negra, do NF160 em 3D ($p<0,05$).

Conclusões: As alterações na expressão de SYS, SYF e NF decorrentes dos diferentes tempos de exercício demonstram o efeito desta atividade sobre a plasticidade de regiões do encéfalo relacionadas a funções cognitivas, memória e aprendizado, além das regiões relacionadas ao controle motor.

Palavras-Chave: exercício, neurofilamentos, proteínas sinápticas, plasticidade

Apoio Financeiro: FAPESP, CNPq

Potenciação sináptica de curto prazo em ratos privados de sono

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Objetivos

Estudos têm demonstrado que a privação de sono (PS) reduz a potenciação sináptica em fatias hipocâmpais. A proposta do presente estudo é verificar a potenciação de curto prazo (PCP) do giro denteado de animais privados de sono por 96 horas, através de uma preparação com anestesia.

Métodos

Ratos Wistar machos (3 meses) foram separados em dois grupos: privação de sono (PS, n = 3) e controle (CT, n = 3). O grupo PS foi submetido à privação de sono por 96 horas através do método de plataforma individual. Ao final do procedimento, os animais foram anestesiados com uretano e operados para implantação de eletrodos de estimulação (via perfurante) e de registro (giro denteado). Uma curva estímulo-resposta (E-R) foi obtida por meio da quantificação da amplitude e inclinação do potencial excitatório pós sináptico (PEPS). A PCP foi quantificada comparando-se as respostas obtidas durante 10 min de registro basal com aquelas obtidas após duas rajadas de estímulos em alta frequência (50 pulsos @ 100 Hz, 10 min de intervalo entre as rajadas).

Resultados

Curva Estímulo-Resposta

Os grupos diferem quanto a amplitude do PEPS (teste de Tukey $p < 0,01$), mas não quanto a inclinação do mesmo ($p = 0,07$). A aplicação de níveis crescentes de intensidade variou de forma significativa para amplitude ($p < 0,01$) e inclinação ($p < 0,01$) do PEPS.

Potenciação de Curto Prazo

Não houve diferença entre os grupos quanto à amplitude ($p = 0,40$) e inclinação do PEPS. ($p = 0,07$), mas houve diferenças significativas entre as sessões para a amplitude ($p = 0,01$) e inclinação do PEPS ($p = 0,01$). Não houve interação entre os fatores ($p = 0,62$ amplitude, $p = 0,12$ inclinação). Análises a posteriori do fator sessão indicaram que após a primeira rajada houve um aumento significativo da amplitude e inclinação do PEPS ($p < 0,01$), e que não houve aumento da potenciação com uma segunda rajada, em relação a primeira ($p = 0,23$ amplitude, $p = 0,93$ inclinação).

Conclusão

Os dados preliminares mostram que a privação de sono não interfere na PCP em neurônios granulares do giro denteado. É plausível que esse efeito esteja relacionado ao aumento de amplitude do potencial de campo observado na curva E-R após PS.

Palavras-Chave: Sono , Privação de Sono , Plasticidade , Hipocampo

Apoio Financeiro: AFIP , FAPESP , CNPq

B.04.005

Interferon beta treatment enhances axonal growth and motor function recovery following peripheral nerve injury

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Recent studies have demonstrated that major histocompatibility complex class I (MHC I) expression by neurons and glia constitutes an important pathway that regulates synaptic plasticity and stability (PNAS, 101(51):17843, 2004). In this sense, lesioned neurons in mutant knockout mice show more extensive synaptic detachments than those in wild-type animals. On the other hand, the upregulation of MHC I after treatment with interferon beta (IFN beta) accelerates the response to injury. Therefore, the present work studied the regenerative outcome after peripheral nerve lesion and treatment with IFN beta aiming at increasing the MHC I upregulation in the spinal cord microenvironment. C57BL/6J adult mice were subjected to unilateral sciatic nerve crush after being treated with 10,000 IU of IFN beta for 1 week. Following injury, they were kept under treatment for two more weeks. After that, the animals were sacrificed and the lumbar spinal cords were processed for immunohistochemistry (anti-MHC class I) and the sciatic nerves for morphometry and counting of regenerated axons. Motor function recovery was monitored twice a week, for three weeks, using the walking track test. The results showed a greater MHC I expression in the motor nucleus of IFN treated animals (placebo 4.74 ± 0.57 and IFN treated 8.03 ± 0.67 , $p = 0.004$). In the peripheral nerve, a significant greater number of regenerated axons was observed (placebo 4.85 ± 1.46 and IFN treated 9.16 ± 2.60 , $p = 0.0004$). The morphological findings indicating improvements in the regenerative process after IFN treatment were in line with the motor behavior test applied to the animals during the recovery process. The present data indicate the role of MHC I upregulation on the response to injury and show that IFN treatment may be beneficial to the motor recovery after axotomy.

Palavras-Chave: synaptic plasticity, nerve regeneration, MHC class I, axotomy

Apoio Financeiro: Fapesp, CNPq, Faepex

GERAÇÃO DE ESPÉCIES REATIVAS DE OXIGÊNIO NO COLÍCULO SUPERIOR E NO NÚCLEO GENICULADO LATERAL DE RATOS ADULTOS APÓS ENUCLEAÇÃO OCULAR.

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No sistema nervoso central, espécies reativas de oxigênio (EROs) são capazes de intermediar desde circuitos de sinalização fisiológicos e patológicos até lesões a constituintes celulares. No entanto, pouco se sabe acerca do envolvimento das EROs na neurodegeneração e/ou neuroplasticidade. Este estudo visou avaliar a geração de EROs em áreas visuais primárias após enucleação ocular.

Ratos Wistar adultos foram submetidos à enucleação ocular e divididos em diferentes grupos experimentais, de acordo com tempos de sobrevivência, que variaram entre 1 hora e 30 dias pós-lesão. Após o término do período de sobrevivência, foi avaliada a geração de EROs no colículo superior (CS) e no núcleo geniculado lateral (NGL) por fluorescência da dihidroetidina (DHE) em cortes histológicos. Para análise estatística utilizou-se o teste ANOVA seguido do teste de Tukey. Adotou-se nível de significância de 5%.

Análise da fluorescência da DHE revelou um aumento na geração de EROs tanto no CS quanto no NGL, entre uma hora e 30 dias pós-lesão. A enzima antioxidante superóxido dismutase (1000U/ml) inibiu significativamente a geração no CS e no NGL (60% e 80% respectivamente), assim como a presença de 7-nitroindolazole (1mM), um inibidor da isoforma neuronal da enzima óxido nítrico sintase (nNOS) (42% e 64%). Um grupo de animais foi tratado por 14 dias com apocinina (60mg/L na água de beber), um inibidor não seletivo da enzima NADPH oxidase, o que causou uma significativa diminuição no sinal da fluorescência nas estruturas desaferentadas (72% no CS e 80% no NGL). A presença de catalase (800U/ml), uma enzima decompositora de peróxido de hidrogênio, inibiu significativamente a geração de EROs apenas no NGL (55%). Além disso, a adição aos cortes de *diphenylene iodonium* (DPI) (10μM), um inibidor da NADPH oxidase, aboliu completamente a geração de EROs tanto no CS quanto no NGL.

Os resultados sugerem que lesões retinianas podem induzir um aumento da geração de EROs tanto no CS quanto no NGL. Sendo a NADPH oxidase uma importante fonte geradora de EROs, a inibição da geração observada após o tratamento com apocinina, bem como a ação do DPI, sugerem a participação desta enzima no fenômeno observado.

Palavras-Chave: neurodegeneração, espécies reativas de oxigênio, sistema visual, neuroplasticidade

Apoio Financeiro: FAPESP e CNPq

ALPHA MONOTONEURON INPUT CHANGES IN THE DYSTROPHIC MDX MICE AFTER SCIATIC NERVE TRANSECTION.

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The aim of this study was to investigate eventual synaptic alterations and glial reactivity changes in the microenvironment surrounding the alpha motoneurons in a Duchenne muscular dystrophy animal model, namely the MDX mice (Int. J. Exp. Pathol., 84:165, 2003). In this sense, six weeks old, male, MDX mice, were subjected to the left sciatic nerve transection. The axotomy was performed after the cycles of muscular degeneration/regeneration, previously described in such model of muscular dystrophy (J. Neurol. Sci., 129:97, 1994). C57BL/10 mice were used as controls. Seven days after surgery, the animals were sacrificed and their lumbar spinal cords processed for immunohistochemistry (anti-MHC I, anti-Synaptophysin and anti-GFAP – *glial fibrillary acidic protein* antisera were used) (Brain Res., 1095:35, 2006). Overall MHC-I expression increased in both strains after axotomy. Nevertheless, MDX mice displayed a significantly smaller MHC I upregulation (MDX, $18,90 \pm 0,60$, mean + se; C57BL/10, $24,34 \pm 1,06$, $p > 0,05$). Regarding GFAP expression, MDX showed a stronger astrogliosis in comparison to C57BL/10 mice (MDX, $16,00 \pm 1,06$; C57BL/10, $9,70 \pm 0,43$; $p < 0,01$). In MDX mice the immunostaining demonstrated significant decreased of synaptophysin expression (MDX, $4,25 \pm 0,30$; C57BL/10, $5,70 \pm 0,28$; $p < 0,05$), indicating a reduction of synaptic activity after axotomy. We conclude that the reduction of active inputs to the alpha motoneurons and increased astrogliosis in MDX mice maybe associated with the cycles of muscle degeneration/regeneration that occur postnatally. Also, the decreased MHC I expression in MDX mice may indicate a lower regenerative potential after lesion.

Palavras-Chave: Duchenne Muscular Dystrophy, Spinal Cord, Motoneuron, Synaptic Elimination, MHC-I

Apoio Financeiro: CAPES

MONOCULAR ENUCLEATION AFFECTS THE EXPRESSION OF AMYLOID PRECURSOR PROTEIN IN THE DEAFFERENTED SUPERIOR COLLICULUS OF JUVENILE AND ADULT RATS

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The amyloid precursor protein (APP) plays a central role in Alzheimer's disease (AD) through its cleavage leading to the accumulation of the amyloid beta peptide. Diffusible oligomeric assemblies of Abeta peptide are thought to induce synaptic dysfunction, an early event in AD. Several evidences point out that APP present biological functions, such as synaptic formation and neuronal plasticity. The superior colliculus has been used as a model to study the neural mechanisms related to formation of synaptic specificity during development and plasticity. In previous studies, we have demonstrated that APP expression is higher until the second postnatal week in the rat superior colliculus, when retinotectal axons undergo extensive axonal/synaptic remodeling leading to precise patterns of connections. These projections can also undergo plasticity under various experimental manipulations, such as monocular enucleation and retinal lesions. In this work we investigated the expression of APP in animals submitted to monocular enucleation during or after the critical period of retinotectal development. Lister Hooded rats were submitted to monocular enucleation at PND10 or PND21. After different survivals, the rats were sacrificed and the superficial layers of the superior colliculus, extracted and processed for western blotting analyses for APP. Our results demonstrate that the monocular enucleation within the critical period induces a transient reduction (57% of control) in APP expression after 48h post-lesion indicating a pre-synaptic location of this protein. APP levels remain low and return to basal values one week after the lesion. Monocular enucleation at the end of the critical period reveals a similar reduction (60% of control) in APP expression with a slow, three-week recovery afterwards. The time course of APP recovery matches the plasticity of the intact uncrossed retinotectal pathway. These results indicate that APP expression is regulated after a monocular enucleation and suggest a possible association with the synaptic rearrangements observed both in normal development and also in the reorganization of intact axonal pathways in lesion conditions.

Palavras-Chave: APP; visual system, plasticity

Apoio Financeiro: FAPERJ, PROPP-UFF, PRONEX-MCT

B.04.009

Spinal motoneuron synaptic plasticity in an animal model of multiple sclerosis

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Recent studies regarding the acute phase of the Multiple Sclerosis (MS) in animal models have shown a significant detachment of inputs to the cell body of alpha motoneurons, directly influencing the excitability and synaptic transmission (Eur. J. Neurosc. 24:3053, 2006). Nevertheless, the impact of exacerbation and remission phases of the MS on the spinal cord microenvironment is still poorly understood. Therefore, the present work investigated the synaptic covering of spinal alpha motoneurons during exacerbation and progressive remissions of the MS. C57BL/6J mice were induced to experimental autoimmune encephalomyelitis (EAE) and divided in 4 groups: control, exacerbation (degree 3), first remission (30th day after EAE induction) e second remission (40th day after EAE). The animals were sacrificed and their lumbar spinal cords were dissected out and processed for immunohistochemistry and transmission electron microscopy. The results indicate an increase of microglial reaction (control 6.68 ± 0.85 ; exacerbation 9.87 ± 0.6) and macroglial reactivity (control 1.86 ± 0.28 ; exacerbation 5.40 ± 0.57) during exacerbation phase. In this period, the ultrastructural analysis showed a reduction of the synaptic covering in relation to motoneurons, which corresponded to a reduction of synaptophysin immunolabeling (control 16.92 ± 0.28 ; exacerbation 7.54 ± 0.4 ; *second remission* 12.5 ± 1.0) and an increase of the major histocompatibility complex class I molecule expression (MHC I) (exacerbation 12.32 ± 1.11 ; *second remission* 6.47 ± 1.59). Such molecule play an important role in the neuronal-glial response affecting the synaptic plasticity process (PNAS, 101(51):17843, 2004). The results observed in both remission groups indicated a return of the terminal contact in relation to the motoneuron surface, depicted by the increase of synaptophysin immunolabeling. In conclusion, this study provided evidences that synaptic plasticity and the glial reactivity during the different phases of the MS disease, play an important role in the motor deficit and recovery that occur throughout the MS development. **Palavras-Chave:** Multiple Sclerosis , motoneurons, synaptic plasticity

Apoio Financeiro: FAPESP

B.04.010

Distinct effects on BDNF and TrkB in retina and visual cortex in mice caused by environmental enrichment

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Objective: Environmental enrichment (EE), a paradigm used to induce plasticity in the central nervous system, involves increasing sensorial complexity by providing novelty with inanimate objects, increasing exercise and adding social interaction. One potential mechanism for the functional changes seen in the visual system could be concurrent with modifications in neurotrophins levels. In the present study, we evaluated changes on BDNF and TrkB immunocontent in retina and visual cortex in mice following exposure to 8 weeks of enriched environment.

Methods: 21 days mice were divided in two groups: a control group (C, n=6) that were maintained in standard condition and EE group (n=6) that were maintained in the enrichment housing apparatus, containing running wheels and a variety of objects, which were changed three times per week. After 8 weeks, both groups were evaluated in relation to [³H] glutamate uptake in retina and the immunocontent of BDNF and TrkB in retina and visual cortex by Western blot.

Results: Our results demonstrated no change in the glutamate uptake in retina (0.576 ± 0.04 C vs 0.529 ± 0.03 EE) in both groups. The immunocontent of BDNF was increased in retina of EE group (8.38 ± 1.68 C vs 13.85 ± 2.58 EE, $p < 0.05$), while TrkB immunocontent did not changed (9.11 ± 1.06 vs 7.69 ± 1.16). In relation to visual cortex, we did not observe change on BDNF immunocontent in EE group (10.40 ± 2.48 vs 7.39 ± 0.87); however, a decrease in TrkB immunocontent was observed (9.71 ± 1.02 vs 6.69 ± 0.42 , $p < 0.05$).

Conclusions: Increased BDNF immunocontent were not associated with a change in TrkB in retina. In visual cortex there was no difference in BDNF immunocontent but a decrease in TrkB was observed. One possible explanation is the region-specific differences in neurotrophins expression and its receptor regulation. This factor is one of the possible mechanisms involved in the different plasticity in these two regions.

Palavras-Chave: retina, environmental enrichment, BDNF, plasticity, visual cortex

Apoio Financeiro: IBNNet, CNPq, CAPES, FINEP, FAPERGS

B.04.011

c-Abl Kinase Regulate Synaptic Contact Number and PSD-95 Accumulation

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The tyrosine-kinase c-Abl is a key regulator of the cytoskeleton dynamics and modulates neuronal morphogenesis by activation of surface receptors. It has been described that this kinase is localized on pre- and post-synaptic compartment in mouse hippocampus and regulates short term synaptic plasticity. Furthermore, Abl kinases participate in the post-synaptic assembly of the neuromuscular junction, being the acetylcholine receptor clustering induced by agrin inhibited by a specific Abl family inhibitor, STI571.

HYPOTHESIS: These studies suggest a role for the Abl family in synapse formation in the central nervous system (CNS).

OBJECTIVES: We decided to study if c-Abl kinase is involved in synaptic contact formation *in vitro*, and identify synaptic proteins that could be c-Abl targets during the synaptogenesis.

RESULTS: We observed an increase in the c-Abl levels in the rat brain the first days after birth and c-Abl clustering in rat hippocampal culture (14 DIV) in later developmental states. This kinase has synaptic localization and interacts with PSD-95 *in vitro* and *in vivo*. The clusters number and protein levels of PSD-95 decrease with STI571 treatment. Moreover, transfected neurons with inactive GFP-c-Abl kinase have a diminished clusters number of PSD-95 than neurons transfected with GFP. Additionally, the contact synaptic between PSD-95 and pre-synaptic markers Synapsin I and SV-2 decrease in presence the specific inhibitor STI571.

CONCLUSIONS: The clustering and levels of PSD-95 are regulated by c-Abl, these results suggest that PSD-95 is a synaptic target of kinase c-Abl. The decrease of synaptic contact by inhibition of c-Abl activity suggests that this kinase could be involved in synaptogenic processes in the CNS.

Palavras-Chave: PSD-95, c-Abl, synapses

Apoio Financeiro: Fondecyt 1080221 / FONDAP 13980001

B.04.012

Synaptic distribution of Acyl-peptide Hydrolase in Central Nervous System

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Acyl-peptide hydrolase (ACPH, EC 3.4.19.1) is a serine-protease that belongs to the family of prolyl-oligopeptidases. This enzyme catalyzes the removal of N-acylated amino acids from short peptides to generate acylated amino acid and a peptide with a free N-terminal. It is present in the Central Nervous System (CNS) but neither its function nor its localisation is known, although it was described recently the degradation of the amyloid beta peptide (Ab) by this enzyme in conditioned media from neuroblastoma cell cultures. **Objective:** to determine the synaptic localisation of ACPH in rat cortex and hippocampus. **Methods:** kinetic analysis of ACPH in different synaptic fractions isolated by differential centrifugation and sucrose gradients at different pH. We used total brain homogenates (H), vesicular fraction (P3), Sinaptosomes (Sin), Light Membranes enriched in synaptic vesicles (LM) and Synaptic membranes after hyposmotic shock of Sin (SM) isolated at pH 8.1, to enrich mainly postsynaptic elements; and at pH 7.4, to enrich presynaptic elements in the synaptic fractions. **Results:** At pH 8.1, the kinetics analysis shown a 40% increase of ACPH activity in Sin compared to H (Sin=0.036 ± 0.0009 mmol min⁻¹ mg prot⁻¹, H=0.024 ± 0.0009 mmol min⁻¹ mg prot⁻¹, p<0.05). When Sin were subjected to hyposmotic shock and SM were analyzed, we found a 60% decrease of ACPH activity compared to H (SM=0.01 ± 0.001 mmol min⁻¹ mg prot⁻¹, H=0.024 ± 0.0009 mmol min⁻¹ mg prot⁻¹, p<0.01). At pH 7.4, the same kinetic analysis shown similar increase of ACPH activity in Sin but it was no decrease in SM. **Conclusions:** These results suggest a presynaptic localisation of ACPH and contribute to the understanding of the physiological role of ACPH on synaptic transmission in the CNS.

Palavras-Chave: Acyl-peptide hydrolase, Alzheimer disease, synaptic distribution, synaptic transmission

Apoio Financeiro: Proyecto Bicentenario PSD-11.

B.04.013

Rol de la proteína MAP1B en la regulación del citoesqueleto de actina en sinapsis

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Objetivos

La proteína asociada a microtúbulos MAP1B puede interaccionar con el citoesqueleto de actina. Esto se ve reflejado en que animales mutantes para MAP1B presentan un citoesqueleto de actina anormalmente polimerizado. Debido a que la integridad del citoesqueleto de actina puede tener efectos sobre el control de las propiedades sinápticas, hipotetizamos que en cerebro adulto la proteína MAP1B forma complejos multiproteicos con moléculas involucradas en remodelamiento del citoesqueleto de actina en las espinas dendríticas y terminales axónicos, lo que regularía los procesos de neurotransmisión, y/o plasticidad neuronal.

Metodología

Para evaluar la hipótesis hicimos una búsqueda de interactores para nuestra proteína, mediante la técnica de doble híbrido. Utilizamos la cadena liviana de la MAP1B (LC1) como cebo contra una genoteca de cerebro de ratón adulto, y detectamos las proteínas que interaccionan con MAP1B. La importancia de MAP1B en la regulación de la dinámica de actina se evaluó en cultivos de neuronas de hipocampo.

Resultados

La dinámica de actina esta alterada en las neuronas deficientes en MAP1B. El análisis de doble híbrido determinó que las proteínas Snapin y la Subunidad B1 de la Na⁺K⁺ATPasa, interaccionan positivamente con la LC1. Estas interacciones fueron posteriormente confirmadas en ensayos bioquímicos y celulares, en sistemas fisiológicamente relevantes.

Conclusiones

La MAP1B puede participar en el control de la dinámica de actina y regular actividad sináptica a través de dos posibles mecanismos: 1) modulación del control de estructuras especializadas en la transmisión sináptica (espinas dendríticas) o 2) a través de la interacción con proteínas que formen parte de multicomplejos proteicos (ej. Snapin) que regulen la actividad eléctrica de las neuronas en el hipocampo.

B.04.014

Calcium and ROS activate NFkB in electrical stimulated hippocampal neurons

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Neuronal activity induces a rise of intracellular calcium (Ca^{2+}_i) and generation of reactive oxygen species (ROS), which in turn participate in synaptic plasticity and memory consolidation. The cross-talk between them might regulate specific signaling pathways and the activity of transcription factors. **OBJECTIVE.** To characterize NFkB activation by electrical field stimulation in hippocampal neurons at two frequencies. **METHODS.** Rat hippocampal neurons in culture (10-14 DIV) were stimulated at 50 or 10 Hz with 1000 pulses. Time series of confocal images for Ca^{2+}_i and H_2O_2 production in a single cell were monitored simultaneously with FuraRed and by Hyper-Cyto protein, respectively. NFkB activation was identified by p65-eGFP nuclear translocation. All images were analyzed by Image J and Huygens software. **RESULTS.** At 50 Hz the increase in Ca^{2+}_i is diminished by DPI incubation, and H_2O_2 generation is dependent on ryanodine receptor activation. Similar results were obtained at 10 Hz. At 50 Hz, the stimulation increases p65 nuclear translocation (Control: nucleus 54%, cytoplasm 46%; 50 Hz: nucleus 72%, cytoplasm 23%), and is inhibited by ryanodine (nucleus 57%, cytoplasm 43%) and NAC (nucleus 59%, cytoplasm 41%). **CONCLUSIONS.** The results suggest that there is an interaction between Ca^{2+}_i and H_2O_2 increased by electrical physiological stimulation of hippocampal neurons. NFkB activation involves both RyR-mediated calcium release and ROS increase.

Palavras-Chave: calcium, ROS, NFkB

Apoio Financeiro: FONDECYT 1060177, FONDAP 15010006

B.04.015

Interferon gamma influences MHC class I expression and synaptic plasticity in alpha motoneurons after sciatic nerve transection

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Interferon gamma (IFN- γ) is a potent cytokine involved in the upregulation of the major histocompatibility complex (MHC I). MHC I has been recently shown to play an important role in the neuronal/glial response to injury, influencing the synaptic plasticity process. In this sense, the aim of the present work was to investigate the glial reactivity as well as the synaptic elimination in the spinal cord of C57BL/6J IFN- γ ^{-/-} mice, after unilateral sciatic nerve transection. One week after lesion, the animals were sacrificed and had their lumbar spinal cord processed for immunohistochemistry (anti-synaptophysin and anti-GFAP antisera were used), Western blotting (WB; synaptophysin and GFAP) and *in situ* hybridization (MHC I). The results showed a decrease of synaptophysin immunolabeling in the microenvironment surrounding large motoneurons (control 0.52 ± 0.06 , IFN- γ ^{-/-} 0.78 ± 0.08 ; $p < 0.05$), which was confirmed by WB (control 0.95 ± 0.07 , IFN- γ ^{-/-} 0.98 ± 0.08 ; $p < 0.05$). Following axotomy, GFAP immunolabeling revealed a decreased reactive astrogliosis in the knockout mice (control 3.91 ± 0.24 , IFN- γ ^{-/-} 3.16 ± 0.14 ; $p < 0.05$). Nevertheless, such down regulation was not detectable by WB (control 1.06 ± 0.04 , IFN- γ ^{-/-} 1.07 ± 0.07 ; $p > 0.05$), possibly because the astrogliosis was confined to the neuropil adjacent to the lesioned motoneurons. The absence of IFN- γ in the knockout mice decreased the expression of MHC I mRNA, as seen by *in situ* hybridization (control 2.89 ± 0.27 , IFN- γ ^{-/-} 1.22 ± 0.41 ; $p < 0.05$). In conclusion, the present results show the importance of MHC I upregulation after lesion to the proper response to injury, regarding astroglial reaction and synaptic elimination.

Palavras-Chave: synaptic plasticity, motoneuron, MHC class I, interferon gamma

Apoio Financeiro: Fapesp

B.04.016

Glatiramer acetate influences MHC class I up regulation and synaptic plasticity in mice subjected to the experimental autoimmune encephalomyelitis

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The occurrence of synaptic changes in the spinal cord has been linked to the clinical signs of the experimental autoimmune encephalomyelitis (EAE). Treatment with glatiramer acetate (GA) reduces the progress of EAE and may attenuate the synaptic elimination process. In the present study, we have investigated the changes of synaptic covering, glial reaction and the major histocompatibility complex (MHC I) expression during the course of EAE. Seven weeks old C57BL/6J female mice were used and distributed in one of three groups: control (normal, n= 5), placebo (EAE+saline solution, n= 5) and EAE+GA treatment (n=5). During the exacerbation of the disease (degree 3; paraplegia) the animals were sacrificed and their lumbar spinal cords were processed for immunohistochemistry (anti-synaptophysin, anti-GFAP, anti-Iba1 and anti-MHC class I antisera were used). The results revealed that GA treatment preserved synaptic contacts in the motor nucleus of the spinal cord (synaptophysin: treated, 15.34 ± 0.69 and placebo, 7.06 ± 0.81 , mean $\pm$ SE; $p < 0.001$), together with a less intensive astroglial (GFAP: GA treatment, 5.57 ± 0.97 and placebo, 13.14 ± 1.32 ; $p < 0.001$) and microglial reaction (Iba1: GA treatment, 6.88 ± 1.15 and placebo, 19.54 ± 0.97 ; $p < 0.001$). Interestingly, MHC class I expression was up regulated following EAE induction during the exacerbation phase. However, GA treated animals displayed a less intense MHC I labeling (GA treatment, 9.83 ± 0.83 and placebo, 13.18 ± 0.93 ; $p < 0.001$). This is in line with the concept that MHC I down regulation decreases the input retraction after lesion (PNAS, 101(51): 17843, 2004). In conclusion our data show that GA treatment acts on the control of glial reaction as well as on the stability of synaptic covering, what may be directly linked to the beneficial results of such drug on the clinical signs of EAE.

Palavras-Chave: EAE, Glatiramer Acetate, synaptic plasticity

Apoio Financeiro: FAPESP

B.04.017

Effects of enriched environment in densitometry of GFAP in the hippocampal astrocytes of mice

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Objectives: Exposure of animals to an enriched environment (EE) has long been known to have beneficial cognitive effects, increased neurogenesis, higher levels of neurotrophins and alterations in neurotransmitters and receptors. Various enrichment protocols have been increasingly used in the study of neuroplasticity, it is important to understand how changes in these factors can affect the effects of enrichment animals. Glial fibrillary acidic protein (GFAP) is an intermediate filament of astroglia and an important cell marker of mature astrocytes. Astrocytic shape is influenced by changes in GFAP expression. Therefore, this study is intended to determinate the shape and densitometry of regional and astrocytic GFAP immunohistochemistry (GFAPir) in the CA1 of hippocampus in mice.

Methods: Swiss albino (CF-1) mice underwent 8 weeks of environmental enrichment immediately after weaning. After 8 weeks, all animals were deeply anesthetized and injected of heparin. Thereafter, they were transcardially perfused with saline and 4% paraformaldehyde. The brains were removed and sectioned. Sections were collected and processed for GFAPir. Lacunoso Molecular (LM) and Radiatum (R) layers (CA1, hippocampus) were analyzed. The EE group was compared to control. It was analyzed mean $\pm$ standard deviation.

Results: The regional densitometry (LM and R) of GFAPir was not significantly different between EE and control ($0,127 \pm 0,0465$ vs $0,121 \pm 0,0422$ and $0,804 \pm 0,0303$ vs $0,0859 \pm 0,0343$, respectively). The astrocytic densitometry (LM and R) was not significantly different between EE ($0,426 \pm 0,0631$ vs $0,412 \pm 0,081$ and $0,341 \pm 0,071$ vs $0,358 \pm 0,087$, respectively). Therefore, the astrocytic shape is different.

Conclusions: Our data shows that there was no difference between EE and control in regional and astrocytic densitometry GFAPir parameters. After 8 weeks of EE, astrocytes shape became different compared to control, indicating morphology modification over hippocampal astrocytes, what suggests developmental and activational effect in synaptic formation and plasticity.

Palavras-Chave: Enrichment environment, Astrocytes, GFAP, densitometry

Apoio Financeiro: IBNNet, CNPq, CAPES, FINEP, FAPERGS.

B.04.018

A plasticidade sináptica e a neurogênese do hipocampo em ratos adultos submetidos a dieta multideficiente e ao ambiente enriquecido.

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O hipocampo tem papel funcional no processo de informação vinda do córtex, além de, modular diversos comportamentos e ser ideal para o estudo da organização e função celular e da formação de circuitos quando se utilizam estímulos ou insultos externos. A inadequação nutricional é um dos principais fatores que afetam o desenvolvimento do SNC. Por outro lado, um ambiente enriquecido (AE) induz neuroplasticidade em cérebro de ratos. O objetivo deste trabalho foi verificar os efeitos da malnutrição, utilizando a dieta básica regional que se mostra desbalanceada e pobre em diversos nutrientes, sobre a neurogênese e a plasticidade do hipocampo durante a fase adulta e se o AE poderia prevenir esses efeitos. Utilizamos a imunocitoquímica para a quantificar a proliferação (anti-BrdU), neuroblastos (doublecortina) e proteínas envolvidas na sinapse (anti-sinaptofisina). Ao MO observamos um aumento do número de células BrdU+ nos animais com dieta normal em AE ($493 \pm 19.9 \text{ mm}^3$) quando comparado aos animais controle (CTR) ($275 \pm 18.7 \text{ mm}^3$), acompanhado pelo aumento das projeções neuronais, conforme observado pelo aumento das células doublecortina+. A malnutrição causou uma diminuição no número de células BrdU+ ($150 \pm 18.0 \text{ mm}^3$) quando comparadas com grupo CTR, mas quando estes animais são também criados em AE há tanto um aumento número de células BrdU+ ($350 \pm 25.8 \text{ mm}^3$) quanto de suas projeções. Através da análise da densidade óptica da proteína sináptica sinaptofisina observamos que o AE causa um aumento da expressão (aumento em 28% – $p < 0.05$) em comparação ao CTR. O insulto da malnutrição causa diminuição desta proteína (diminuição em 42% – $p < 0.05$) mas quando é associada ao AE previne a diminuição mantendo a expressão da sinaptofisina como são observados no CTR. Estes dados sugerem que no ambiente enriquecido há um aumento tanto de neurogênese quanto da sinaptogênese e indicam que a atividade é um fator importante não somente na geração de novas células como na sobrevivência destas, tornando-as competentes para a formação de sinapses e integradas aos circuitos já existentes garantindo que não sejam extintas.

Palavras-Chave: malnutrição, ambiente enriquecido, neurogênese, plasticidade sináptica

Apoio Financeiro: CNPq

BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) AND SPATIAL MEMORY TRAINING INCREASE RYANODINE RECEPTOR EXPRESSION IN HIPPOCAMPAL NEURONS

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BDNF and Ca²⁺ contribute to hippocampal spatial memory formation. BDNF downstream signaling activates Ca²⁺ mobilization, transcription factors, gene expression¹, and dendritic spine growth². Ryanodine receptor (RyR)-mediated calcium-induced calcium release promotes dendritic spine growth³, while RyR inhibition blocks transcription involved in hippocampal synaptic plasticity. **Objectives:** To study RyR expression in hippocampal cells in primary culture incubated with BDNF, and in the hippocampus of rats trained in Morris Water Maze (MWM). **Methods:** Hippocampal cells were incubated with 50 ng/ml BDNF for 6 h. RyR were selectively inhibited by preincubating cells with 50 mM ryanodine for 60 min. Rats were trained for spatial memory (5d, 2d rest, 1d platform free) in the MWM; the hippocampus was dissected 6 h after the last behavioral task. Expression of RNA and proteins was determined by RT-PCR, Western blot and immunocytochemistry. RyR-mediated calcium release was analyzed by confocal microscopy in cells preloaded with FLUO-4 AM and exposed to 4-chloro-methyl cresol, a RyR agonist. **Results:** Neurons incubated with BDNF increased RyR2 (2.1 ± 0.2; 4.5 ± 0.3) and RyR3 (1.59 ± 0.04; 2.1 ± 0.3) expression at the mRNA and protein level, respectively (Mean±SE); RyR inhibition abolished these stimulatory effects. BDNF also increased RyR activity, reflected as an increment in Ca²⁺ signal amplitude, and increased TrkB expression. The hippocampus of trained rats showed increased protein levels of RyR2 as described⁴, and RyR3. **Conclusions:** Our results suggest that BDNF and spatial memory training promote the expression of RyR isoforms that may provide the calcium signals required for new synapsis formation and memory consolidation.

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2.- Learn Mem. 11:172, 2004.

3.- Proc Natl Acad Sci. 96:12068,1998.

4.- FASEB J. 14: 290, 2000.

Palavras-Chave: Ryanodine receptor, BDNF, calcium, spacial memory.

Apoio Financeiro: FONDAP 15010006; FONDECYT 1060177; FONDECYT-Post-Doc 3070035; AT-24080073

B.04.020

Zif-268 induction in response to novelty in the medial cortex of the tropical lizard *Tropidurus hispidus*

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Objectives: The cerebral lizard cortex is divided in four regions: medial, dorso-medial, dorsal and lateral cortex, and have 3 separated layers, as follows: inner plexiform, cellular layer, and outer plexiform layer. The reptile medial and dorsal cortices are homologous to the mammalian hippocampus, and are thought to be responsible for spatial memory formation due to environmental inputs in lizards. These brain areas are expected to share with mammals similarities in mnemonic processes considering inter-specific behavioral comparisons. Regarding intracellular pathways linked to memory consolidation, one important step is synaptic plasticity mediated by the protein Zif-268. Here we used immunohistochemistry to test whether exposure to a novel environment elicits zif-268 induction in the lizard medial cortex.

Methods: We used specimens of the tropical lizard *Tropidurus hispidus*, captured in the UFS campus, with environmental authority (IBAMA) approval. Two of them were used as controls (two hours in a dark room at 18° C), and two were tested in a new object arena interaction task (a round arena with different rocks of similar size) measuring 1m diameter at 35-38° C. After two hours, animals were perfused with paraformaldehyde, brain sections were obtained in a cryostat, and immunohistochemistry for zif-268 was performed (EGR-1 c-19; Santa CruzTM).

Results and Conclusions: Our preliminary results suggest that zif-268 is induced in the dorsomedial cortex of novelty-exposed lizards, in comparison with controls.

Palavras-Chave: lizard; memory; neural plasticity; zif-268; immediate-early genes

Apoio Financeiro: AASDAP; CAPES; CNPq

B.04.021

NEURONAL PLASTICITY IN CA1 HIPPOCAMPAL NEURONS AFTER HIPOXIA-ISCHEMIA

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During hypoxia episode, synaptic dysfunction observed has been related with neuronal death, although this mechanism is not fully understood yet. In previous work we have shown modifications in neurons and synapses in the neostriatum of rat subjected to hipoxia. In this work we study the effects of hypoxia-ischemia on the actin cytoskeleton of Hippocampal CA1 post-synaptic densities (PSD) and the actin binding proteins in 30 days olds rats, by combining photooxidation, immunofluorescent, 3D reconstruction and Western blot. In addition, we test the effects of reduced temperature from 37°C to 15°C on dendritic spines. Staining with phalloidin-alexa 568 followed by confocal microscopy analysis showed an increase of F actin fluorescent staining in the CA1 Hippocampal dendritic spines (Relative fluorescent intensity: control 15.1 ± 3 vs hypoxic animals 33.5 ± 6 $p < 0.001$). Electron microscopy and 3-D reconstruction confirmed these observations showing an increment in the number of F-actin staining spines in CA1 hippocampal excitatory synapses subjected to severe hypoxia (control 2 ± 0.1 vs. hypoxic animals 3.6 ± 0.2 $p < 0.001$). Western blot revealed β -actin increase in hypoxic animals, consistent with the microscopy data (D.O.R: control: 1.43 ± 0.05 vs. hypoxic animals 2.98 ± 0.41 $p < 0.001$). Staining with profilin-alexa 488 followed by confocal microscopy analysis showed an increase of pyramidal neurons immunostaining, located in the stratum radiatum (Relative fluorescent intensity: control 20.4 ± 3 vs hypoxic animals 35.9 ± 6 $p < 0.005$). When hypoxia was induced under hypothermic conditions the changes observed were blocked. These results suggest that the cellular and subcellular alterations in CA1 hippocampal PSDs could be related to F-actin increase probably due to an alteration in profiling dynamic inducing more actin polymerization and changes in the dendritic synaptic cytoarchitecture.

Palavras-Chave: Dendritic Spine, Hypoxia, Profilin, Actin cytoarchitecture, Hippocampal

Apoio Financeiro: ANPCyT 15001, PID 5782, M020.

Differential effect of Zn^{2+} on long-term potentiation through $P2X_4$ receptors in CA1 area of rat hippocampus

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Zn^{2+} is an essential metal abundant in the CNS especially in glutamatergic synapses from hippocampus. However, the role that this metal plays in synaptic plasticity processes as long-term potentiation (LTP) is still far to be understood. The aim of this work was to study how different Zn^{2+} concentrations affect the induction of LTP and to know the molecular mechanisms involved in these effects. Slices of 400 μ m thick were obtained from 2-4 week-old Sprague-Dawley rats. Field excitatory postsynaptic potentials were recorded in CA1 area of hippocampus. LTP was induced by theta burst stimulation (TBS) applied from Schaffer collaterals. Zn^{2+} superfused for 10 min before and 10 min after TBS, showed a biphasic bell-shaped effect on the TBS-induced LTP. Low concentrations of Zn^{2+} facilitate in a concentration-dependent manner the LTP. Thus, 10 and 50 μ M of Zn^{2+} potentiated from 239.5 $\pm$ 22.1% to 401.4 $\pm$ 55.2% (n=15-18, p<0.01) and from 209.6 $\pm$ 14.2% to 307.3 $\pm$ 47.8% (n=7, p=0.08) the LTP, respectively. In contrast, 100 μ M Zn^{2+} inhibited the TBS-induced LTP from 277 $\pm$ 36.8 to 113.1 $\pm$ 54.9 (n=3, p<0.05). A similar effect was observed with 300 μ M. On the other hand, a biphasic effect also was obtained with extracellular ATP. Low micromolar concentrations of ATP (0.1-1 μ M) augmented TBS-induced LTP. A significant increase, from 172.3 $\pm$ 15.8% in absence to 236.1 $\pm$ 26.3% in presence of 1 μ M ATP (n=12-13, p<0.05) was observed. ATP at higher concentrations inhibited LTP from 209.1 $\pm$ 22.5% to 123.3 $\pm$ 24.7% with 5 μ M and from 180.8 $\pm$ 21.4% to 95.8 $\pm$ 12.3% with 10 μ M (both, n=9-11, p<0.01). Furthermore, a displacement to the left of the ATP concentration-response curve was observed in presence of 5 μ M Zn^{2+} , a concentration which did not affect the LTP. Finally, we found that oxidized ATP, a $P2X$ receptors antagonist, fully prevented the Zn^{2+} -induced facilitation of LTP. Ivermectin, a selective $P2X_4$ receptor modulator, increased in a similar manner to Zn^{2+} the TBS-induced LTP. In conclusion, Zn^{2+} exerts a differential effect on LTP. We demonstrate for the first time that Zn^{2+} , at low concentrations, facilitates the TBS-induced LTP in CA1 of hippocampus probably through modulation of $P2X_4$ receptors. **Palavras-Chave:** long-term potentiation; zinc; purinergic receptors; $P2X$; hippocampus; synaptic plasticity

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IMPACT OF ESSENTIAL FATTY ACIDS RESTRICTION IN MAINTENANCE OF CONNECTIONS IN THE RAT VISUAL SYSTEM

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The development of sensory neuronal circuitry occurs during a critical period when extensive axonal elimination and plasticity precede the stabilization of topographical representation. Among the mechanisms of synaptic maintenance, essential fatty acids (EFA) appear as important messengers, since the blockade of arachidonic acid synthesis, induces sprouting in the retinotectal pathway of adult rats (Neuroscience, 139:979, 2006). In the present study, we tested the effect of EFA restriction in the long-term stabilization of connections in the visual system and in a plasticity model following a contralateral retinal lesion. Lister Hooded female rats were fed during 5 weeks before mating (30g/day) with either a control (soy oil) or a restricted (coconut oil) diet. After birth, litters were fed until postnatal day 28 (PND 28) or PND 42 when they received an intraocular injection of HRP. Another group received a single retinal lesion at the temporal periphery at PND 21. Serial coronal brain sections (40µm) were processed according to Mesulam's protocol (1978). For statistical analysis we considered as significant differences of $p < 0.05$. EFA restriction produced a significant increase in the optical density in the superficial layers of the superior colliculus, as a result of axonal sprouting outside the main terminal zones. In the intermediate aspect of the stratum griseum superficiale (SGS) we observed an increase in optical density of fibers at PND 28 and in PND 42. We also observed increased fiber density in the intercluster region indicating an intralaminar sprouting as well. After a contralateral retinal lesion, the EFA restricted group displayed increased sprouting of intact axons at the lesion site as compared with the control group. The data indicate that food restriction of arachidonic acid (AA) and docosahexaenoic acid (DHA) induces a disruption in the topographical map organization and enhanced sprouting capacity of retinal axons, consistent with a role for EFA as precursors of stabilizing synaptic agents in the visual system of adult rats.

Palavras-Chave: essential fatty acids; plasticity; synaptic maintenance; topographic maps; retinotectal

Apoio Financeiro: CNPq, FAPERJ, CAPES, PRONEX-MCT

MUSCARINIC MODULATION OF LONG-TERM DEPRESSION IN THE HIPPOCAMPUS-PREFRONTAL CORTEX PROJECTIONS IN VIVO

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Objectives: The brain cholinergic system modulates perception and memory, besides long-term potentiation (LTP) and long-term depression (LTD) involving the hippocampus and cortex. Moreover, impairments of this system are associated with psychiatric and cognitive disorders. Here, we investigated the muscarinic modulation of LTD induction and maintenance in the hippocampal-medial prefrontal cortex (mPFC) pathway. **Material and Methods:** Urethane-anaesthetized rats were implanted with CA1 and mPFC microelectrodes for stimulation and recording of postsynaptic potentials (fEPSP), while metal cannulae positioned above the lateral ventricle was used for drug injection, under body temperature control. After 30min of fEPSP baseline recordings, rats received either a microinjection of the muscarinic agonist, pilocarpine (PILO; 40nmol; 1mL, in artificial spinal fluid, aCSF), or an injection of aCSF (control animals). LTD was induced immediately after the injections and fEPSPs were monitored for an additional 240min. In pilot experiments, CA1 and mPFC oscillatory activities (delta, theta, sigma, gamma relative power) were monitored after different PILO doses in order to determine the latency and duration of the cholinergic desynchronization. **Results:** Our results showed that PILO significantly facilitates the induction of CA1-mPFC LTD ($-24.8 \pm 2.6\%$ of baseline; $n=5$) compared to control rats ($-10.5 \pm 3.0\%$ of baseline; $n=5$; paired Student's t-test; $p<0.05$). PILO also enhanced the maintenance of LTD at 60min ($-16.5 \pm 2.5\%$ of baseline) compared to control rats ($+6.0 \pm 2.4\%$ of baseline; Two Way ANOVA, Newman-Keuls, $p<0.05$). Besides, PILO effects were associated to a stable EEG desynchronization for 11.6 ± 4.83 min. **Conclusion:** Together with recent data from our lab [1], these findings indicate that muscarinic cholinergic activation promotes a bidirectional (LTP and LTD) regulation of synaptic strength in the CA1-mPFC pathway. Such regulation may underlie some of the effects of arousal, attention and REMS on memory consolidation processes. **References:** [1] Neuroscience 153:1309, 2008.

Palavras-Chave: Memory; Long-term depression; Cholinergic system; Prefrontal cortex; Synaptic Plasticity

Apoio Financeiro: Fapesp; Fapesp - Cinapce; CNPq; CAPES - Prodoc; FAEPA.

MONOCULAR ENUCLEATION INDUCES DIFFERENTIAL PLASTICITY AND MICROGLIAL ACTIVATION IN AND OUT THE CRITICAL PERIOD.

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Objectives: Several types of lesions are able to induce plasticity of intact circuits in the central nervous system. Meanwhile too much progress has been made to understand the mechanisms shared between immune system activation and lesion-induced plasticity. We used the retinotectal pathways to study the correlation between plasticity and microglia activation during the critical period of synaptic development. We also investigated the role of calcineurin (CaN), involved both in long-term depression and immunological activation. **Methods:** Lister Hooded rats were submitted to monocular enucleation at postnatal day 10 (PND10) or PND21 with different survival-times. Animals received intraocular injections of horseradish peroxidase (HRP) as an anterograde tracer. The timing of microglial activation after monocular enucleation at PND10 was evaluated through immunohistochemistry with isolectin B4. Yet, animals received local drug delivery treatment using ELVAX implants filled with CaN antagonists cyclosporine A (CsA - 1mM) and CaN inhibitory peptide (CIP - 285µM). **Results:** During the critical period (PND10), monocular enucleation induced in the contralateral superior colliculus (SC) an unspecific sprouting of intact axons 24h after denervation. In later stages (PND21) the deafferentation induced a more limited intralaminar expansion of ipsilateral axons. Microglial activation was detected as soon as 6h after enucleation and showed a vigorous increase 1 week after the lesion in the contralateral SC. Local delivery of CaN antagonists did not interfere with microglial activation. **Conclusions:** The present results point out that a critical period is determinant to the magnitude and pattern of plasticity after a monocular enucleation. Also, the progressive increase in microglia activation in the SC correlates with the limitation in plasticity of intact axons 1 week after the lesion. The data also suggest that the local delivery of CsA may be insufficient to guarantee global microglia inhibition over the brain. CsA systemic administration is needed to verify the effect of global CaN inhibition in the timing of microglia activation and plasticity enhancement. **Palavras-Chave:** monocular enucleation, plasticity, microglia, calcineurin

Apoio Financeiro: CAPES, CNPq, FAPERJ, PRONEX-MCT

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Comparación de dos formas de inducción de potenciación prolongada en la sinapsis CA3-CA1 de hipocampo. Efecto del antagonista muscarínico escopolamina.

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La potenciación de larga duración (LTP) que ocurre en las sinapsis glutamatérgicas CA3-CA1 del hipocampo ha sido utilizada como modelo para el estudio de plasticidad sináptica. Se han usado varios tipos de protocolos de estimulación eléctrica para su inducción, como la estimulación por alta frecuencia (HF) y por frecuencia theta (TF).

Previamente estudiamos los efectos del antagonista muscarínico escopolamina sobre la neurotransmisión basal y la potenciada por HF (3 trenes de 1 segundo a 100 Hz, con intervalos de 10 min.).

Objetivo y métodos: Utilizamos un protocolo TF (3 trenes de 25 ráfagas c/u de 4 pulsos a 100 Hz cada ráfaga, con intervalos de 10 seg., separadas por 200 miliseg.) para evaluar posibles diferencias entre ambas formas de potenciación en registros de campo en rebanadas de hipocampo de rata, utilizando como parámetro la pendiente del potencial excitatorio postsináptico de campo (fEPSP).

Resultados: Hubo diferencias entre los resultados obtenidos por aplicación de las diferentes formas de estimulación. La inducción por HF produjo una potenciación de $78 \pm 4\%$, mientras que la inducción por TF potenció $38 \pm 3\%$.

Utilizando estimulación con pulsos pareados se evaluó la facilitación sináptica. La estimulación HF no modificó significativamente la facilitación observada en condiciones basales. Pero la estimulación TF incrementó levemente dicha facilitación ($6 \pm 3\%$).

Escopolamina 10 mM afectó la transmisión basal (reduciéndola en $15 \pm 7\%$) e impidió la potenciación inducida por HF; a concentraciones menores, sólo afectó la potenciación, en forma dosis-dependiente. En cambio, cuando se estimuló con TF, escopolamina 25 μ M impidió la potenciación ($p < 0.01$) sin afectar la transmisión basal. La facilitación, tanto en condiciones basales como luego de la inducción por HF o por TF, no resultó afectada por escopolamina (2,5 μ M-10 mM para HF; 5 y 25 μ M para TF).

Conclusiones: Mientras que parece haber un efecto leve sobre la facilitación por pulsos pareados, los resultados de las dos formas de inducir LTP muestran diferencias significativas en cuanto a la magnitud de la potenciación y al efecto de escopolamina, lo que sugiere que HF y TF pondrían en evidencia mecanismos diferentes.

Palavras-Chave: LTP, escopolamina, muscarínicos, hipocampo, frecuencia theta

Apoio Financeiro: UBA, CONICET

CALCIUM-DEPENDENT-KINASES AND CALCINEURIN MODULATE THE SPECIFICITY OF SENSORY VISUAL CONNECTIONS IN DEVELOPING RATS.

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We studied the functional roles of Calcium/calmodulin-dependent protein kinase II (CAMKII), Protein kinase C (PKC) and Calcineurin (CaN), recognized as important modulators of synaptic efficacy, in the development of the refinement and specificity of visual retinotectal maps. At postnatal day 7 (PND7), Lister Hooded rats were submitted to intracranial delivery of CAMKII, PKC and/or CaN inhibitors, through ELVAX implants containing, respectively, KN-93 (1,5mM), Chelirithrine Chloride (CHE – 10,0mM) and/or Ciclosporin A (CsA – 1,0mM) or vehicle (DMSO). The distribution of the uncrossed retinotectal terminal fields was examined following intraocular injections of horseradish peroxidase (HRP 30%) in aldehyde-fixed, free floating sections (40µm) through the superior colliculus at PND14. Animals treated with CsA during the second postnatal week showed a dispersed distribution of uncrossed axons with several fibers located outside the main clusters of terminal zones labeling as compared to vehicle-treated animals. On the other hand, the treatment with protein kinase inhibitors, KN-93 and/or CHE, induced an increased convergence of fibers into terminal zones, with a resulting sharpening of clusters. Additionally, the administration of KN-93 or CHE plus CsA was able to partially reverse the effects of kinase or phosphatase inhibitors, resulting in a distribution pattern similar to that observed in controls. The present results indicate that the balance between kinase and phosphatase systems is able to modulate the topographical specificity during the initial stages of the visual system development, when the connections are more sensitive to use-dependent influences – the critical period. We suggest that a shift towards phosphorylation may result in axonal sprouting and synaptic stabilization in inappropriate target regions. On the other hand, a shift favoring dephosphorylation might induce an excess in axonal elimination and therefore an increase in the sharpness of terminal fields.

Palavras-Chave: calcineurin, pkc, CAMKII, synaptic plasticity, retinotectal development

Apoio Financeiro: FAPERJ, PRONEX-MCT, CNPq, CAPES

5-HT₂ and D₁ receptors participate in the modulatory effect of MDMA on synaptic plasticity

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3,4-methylenedioxymethamphetamine (MDMA) is an amphetamine analog drug illegally used with recreational aims, that in chronic and acute treatment affect cognitive process. Previously, we demonstrate for the first time that MDMA modulate differentially the long-term potentiation (LTP) and long term depression (LTD); synaptic plasticity models widely accepted as may occur learning and memory. In this work we studied the cellular and molecular mechanisms of how MDMA exerts its modulatory effect on LTP and LTD in CA1 area from rat hippocampus. Slices 400 µm thick were obtained from 3-5 week-old Sprague-Dawley rats. Field EPSPs were evoked with electrical stimulation from Schaeffer collaterals. LTP or LTD were recorded after either theta burst (TBS, 10 trains) or low frequency (LFS, 1Hz/900 pulses) stimulation protocols, respectively. MDMA, agonists and antagonists were superfused 10 min before and 10 min after stimulation. MDMA increased in a concentration dependent manner the LTP induced by TBS with an EC₅₀ of 18.39 ± 0.03 mM. 50 µM MDMA remarkably increase the magnitude of the TBS-induced LTP from +147 ± 34 % to +404 ± 125% (n=6,6; p<0.05). Although a small effect was observed with 50 µM MDMA on LFS-induced LTD (p=0.39), we not found a significant differences in any of the concentrations applied. To know the mechanism we used a known antagonist of 5-HT₂ receptors. 20 µM ketanserin abolished the increase of 50 µM MDMA from +395 ± 105% to +52 ± 7% (n=5,6; p<0.05). Since previous studies showed that serotonin inhibit the LTP, we hypothesized that in the enhanced of MDMA-dependent LTP could be involved the dopamine neurotransmitter. 5 µM SCH23390, an antagonist of D₁/D₅ receptors subtype, prevented the increasing of LTP induced by 50 µM MDMA from +347 ± 99 % to +245 ± 82% (n=6,6; p<0.05). Moreover, this was recently confirm by preliminary results in where is co-applied serotonergic agonists with D₁ antagonists. These results suggest that serotonergic receptors of the 5-HT_{2A/2C} subtypes and dopaminergic receptors of the D₁ subtype participate in the mechanism which MDMA modulate the synaptic plasticity.

Palavras-Chave: long-term potentiation, long-term depression, MDMA, 5-HT, Dopamine, hippocampus, synaptic plasticity

Apoio Financeiro: FONDECYT grant 1080652 and grant DICYT-USACH

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Retinotectal plasticity and pGAP-43 modulation

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The rat visual system presents a precise retinotopic organization, that develops, from diffuse projections, within the first two weeks after birth. This precise retinotopic map is generated and maintained by acoplated pre- and post-synaptic activity, which could be related to retrograde signaling.

Previously, our group showed that both retinal lesions and the inhibition of retrograde messengers synthesis such as nitric oxide (NO) and arachidonic acid (AA) induce and expansion of uncrossed retinotectal axons toward the superior colliculus surface. The axon growth phenomena is frequently associated to reorganization of growth cone and pGAP-43 up-regulation. In these context, we decided to study pGAP-43 expression and time-course modulation of this protein after different retinotectal induced-plasticity models.

To achieve these goals, Lister Hooded rats were submitted to monocular enucleation protocols or retrograde messengers blockade. One group of animals received a monocular enucleation at PND10 or PND21 with different survival-time. Another group of animals received a daily injection of either nitric oxide synthase (L-Narg 50 mg/kg, ip) or phospholipase A2 (Quinacrine – 40 mg/Kg, ip.) inhibitor between PND9-12 or PND20-27, respectively. Control animals were treated with saline (vehicle). Afterwards, the visual layers of superior colliculus were extracted and submitted to western blot analysis.

Our results show that monocular enucleation induces an up-regulation in pGAP-43. At PND10 the great increase was observed 24 hours after lesion. No difference was detected 72 hours after-lesion. In contrast, at PND21 the increase was obtained one week after lesion. These results are in concordance with the retinal lesion induced retinotectal plasticity time-course, which are faster inside the critical period than afterwards. Moreover, animals submitted to L-Narg treatment presented a decrease in pGAP-43 expression, whereas Quinacrine treatment leads to an up-regulation in pGAP-43.

Taken together, these data suggest that the modulation of pGAP-43 expression is an important event that could be associated with the sprouting observed in these models of retinotectal plasticity.

Palavras-Chave: GAP-43, plasticity, nitric oxide, arachidonic acid, phospholipase A2

Apoio Financeiro: Capes, CNPq, FAPERJ, PROPP-UFF

MODULATION OF MÜLLER CELLS ACTIVATION BY IPSILATERAL INTRAVITREOUS IL-2 INJECTION AND CONTRALATERAL RETINAL LESION

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Objective: Glial cells play an important role in the development and regeneration of the nervous system. Müller cells, the predominant glia in the retina, synthesize and respond to many signaling molecules. During the development and following different injury, these cells up-regulate glial fibrillary acidic protein (GFAP) which has been associated with a switch to an activated state. This change possibly represents a Müller cell's mechanism to protect the retina from damage and it can be modulated by factors like cytokines. The aim of this work was to study the regulation of rat retinal Müller cells reactivity after ipsilateral intravitreal interleukin (IL)-2 treatment and contralateral retinal injury. The retinal lesions represent an important model to study retinotectal plasticity.

Methods: Lister Hooded rats were divided in 2 groups. A group was submitted to the left temporal retinal lesion (TRL) at PND7 and the other, not lesioned, was used as control. Both groups received three IL-2 or PBS injections in the right eye between the PND7-13. The rats were fixed in PND14 with paraformaldehyde 4%. After cryoprotection, the eyes were cut in 10µm sections. For immunohistochemistry, the eyes sections were incubated with anti-GFAP (1:200), biotinylated antibody, avidin-biotin-HRP complex and the staining visualized after incubation with DAB. A group of normal treated-animals were used for western blotting.

Results: The IL-2 treated retinas presented an increase in GFAP expression compared to PBS injected retinas. Moreover, after contralateral TRL, Müller cells in the intact retina also up-regulated GFAP protein. In contrast, after contralateral TRL and ipsilateral IL-2 injection, a reduction in the GFAP expression was observed.

Conclusion: The IL-2 *in vivo* treatment increases the Müller cells reactivity, as demonstrated by the increase GFAP expression. So, our results strengthen the hypothesis that the Müller cells are active elements in the visual information processing. Likewise, TRL induced activation in the contralateral intact retina suggesting a binocular interaction which could involve superior visual targets as superior colliculus. **Palavras-Chave:** IL-2, retina, GFAP, muller cells, retinal lesion

Apoio Financeiro: CAPES, FAPERJ, PROAP-UFF, PRONEX-MCT

B.04.031

MODULATION OF GLIAL REACTIVITY IN THE SUPERIOR COLLICULUS BY *IN VIVO* IL-2 TREATMENT IN NORMAL OR RETINAL LESIONED RATS

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Objectives: Glial cells are key elements on development and regeneration of the CNS and are able to produce and respond to cytokines. They undergo change in cell shape during the development and after CNS injuries. In the visual system, retinal lesions, within the critical period of development, induce a rapid plastic response characterized by an expansion of ipsilateral pathway of the intact eye to superficial layers of superior colliculus (SC). The aim of this work was to study the modulation of astrocytes reactivity in the SC after intravitreal IL-2 treatment and contralateral retina injury.

Methods: Lister Hooded rats were submitted to left temporal retinal lesion (TRL) at PND10. Normal rats were used as control. Both groups received IL-2 or PBS injections in the right eye at PND7/10/13. For immunohistochemistry, brain sections were incubated with anti-GFAP antibody (1:100) and fluorescent (Alexa 488) secondary antibody. A group of unlesioned treated-animals were used for western blotting.

Results: Intravitreal IL-2 treatment leads to an increase in GFAP immunoreactivity in the superior colliculus compared to PBS-treated rats. This effect was confirmed after the quantitative analysis of GFAP expression performed by western blot assay. Likewise, temporal retinal lesion induced an up-regulation of GFAP expression in the superior colliculus. In contrast, after TRL, the SC of IL-2 treated rats presented a reduction of GFAP expression compared to PBS-treated group.

Conclusion: Our results indicate that GFAP expression is modulated by interleukin-2 in the visual system and support the hypothesis that retinotectal development and plasticity could be related to glial cells activation.

Palavras-Chave: IL-2, GFAP, astrocytes, plasticity

Apoio Financeiro: CAPES, FAPERJ, PROAP-UFF, PRONEX-MCT

B.05.001

Atividade da Cadeia Respiratória Mitocondrial em Cérebro de Ratos Após a Indução de Sepses por Ligação e Perfuração Cecal

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Objetivos: Diversos mediadores inflamatórios potencialmente neurotóxicos são liberados durante o desenvolvimento da sepse. Esta liberação está associada a uma resposta inflamatória do sistema imunológico à infecção (J Neurochem, 100:1375, 2007). Estudo recente mostra que a sepse induz a danos oxidativos em tecido cerebral nas primeiras horas após a técnica de ligação e perfuração cecal (CLP) (Crit Care Méd, 34:886, 2006). Neste contexto, o objetivo deste trabalho foi avaliar a atividade da cadeia respiratória mitocondrial no cérebro de ratos após indução de sepse por ligação e perfuração cecal (CLP).

Métodos: Ratos Wistar machos (300-350g) foram submetidos a CLP (grupo sepse; n=6, ressuscitação volêmica + antibióticoterapia) e ao sham (grupo controle; n=6; ressuscitação volêmica). Alguns tempos (0, 6, 12, 24, 48, e 96 horas) após CLP os animais foram mortos por decaptação e as estruturas cerebrais (cerebelo, hipocampo, estriado e córtex) foram retirados e armazenados a -80 graus celcius para posterior análise da atividade da cadeia respiratória mitocondrial e da enzima succinato desidrogenase. Para análise dos dados, foi utilizado ANOVA post-hoc Tukey com significância de 0.05.

Resultados: Foi observado que os animais submetidos ao CLP apresentaram diminuição significativa na atividade do complexo I em cerebelo ($F_{(6-30)}=9,370$; $p<0,05$), hipocampo ($F_{(6-29)}=4,704$; $p<0,05$) e córtex ($F_{(6-34)}=6,602$; $p<0,05$) e da enzima succinato desidrogenase em cerebelo ($F_{(6-33)}=4,086$; $p<0,05$), hipocampo ($F_{(6-32)}=2,659$; $p<0,05$) e córtex ($F_{(6-34)}=4,166$; $p<0,05$) da cadeia respiratória mitocondrial nas horas mais tardias (24, 48 e 96 horas) após a indução em comparação ao grupo sham. Não houve alteração significativa na atividade nos complexos II, III e IV em relação ao grupo sham.

Conclusão: A sepse associada a lesão cerebral pode induzir disfunção na cadeia respiratória mitocondrial e na enzima succinato desidrogenase nos tempos posteriores a indução da técnica.

Palavras-Chave: Sepses, Cadeia Respiratória Mitocondrial, Succinato Desidrogenase

Apoio Financeiro: CNPq, UNESC; Instituto Cérebro e Mente

CHARACTERIZATION OF LIPID RAFTS IN OLFACTORY ENSHEATHING CELLS

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Olfactory ensheathing cells (OEC) are candidates for therapeutic approaches for neural regeneration due to their ability to assist axon regrowth in central nervous system lesion models. However, little is understood about the processes and mechanisms underlying migration of these cells. For this reason, the characterization of lipid rafts and descending events involved in OEC's migration may become a central issue on the wider use of these cells in regeneration studies. Lipid rafts are cholesterol-rich, and detergent-resistant microdomains found in plasma membranes of many cells. These domains play a role in a wide range of important biological processes, including signal transduction pathways, apoptosis, cell adhesion, migration and organization of cytoskeleton. Our aim is the purification and characterization of OEC's lipid rafts, verifying the presence of CNPase (non-compact myelin protein 2' 3' cyclic nucleotide 3' phosphodiesterase), 9-*O*-acetyl GD3 and other gangliosides recognized by the A2B5 antibody, which are commonly expressed by high motility neural cells, such as young neurons, oligodendrocyte progenitors and glioma cells.

Purified cells were sonicated, treated with 1% Triton X-100 and submitted to a sucrose gradient. The detergent resistant fraction was collected, assayed for cholesterol and protein level and submitted to dot blot analyses. Lipid rafts markers (cholera B toxin and anti-flotilin-1 antibody) showed positive staining in dot blots in cholesterol-rich/protein-poor membrane sub-fractions. Immunoblottings were also performed focusing on the gangliosides mentioned above and on CNPase, resulting in raft fractions positive immunostaining. The positive dot blot fraction was submitted to lipid composition analysis, showing composition similar to that of raft fractions of other eukaryotic cells.

Our data suggest the OEC rafts appear to be at least partly involved in migration, since these microdomains express CNPase, that is crucial for axon ensheathment by oligodendrocytes, 9-*O*-acetyl GD3 and A2B5-reactive gangliosides.

Palavras-Chave: Olfactory ensheathing cells, lipid rafts

Apoio Financeiro: Capes, CNPq, FAPERJ

B.05.003

Mannose receptor is involved in the phagocytosis of *Streptococcus pneumoniae* by olfactory ensheathing cells

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Olfactory ensheathing cells (OECs) is a special glial type derived from progenitors in the olfactory mucosa that ensheath olfactory receptor axons. Axonal bundles extend toward the cribriform plate and enter the brain via the olfactory phila, thus, providing a potential route for access of pathogens to the brain. *Streptococcus pneumoniae* is the most common cause of acute bacterial rhinosinusitis that may evolve to meningitis. These Gram⁺ bacteria have a polysaccharide capsule, rich in mannosyl residues, which can act as a virulence factor for cell infection. Since OECs express the mannose receptor (MR – Carvalho LA, 2008), we tested whether OECs *in vitro* could internalize *S. pneumoniae* via MR.

OECs cultures were infected by a suspension of *S. pneumoniae* (ATCC 49619) in a ratio of 1:100 bacteria/cells. For the dark field microscopy analyses, the infected cultures were washed, fixed with paraformaldehyde and stained with DAPI. Competition assays were performed by infecting cultures in the presence of 100 mg/mL of mannan. For immunoelectron microscopy assay, infected cultures were washed, scraped out from the culture dishes and fixed in a mixture of aldehydes. After fixation, the samples were dehydrated and embedded in LR-White resin. Thin sections were incubated in blocking buffer and then in a primary anti-cMR antibody (Ab) produced by Régnier-Vigouroux. The secondary Ab was a 10 nm gold-labeled goat anti-rabbit IgG. Sections were stained in uranyl-lead and examined in a Zeiss EM 900 electron microscope (EM).

EM analyses showed OEG tagged by old particles close to the phagosomes containing internalized bacteria. This was confirmed by competition analyses showing a substantial reduction in the number of internalized bacteria by OEG when incubated in the presence of excess mannan.

Our data suggest a possible involvement of MR in the initial steps of the bacterial adhesion on OECs cellular surface. They also point out to a prospectively important role of OECs as a host cell during the bacterial invasion of the brain via the olfactory route.

Palavras-Chave: Mannose Receptor, *Streptococcus pneumoniae*, Olfactory ensheathing glial

Apoio Financeiro: FAPERJ, CNPq

B.05.004

Matching between the computational properties of the neurons and the circuits that they form.

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Objetives: This study was designed to provide evidences in favor the hypothesis that dynamic properties of the circuits and their constitutive neurons are well correlated. We used the electrosensory system of pulse weakly electric fish because it has two main parallel pathways with different dynamic properties that evaluate the same series of discrete images: a) the fast pathway (FEP) encode pulse to pulse the images using a latency code, without any temporal integration(1); b) the slow pathway (SEP) integrate series of images and detect differences between actual images and the moving averages of the recent past images (2). **Methods:** To analyze output cells intrinsic properties in the two pathways, we used *in vitro* whole cell patch combined with computer modeling. **Results:** The spherical cells of the FEP respond with a single spike at the onset of square current pulses independently on the duration and the intensity, and follow one to one sine waves currents between 10 and 150 Hz. White noise injection caused excitability depression. Computer modeling and pharmacological experiments indicate that the intrinsic properties of these cells are mainly based on a low threshold, non inactivating potassium conductance, and a mixed cationic conductance (I_h). The basilar pyramidal cells of the SEP respond to depolarization with a bursty train of spikes which firing rate depends on current intensity. With square pulse the first spike is delayed suggesting the presence of an I_A potassium current. These cells responds to sine waves as a low pass filters, ceasing to respond above 30 Hz and increasing their firing rate with amplitude and period of the stimulus sine wave. Mehaffey et al.(3) simulated the interactions soma-dendrites to show the importance of channel repertoire in the bursty behavior. We used a similar simulation to reproduce the low pass filter type of responses. **Conclusions:** We show that the intrinsic properties of the output cells are tuned and probably contribute to determine a different temporal code of two types of signals extracted from the same sensory image.

1-J Comp Neurol 401:549, 1998; 2-J Exp Biol 206:999, 2003; 3-J Neurosci 25:9968, 2005.

Palavras-Chave: electrosensory system, intrinsic properties, computer modeling

Apoio Financeiro: UdelaR, NIH, PEDECIBA

B.06.001

Thy-1/ $\alpha_v\beta_3$ integrin interaction on cell contact between neurons and astrocytes

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Background: Thy-1 is a highly expressed neuronal glycoprotein of poorly implicated in inhibition of neurite outgrowth. Our findings indicate that $\alpha_v\beta_3$ integrin is a receptor for Thy-1 on astrocytes, which upon engagement triggers integrin clustering, tyrosine phosphorylation and activation of RhoA thereby increasing astrocyte adhesion.

Objectives: To study whether adhesion of DITNC1 astrocytes stimulated by the neuron-like CAD cells is due exclusively to Thy-1/ $\alpha_v\beta_3$ integrin interaction. To evaluate if $\alpha_v\beta_3$ integrin on astrocytes acts via Thy-1 to inhibit neurite outgrowth.

Methods: Focal adhesion (FA) formation was evaluated by confocal microscopy in DITNC1-CAD co-cultures. Neurite outgrowth was induced by serum deprivation of CAD cells seeded over DITNC1 cells or plastic coated either with the fusion protein $\alpha_v\beta_3$ -Fc or control supernatant.

Results: Visualization of the cell contact area in the xz or yz planes showed FA formation around the area of DITNC1-CAD association. Interestingly, antibodies against Thy-1 inhibited FA formation but not the binding between CAD and DITNC1 cells. Additionally, neurites of CAD cells seeded over $\alpha_v\beta_3$ -Fc were fewer in number and shorter compared those seeded over the control matrix. Moreover, when CAD cells were seeded over DITNC1 cells—but not over DITNC1 cells pre-treated with Thy-1-Fc protein or antibodies against α_v or β_3 integrins—neurite outgrowth was also inhibited.

Conclusions: Although many molecules mediate neuron-astrocyte communication, changes described in astrocytes depended solely on the Thy-1/ $\alpha_v\beta_3$ integrin interaction. Also, $\alpha_v\beta_3$ integrin expressed by DITNC1 cells is responsible for inhibition of neurite outgrowth. Thus, Thy-1/ $\alpha_v\beta_3$ integrin interaction represents a novel mechanism of bi-directional cell communication between neurons and astrocytes that triggers morphological changes in both cells.

Palavras-Chave: Thy-1, $\alpha_v\beta_3$ integrin, neurite outgrowth, focal adhesion, stress fibers

Apoio Financeiro: Ph.D.CONICYT fellowships (R H-M), FONDAP #15010006 (AFGQ), FONDECYT #1070699 (LL)

B.06.002

Glial networks and the role of astrocytic connexins in neuroglial interaction

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Objectives: There is now a number of evidence for dynamic interaction between neurons and glial cells, in particular astrocytes. A characteristic of astrocytes is their high level of connexins expression and their strong coupling through gap junction channels. Such intercellular pathway provides the basis for ionic and biochemical coupling between cells identified as astrocytes (>80%). While it was initially proposed that communicating astrocytes form a glial syncytium, there are now increasing evidence arguing for a network organization with specific rules governing their extent, shaping and permeability properties.

Results: Firstly, gap junction-mediated communication in astrocytes is under the control of active compounds, including neurotransmitters and inflammatory factors, as demonstrated by dye coupling experiments and patch-clamp recording of junctional currents. Secondly, not all astrocytes identified on the basis of their GFAP expression, are involved in these networks suggesting a selectivity in such partnership. Thirdly, in brain regions characterized by strong anatomic-functional neuronal compartments, like the barrel cortex or the olfactory glomeruli, the expression of connexins and dye coupling in astrocytes are also compartmentalized. Moreover, the use of fluorescent glucose derivatives (2- and 6-NBDG) demonstrates that astrocyte gap junctions are permeable to energetic compounds. In the CA1 region of the hippocampus, the shape and extent of such metabolic networks are regulated by neuronal activity, being reduced in the presence of TTX and increased when epileptic-like discharges are induced by 0 Mg⁺⁺ and picrotoxin treatments. Finally, the intercellular dialysis of glucose through these astrocytic networks rescues neuronal activity that is abolished by the withdrawal of external glucose. **Conclusions:** Altogether the above listed properties indicate that now astrocytes should not be solely considered as independent individualities but also as groups of cells organized in dynamic networks that interact with neuronal circuits.

Palavras-Chave: Gap junctions, glial cells, brain metabolism, connexins

Apoio Financeiro: INSERM

GLUTAMATE ACTIVATES GFAP GENE PROMOTER THROUGH TGF- β 1 SMAD-DEPENDENT AND -INDEPENDENT PATHWAYS

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The close proximity of astrocytes to the synaptic cleft suggests that these cells might be potential targets for neuronal-released molecules although this issue has been less addressed. We recently demonstrated that cortical neurons activate the gene promoter of the astrocyte maturation marker, GFAP (glial fibrillary acidic protein) of cerebral cortex (Cc) astrocytes by inducing TGF- β 1 (transforming growth factor beta 1) secretion *in vitro*. Here, we evaluated the role of TGF- β 1 as mediator of the excitatory neurotransmitter, glutamate, in astrocyte differentiation. By using a transgenic mice bearing the beta-Galactosidase (beta-Gal) reporter gene under the regulation of the GFAP gene promoter, we reported that 100 μ M glutamate increased by 150% the number of beta-Gal positive astrocytes. Neutralizing antibodies against TGF- β 1 completely prevented glutamate and neuronal-induction of GFAP gene. Further, induction of GFAP gene in response to glutamate or neurons was followed by nuclear translocation of the Smad transcription factor, a hallmark of TGF- β 1 pathway activation. Addition of the sodium blocker, tetrodotoxin (300 μ M) did not impair neuronal effect on GFAP gene, thus ruling out the involvement of Na channels on this event. The antagonist of the metabotropic glutamate receptors, MCPG, inhibited neuronal effect suggesting that neuronal activation of GFAP gene involves these receptors. MAPK (PD98059) and PI3K (LY294002) inhibitors fully prevented activation of GFAP gene promoter by glutamate/neurons. Surprisingly, these inhibitors also abrogated TGF- β 1 direct action on GFAP gene although they did not inhibit Smad-2 phosphorylation, suggesting cooperation between canonical (Smad) and noncanonical (MAPK/PI3K) TGF- β 1 pathways. Our results suggest that glial metabotropic glutamate receptor activation by neurons induces TGF- β 1 secretion, leading to GFAP gene activation and astrocyte differentiation. Our work provides evidence that astrocytes surrounding synapses are target of neuronal activity and might shed light into the role of glial cells into neurological disorders associated with glutamate neurotoxicity. **Palavras-Chave:** neuron-glia interaction, GFAP gene, CNS development, TGF- β 1, astrocyte, glutamate, synapse.

Apoio Financeiro: CNPq, CAPES, FAPERJ

B.06.004

Efeito do fator de crescimento transformante beta1 e do fator de crescimento epidermal, na migração neuronal cerebelar e morfologia da glia de Bergmann.

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Introdução: Os neurônios granulares cerebelares originam-se a partir de precursores proliferativos localizados na camada granular externa (EGL). Após sua geração, estes migram através das fibras radiais da glia de Bergmann, um tipo glial específico do cerebelo, dando origem à camada granular interna (IGL). Os fatores e mecanismos que modulam esse processo ainda não são completamente elucidados. O fator de crescimento transformante beta1 (TGF-beta1) e o fator de crescimento epidermal (EGF) são conhecidos moduladores da migração neuronal no córtex cerebral.

Objetivos: Caracterizar o efeito de TGF-beta1 e EGF e suas vias de sinalização na migração neuronal cerebelar e na morfologia da glia de Bergmann *in vitro*.

Métodos e Resultados: Explantes de cerebelo derivados de ratos com sete dias de nascidos foram cultivados sobre laminina adicionando-se TGF-beta1 ou EGF (ambos a 10 ng/ml) e a cultura foi mantida por 48 horas. As análises do halo migratório foram realizadas após 6, 12, 24 e 48 horas de tratamento. Após 24 horas, os explantes tratados por EGF mostraram um aumento de 50% na migração cerebelar em contraste com os tratados com TGF-beta1. Estes resultados se mantiveram nas análises após 48 horas. A morfologia radializada da glia de Bergmann é uma condição essencial para o processo migratório. Desta forma, analisamos o efeito de EGF e TGF-beta1 na morfologia deste tipo celular através de imunocitoquímica para o marcador glial, GFAP. A análise de culturas dissociadas de glia de Bergmann revelou um aumento de 50% nos prolongamentos deste tipo celular em resposta a EGF. Ensaios utilizando inibidores de diversas vias de sinalização: PD98059 (inibidor de MAPK), KT5720 (inibidor de PKA), Staurosporina (inibidor de PKC) e LY294002 (inibidor de PI3K) revelaram que EGF induz o processo e alongamento e migração neuronal pela ativação da via de MAPK.

Conclusão: Nossos resultados sugerem que EGF seja um importante modulador da migração neuronal no cerebelo, indicando um novo papel para este fator durante a ontogênese cerebelar. **Palavras-**

Chave: Migração, cerebelo, EGF e TGF-beta1

Apoio Financeiro: CNPq; FAPERJ; CAPES.

B.06.005

GAP junction blockers increase S100B secretion in primary rat astrocyte culture and hippocampal slices.

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Objectives: The aim of this study was to investigate the influence of GAP junction communication on S100B secretion in rat astrocyte cultures and acute hippocampal slices.

Methods: Primary astrocyte cultures from Wistar rats were prepared from cerebral cortex of newborn Wistar rats. C6 lineage cells (clone 2B) were purchased from the American Type Culture Collection (Rockville, MD, USA). Cultures were allowed to grow to confluence and the medium was replaced by DMEM without serum and GAP junction blockers (carbenoxolone or endothelin-1) were added at 100 mM for 1 and 6 h. Hippocampi of Wistar rats were dissected and cut into 0.3 mm slices, which were stabilized in BSS/HEPES buffer for 2 h. The medium was replaced by BSS/HEPES buffer and GAP junction blockers (carbenoxolone or endothelin-1) were added at 100 mM for 1 h. S100B measurement was carried out by ELISA. Cell viability was measured by LDH release, neutral red assay and MTT.

Results: Carbenoxolone induced an increase in S100B secretion in astrocyte cultures (210 ± 32.6 in 1 h and 132.6 ± 15.3 in 6 h of exposure) as well in slices (216.1 ± 40.1). On the other hand, endothelin-1, which specifically blocks astrocyte GAP junctions, induced an increase in S100B secretion (173.1 ± 27.2) in 1 h of exposure and a decrease in 6 h of exposure (51.9 ± 10.8) in astrocyte cultures, and as well as in slices (64.8 ± 13.3). C6 glioma lineage, which does not exhibit GAP junctions, was used as a negative control and no alteration in S100B secretion was observed in carbenoxolone or endothelin-1 exposure, indicating that these effects are due the GAP junction blockage. No decrease in cell viability was observed.

Conclusions: This work shows that cellular communication mediated by GAP junction regulates S100B secretion in astrocyte cultures and hippocampal slices, possibly involving different signaling ways.

Palavras-Chave: GAP junctions, S100B, astrocytes.

Apoio Financeiro: CNPq, CAPES, FINEP/ Rede IBN 01.06.0842-00, FAPERGS.

B.06.006

IMUNORREATIVIDADE AO GLUT-1 E GLUT-3 NO GÂNGLIO DA RAIZ DORSAL E MEDULA ESPINAL DE RÃS *Rana catesbeiana*.

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Objetivos: O tecido nervoso de mamíferos apresenta predomínio dos transportadores de glicose dos tipos GLUT-1 e GLUT-3. No entanto, ainda é desconhecido se estes transportadores prevalecem no tecido nervoso de outras espécies de vertebrados, incluindo as rãs, modelo muito empregado em estudos fisiológicos e farmacológicos. Assim, o objetivo desse estudo foi determinar a distribuição do padrão de imunorreatividade de GLUT-1 e de GLUT-3 em gânglio da raiz dorsal (GRD) e medula espinal lombossacral de *Rana catesbeiana*.

Métodos: Rãs *Rana catesbeiana* (n=5), adultas, machos, em condições basais, foram submetidas à perfusão intra-cardíaca com solução de paraformaldeído 4% diluída em tampão fosfato 0,1M, pH 7,4, para obtenção dos GRDs do nervo ciático e do segmento lombossacral da medula espinal. Após secção destes tecidos em criostato (50 mm), os cortes foram submetidos à técnica de imunoistoquímica, sendo utilizados anticorpos primários policlonais para GLUT-1 e GLUT-3, na concentração de 1:1000.

Resultados e Conclusão: Forte imunorreatividade ao GLUT1 ocorreu na parede endotelial de vasos sangüíneos localizados no GRD e medula espinal, sendo estes escassos no GRD. Nesta estrutura observou-se ainda reação positiva em seu envoltório. A imunorreatividade ao GLUT3 foi observada em somas neuronais. No GRD, a reação localizou-se em neurônios de grande (55 mm), médio (32 mm) e pequeno (10 mm) diâmetro, sendo maior o número de somas de médio e grande diâmetro. Na medula espinal, os neurônios imunorreativos ao GLUT3 ocorreram nos campos terminais dorsal e ventral, banda mediolateral do corno dorsal, região mediana do corno ventral, e em neurônios motores laterais. O neuropilo destas regiões foi intensamente imunorreativo. Esta distribuição é similar àquela descrita em mamíferos, porém ainda não se pode determinar se estes são os tipos prevalentes de transportadores de glicose no tecido nervoso de rãs. Estudos complementares já em desenvolvimento contribuirão para o esclarecimento desta questão.

Palavras-Chave: GLUT-1 - GLUT-3-GRD- Medula Espinal

Apoio Financeiro: CNPq, FAPERGS

B.06.007

Identification of genes involved in axonal outgrowth by cerebellar granule cell precursors using *Representational Differential Analysis* (RDA)

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Introduction: The role played by glial cells in the development of the central nervous system is not completely understood and the interaction of those cells with neurons is extremely important to the correct development of the brain. Our previous results showed that glial cells secrete factors that influence axonal outgrowth cerebellar granular precursors during early postnatal development on an age-dependent manner.

Objective: The aim of the present work was to find candidate genes with expression controlled by glial cells that would be involved in the control of axonal outgrowth during cerebellar development.

Methodology: Using Representational Differential Analysis (RDA) we started by comparing the gene expression profile of granule cell precursors from 3 days old (P3) with P9 RNA extracted from P3 and P9 isolated granule cell precursors were reverse-transcribed into cDNA using the SMART PCR cDNA Synthesis kit. The cDNAs were digested using Bsp143I and the DNA fragments were called "representations". Using the cohesive ends, adaptors were added on each end of the representations and three rounds of subtractive hybridizations were performed (1st round ratio 1:10; 2nd round ratio 1:50, and 3rd round ratio 1:500). By the end of the third round of subtractive hybridization, the DNA representations were cloned into a pGEM-T easy plasmid and DH5-a bacteria were transformed with the vectors obtained. The bacterial clones were selected and the representations were sequenced.

Results: From sequencing we found two candidate genes so far, stathmin1, upregulated in P3 granule cell precursors when compared to P9, and septin11, upregulated in P9, when compared to P3. We are currently validating the results by real time RT-PCR.

Conclusions: We have identified two candidate genes that are likely to be regulated in granule cell precursors and may participate in the events of early postnatal cerebellar development, stathmin1, known to be involved in the organization of microtubules, and septin11, that plays a role in cytokinesis.

Palavras-Chave: glia, neuron, cerebellum, axonal outgrowth

Apoio Financeiro: Supported by FAPESP, CNPq and FADA-UNIFESP.

B.06.008

Protein kinase C phosphorylates serine racemase and regulates the availability of D-serine in the brain.

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Objectives: Serine racemase is a brain-enriched enzyme that converts L-serine to D-serine and pyruvate. D-serine is an endogenous modulator of the N-methyl D-aspartate (NMDA) receptors involved in synaptic plasticity, neurodegeneration and brain aging. Serine racemase activity can modulate D-serine availability. Protein kinase C (PKC) is also correlated to synaptic plasticity, neurodegeneration and aging in the brain. In this study we investigate whether PKC regulates serine racemase activity and D-serine availability in the brain.

Methods: PKC modulation of the serine racemase activity was evaluated using purified mouse recombinant serine racemase, rat astrocyte and neuronal primary cultures. The enzyme products pyruvate and D-serine were measured by 2,4 DPNH colorimetric assay and high performance liquid chromatography (HPLC), respectively.

Results: We studied the interaction between PKC and serine racemase in vitro, in astrocytic and neuronal cultures and in vivo. Active PKC phosphorylated the recombinant serine racemase in vitro and decreased both the formation of D-serine by 62.5% and the formation of pyruvate by 60.8%. The activation of PKC in astrocytic and neuronal cultures increased the phosphorylation of serine racemase and reduced the D-serine availability by 41.7% in astrocytes and 21.6% in neurons. On the other hand the inhibition of PKC decreased the phosphorylation of serine racemase and increased the D-serine availability by 47.2% in astrocytes and 29.2% in neurons. Serine racemase and PKC co-immunoprecipitated from astrocytes, neurons and whole rat brain together with the protein interacting with C-kinase (PICK1). Furthermore serine racemase, PKC and PICK1 partially co-localized in astrocytic and neuronal cultures. Finally, in vivo modulation of PKC activity in the frontal cortex of rats regulated the phosphorylation of serine racemase and the D-serine availability.

Conclusion: We conclude that PKC can phosphorylate serine racemase and regulate D-serine availability in the brain. This interaction may be relevant for the modulation of the NMDA receptors in synaptic plasticity and neurodegeneration.

Palavras-Chave: Serine racemase, protein kinase C, D-serine, neuro-glial communication

Apoio Financeiro: CNPq, FAPERJ, FUJB, CAEN-International Society of Neurochemistry

B.06.009

Alterações na oligodendroglia em ratos submetidos à hipóxia-isquemia perinatal

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Objetivo:

O cérebro infantil humano submetido a hipóxia apresenta perda de oligodendrócitos, hipomielinização, astrogliose e alterações no desenvolvimento cortical. Robinson e cols. (2005) usando modelo de hipoxia-isquemia (HI) através do clampeamento das artérias uterinas da rata grávida, observaram astrogliose na substância branca, morte de oligodendrócitos, e ruptura axonal, alterações similares às observadas em cérebros humanos que passaram por hipoxia perinatal. Neste trabalho avaliamos a expressão da 2'3' nucleotídeo cíclico 3' fosfodiesterase (CNPase) e proteína básica de mielina (MBP) no encéfalo de ratos submetidos a hipoxia-isquemia durante o desenvolvimento.

Métodos:

Ratas grávidas no 18º dia de gestação são anestesiadas com tribromoetanol intra-peritoneal Após exposição dos cornos uterinos em um grupo de animais as 4 artérias uterinas são clampeadas (HI), por 45 minutos, e em outro (SHAM) nenhum clampeamento é realizado. Após a cirurgia o desenvolvimento da gestação prosseguiu. Somente os animais nascidos a termo (23 dias) são utilizados neste estudo. Um terceiro grupo de animais não sofreu nenhum tipo de manipulação até o nascimento (NM). Os animais são anestesiados e perfundidos via intra-cardíaca com solução salina 0,9%, paraformaldeído (PF) a 4% e PF 4% com 10% de sacarose. Cortes de 20 µm são submetidos à imunohistoquímica utilizando anticorpos monoclonais contra a CNPase e MBP.

Resultados:

Observamos uma menor imunorreatividade a CNPase no corpo caloso dos animais HI. Nos grupos NM e sham observa-se uma melhor organização das fibras CNPase+ aos 9 dias de vida. Observamos também uma menor imunorreatividade para a MBP no córtex motor, córtex insular e giro dentado do hipocampo, assim como uma diminuição no número de fibras marcadas no córtex amigdalóide e substância branca subcortical dos animais do grupo HI.

Conclusões:

Nossos resultados demonstram alterações que podem ser decorrentes de uma menor expressão da proteína e/ou alterações no número de oligodendrócitos durante a mielinização, as quais podem originar distúrbios motores observados em ratos jovens adultos que foram submetidos à HI. **Palavras-Chave:** Hipóxia-isquemia, ratos, oligodendroglia, perinatal, CNPase

Apoio Financeiro: CNPq, Faperj, SR2/UERJ

ENVOLVIMENTO DAS CÉLULAS DA GLIA EM MODELO DE LESÃO MUSCULAR EM RATOS

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Objetivo do estudo: As células da glia estão diretamente envolvidas com a indução e manutenção dos processos dolorosos, especialmente sob condições de dor crônica. No entanto, a participação das células gliais no processo de dor muscular ainda não está esclarecida. No presente estudo, avaliamos a participação de astrócitos e da microglia no corno dorsal da medula espinhal durante modelo de miosite aguda em ratos.

Métodos: A miosite aguda foi induzida pela administração de 200 ou 400µg de carragenina no músculo gastrocnêmio (GC) direito de ratos. Animais que receberam injeção de salina (150µL) foram usados como controle. O limiar nociceptivo foi avaliado utilizando-se o sistema de Von Frey eletrônico. Neste teste, o músculo GS foi avaliado pela medição (em gramas) do limiar de retirada do membro. O teste foi aplicado antes e 2 h depois a indução de miosite. Análise imuno-histoquímica para avaliação da ativação de astrócitos e da microglia (GFAP e OX-42, respectivamente, 1:1000) foram realizadas no corno dorsal lombar da medula espinhal.

Resultados: Ambas as doses de carragenina no músculo GS foram capazes de reduzir o limiar nociceptivo dos ratos, causando hiperalgesia mecânica quando comparado com os controles (grupo salina). Os dados da imuno-histoquímica mostraram um aumento da expressão dos astrócitos e da microglia após indução de miosite nos animais tratados com carragenina quando comparada aos animais controle. Não foram encontradas diferenças significativas na imunorreatividade para GFAP e OX-42 entre animais controle e o lado contralateral dos animais com miosite.

Conclusões: Nossos resultados sugerem que células gliais (astrócitos e microglia) estão envolvidas no desenvolvimento da resposta nociceptiva induzida pela inflamação aguda, uma vez que foi possível observar alteração destas células após miosite aguda. Uma melhor compreensão do papel das células da glia durante a nocicepção induzida por miosite nos levará a novas estratégias de tratamento para a dor músculo-esquelética, um dos tipos mais frequentes de dores clinicamente relevantes.

Palavras-Chave: Medula espinhal, dor muscular, microglia, astrócito, carragenina

Apoio Financeiro: Fundação de Amparo à Pesquisa do Estado de São Paulo- FAPESP no: 07/58136-4, 08/51230-0 e Instituto de Ensino e Pesquisa, Hospital Sírio-Libanês, Brasil.

B.06.011

TGFbeta1 REGULA OS NÍVEIS DE D-SERINA EM ASTRÓCITOS E NEURÔNIOS DE CÓRTEX CEREBRAL

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Objetivo: D-serina é um co-agonista endógeno dos receptores de glutamato do tipo NMDA. Está molécula, produto da enzima serina racemase, está envolvida em eventos como migração e morte neuronal. A ativação de receptores de glutamato aumenta os níveis de D-serina extracelular por mecanismo ainda não esclarecido. A ativação de receptores de glutamato também induz síntese do fator de crescimento transformante beta1 (TGFbeta1), sugerindo uma interação entre essas moléculas. Neste trabalho, analisamos o efeito de TGFbeta1 na modulação dos níveis de D-serina em astrócitos e neurônios do córtex cerebral.

Métodos: Culturas primárias de astrócitos corticais de camundongos neonatos foram tratadas com TGFbeta1 (10ng/ml) por 15h. Posteriormente, adicionou-se L-serina (7mM) à cultura e após 24h, o meio e os extratos protéicos foram recolhidos. Culturas primárias de neurônios corticais de camundongos com 14 dias embrionários foram submetidas ao mesmo tratamento. As dosagens de D-serina e glutamato foram feitas por cromatografia líquida de alta resolução (HPLC). Os níveis de serina racemase foram analisados por *western blotting* e sua localização por imunocitoquímica.

Resultados: O tratamento com TGFbeta1 induziu um aumento de 1,5 vezes na concentração de D-serina extracelular em astrócitos e neurônios. Esse evento não foi acompanhado por alteração nos níveis de serina racemase. Os níveis de glutamato extracelular não foram afetados pelo tratamento com TGFbeta1.

Conclusões: TGFbeta1 aumenta a formação de D-serina e sua disponibilidade no meio extracelular em astrócitos e em neurônios. Dados prévios mostram que glutamato aumenta os níveis de D-serina e de TGFbeta1. Em conjunto, esses dados sugerem que TGFbeta1 possa ser um mediador das ações glutamatérgicas na disponibilidade de D-serina.

Palavras-Chave: D-serina, Glutamato, TGFbeta1

Apoio Financeiro: Apoio financeiro: CNPq, CAPES, FAPERJ.

B.06.012

Secreção de S100B em fatias cerebrais agudas: Modulação por níveis extracelulares de Ca^{2+} and K^{+}

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Objetivos: Fatias hipocâmpais são amplamente utilizadas para investigar parâmetros neuronais bem como parâmetros de atividade astrocitária. Nosso objetivo foi caracterizar e padronizar a secreção de S100B sob condições basais e na presença de diferentes concentrações de K^{+} , Mg^{2+} e Ca^{2+} além de avaliar a integridade celular em fatias hipocâmpais agudas.

Métodos: Para preparação e incubação das fatias hipocâmpais agudas foram utilizados ratos Wistar machos de 30 dias. Os ratos foram mortos por decapitação e seus cérebros foram rapidamente removidos. Os hipocâmpos foram dissecados e fatias transversas de 0,3mm foram obtidas utilizando-se um *chopper*. As fatias foram imediatamente transferidas para placas de cultura. A quantificação de S100B foi realizada por ELISA. A integridade celular foi determinada por LDH através de um kit comercial colorimétrico, pelo conteúdo de GFAP por ELISA e pelo ensaio de exclusão do Trypan-blue.

Resultados: Como esperado, houve diminuição significativa na viabilidade celular no início da incubação (LDH e Trypan-blue) e não houve alteração no conteúdo de GFAP durante toda a incubação. Nas condições de estabilização, a S100B extracelular diminuiu significativamente durante a primeira hora e atingiu um platô a partir dos 75 minutos. Uma diminuição significativa na secreção de S100B foi observada em fatias incubadas em meio contendo $\text{Ca}^{2+}/\text{Co}^{2+}$ ($28 \pm 3,8\%$) ou em meio contendo alto K^{+} ($53 \pm 6,9\%$). A remoção de Mg^{2+} não alterou a secreção basal de S100B ($99 \pm 8,4\%$) e um aumento da secreção foi observado em meio contendo baixo K^{+} ($126 \pm 7,1\%$).

Conclusões: A padronização do modelo *ex-vivo* em fatias hipocâmpais, resultou em células viáveis, com capacidade de secreção de S100B, tanto em condições basais, quanto após estímulo. Este estudo sugere que a exposição de fatias hipocâmpais agudas a baixo e alto K^{+} poderia ser usada como um ensaio para avaliar, respectivamente, a secreção de S100B regulada positivamente e negativamente. Nossos dados confirmam que fatias hipocâmpais complementam e, às vezes, sobrepõem limitações de culturas gliais isoladas.

Palavras-Chave: Astrócito, Fatia Cerebral, Hipocampo, S100B

Apoio Financeiro: CNPq, CAPES, Finep/Rede IBN 01.06.0842-00, FAPERGS

B.06.013

Viabilidade Metabólica de Fatias Hipocampais Agudas: liberação de lactato, redução de MTT, captação de glutamato e conteúdo de glutatona

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Objetivos: Fatias cerebrais agudas, particularmente, fatias hipocampais têm sido utilizadas para investigar parâmetros neuronais metabólicos e eletrofisiológicos além de parâmetros de atividade astrocítica. O objetivo deste trabalho foi padronizar e caracterizar a viabilidade metabólica de fatias hipocampais incluindo, liberação de lactato, redução de MTT, captação de glutamato e conteúdo de glutatona.

Métodos: Para preparação e incubação das fatias hipocampais agudas foram utilizados ratos Wistar machos de aproximadamente 30 dias. Os ratos foram mortos por decapitação e seus cérebros foram rapidamente removidos. Os hipocampus foram dissecados e fatias transversas de 0,3mm foram obtidas utilizando-se um *chopper* McIlwain. As fatias foram imediatamente transferidas para placas de cultura de 24 poços sendo que cada poço continha 0,3mL de meio fisiológico e 1 fatia. O conteúdo extracelular de lactato foi medido utilizando-se um kit comercial (Katal Biotecnologica, Brasil), a redução de MTT foi dosada através de método colorimétrico, a captação de glutamato foi medida usando-se L-{2,3-³H} glutamato e o conteúdo de glutatona foi medido por fluorescência com o-ftaldeído.

Resultados: A curva de lactato espelhou a curva do ensaio do MTT demonstrando um dano celular significativo após decapitação seguida por recuperação. A captação de glutamato, medida em 2 e 3h, aumentou ($0,93 \pm 0,2$ e $1,01 \pm 0,5$ nmol/mg proteína) quando comparada a atividade imediatamente após decapitação ($0,68 \pm 0,03$ nmol/mg proteína) e o conteúdo de glutatona diminuiu durante a primeira hora ($28 \pm 5,9$ p/ $13 \pm 2,0$ nmol/mg proteína), mas depois permaneceu inalterado ($13 \pm 2,0$ e $14 \pm 3,4$ nmol/mg proteína).

Conclusões: Com relação ao lactato é possível observar uma significativa variação energética durante a primeira hora e uma recuperação a partir desta. Os astrócitos são funcionantes ativos nestas preparações e apresentam uma atividade metabólica menor durante a primeira hora *ex-vivo*. Nossos dados sugerem que, sob estas condições, parâmetros de atividade glial podem ser avaliados em fatias cerebrais agudas.

Palavras-Chave: Fatias de Hipocampo, S100B, captação de glutamato

Apoio Financeiro: CNPq, CAPES, Finep/Rede IBN 01.06.0842-00, FAPERGS

LOS METABOLITOS DE LA ACIDEMIA GLUTÁRICA I INDUCEN DEPOLARIZACIÓN MITOCONDRIAL Y PROLIFERACIÓN ASTROCITARIA

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INTRODUCCIÓN: El bloqueo congénito del catabolismo del L-triptófano, L-lisina e L-hidroxilina por deficiencia de actividad glutarilCoA deshidrogenasa provoca la acumulación de metabolitos orgánicos [ácidos glutárico (GA) y 3-hidroxiglutarico (OHGA)] que a través de mecanismos desconocidos producen la acidemia glutárica I (GAI), una enfermedad neurometabólica que afecta a niños pequeños que sufren macrocefalia y posterior degeneración estriatal y cortical.

OBJETIVOS Y MÉTODOS: Como los astrocitos brindan soporte estructural y trófico a las neuronas y participan en la neurodegeneración, se estudiaron los efectos de concentraciones fisiopatológicas de GA y OHGA sobre astrocitos corticales en cultivo e *in vivo*.

RESULTADOS: En cultivo, GA y OHGA no afectaron la viabilidad de los astrocitos pero produjeron hasta un 50% ($\pm 10\%$) de depolarización mitocondrial y una proliferación aumentada de $250 \pm 10\%$ y $200 \pm 12\%$ en subconfluencia y confluencia, respectivamente. Ambos efectos fueron bloqueados por antioxidantes y ferroporfinas. La aplicación intraventricular de GA a ratas SD recién nacidas, produjo al día 5 un aumento de la proliferación celular y astrocitaria del $225 \pm 20\%$ y $170 \pm 12\%$, respectivamente. No se observaron cambios en el fenotipo astrocitario ni muerte neuronal significativa en las áreas estriatales afectadas en GAI. El pretratamiento con la ferroporfirina FeTCPP bloqueó el aumento de proliferación inducido por GA y disminuyó moderada pero significativamente la proliferación basal en animales control ($78 \pm 10\%$).

CONCLUSIONES: GA y OHGA promueven una respuesta astrocitaria previa a la muerte neuronal significativa, lo que podría ser relevante para entender la macrocefalia y la neurodegeneración características de la enfermedad y para una posible acción terapéutica focalizada sobre las células gliales.

Palavras-Chave: astrocitos, acidemia glutárica I, neurodegeneración

Apoio Financeiro: PDT Salud 76/23 y PEDECIBA Biología

LAS ALTERACIONES MITOCONDRIALES ASTROCITARIAS PRECEDEN LA MUERTE NEURONAL EN UN MODELO DE ACIDEMIA GLUTÁRICA I

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INTRODUCCIÓN: La acidemia glutárica I (GAI) es una enfermedad invalidante de la infancia temprana, caracterizada por neurodegeneración estriatal y cortical. La disfunción congénita de la enzima mitocondrial glutarilCoA deshidrogenasa produce concentraciones milimolares de ácidos glutárico (GA) y 3hidroxiglutarico (OHGA). Como GA y OHGA podrían actuar como toxinas mitocondriales, propusimos que ambos ácidos alteran la funcionalidad mitocondrial de astrocitos afectando la viabilidad neuronal.

OBJETIVOS: Estudiar los efectos de GA y OHGA sobre astrocitos y su posible repercusión sobre la viabilidad neuronal *in vivo* y en co-cultivos neuronas-astrocitos

RESULTADOS: Sobre cultivos de astrocitos corticales de rata, GA y OHGA produjeron una disminución concentración-dependiente de la actividad deshidrogenasa mitocondrial medida por reducción de 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolio (MTT) y descendieron el potencial mitocondrial al 50% ($\pm 10\%$) respecto de los controles, estimado por pruebas fluorescentes vitales (JC1, MitoFluorGreen y MitoTrackerRed). Las tasas de muerte de neuronas corticales y estriales co-cultivadas sobre astrocitos pretratados con GA y OHGA fueron mayores que las producidas por adición directa de dichos ácidos, sugiriendo un papel neurotóxico mediado por los astrocitos. Sin embargo, luego de 5 días de la administración sistémica de GA a ratas de 1 día, el número de neuronas se mantuvo incambiado aunque el número de astrocitos se duplicó sin que se experimentaran cambios fenotípicos significativos. Cuando la sobrevida de las ratas inyectadas con GA fue de 21 días, la población neuronal estriatal disminuyó significativamente y las alteraciones morfológicas se extendieron a todas las poblaciones celulares incluyendo astrocitos.

CONCLUSIONES: Estos datos sugieren que los astrocitos son capaces de responder en forma temprana a GA y OHGA lo que podría ser relevante para la muerte neuronal posterior. Aparte de colaborar con la dilucidación de los mecanismos patogénicos de GAI, la exploración de los mecanismos subyacentes a los efectos de GA y OHGA sobre astrocitos podría ser útil en el desarrollo de nuevas alternativas terapéuticas.

Palavras-Chave: astrocytes, mitochondria, neuronal death

Apoio Financeiro: PDT Salud 76/23 y PEDECIBA Biología

MÜLLER GLIA FACTORS PROTECT PERIPHERAL AND CENTRAL NEURONS FROM NEUROTOXIC AGENTS

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Objectives: Glial cells are essential elements in the nervous system, and Müller glia is the major macroglia found in the retina. Several functions are related to these cells such as extracellular homeostasis and synaptogenesis. Here we investigated if soluble trophic factors secreted from Müller glia or contact-mediated effects maintain and protect peripheral (dorsal root ganglia –DRG- and sympathetic neurons) or central retinal neurons against toxic insults such as Methyl-mercury (MeHg) or kainate.

Methods and Results: Paravertebral sympathetic ganglia chains or DRGs from embryonic day 9 (E9) chick were dissected, dissociated, and plated on poly-L-lysine-laminin substrate on DMEM media + fetal calf serum (FCS) or on Muller glia carpets for 48-72hs. E8 retinal neurons were plated on a low ($< 5 \times 10^6$ cells/well) or high (20×10^6 cells/well) density manner for 72hs. Exposure of E9C2 sympathetic neurons to MeHg 1 and 10 μ M induced $54 \pm 5\%$ and 100% neuronal death after 24 h. However, incubation of MeHg 1 μ M in the presence of conditioned media prepared from avian Müller glia (AMG 1:1) protected $90\% \pm 8\%$ sympathetic neurons. Also, sympathetic or DRG neurons plated on Müller glia developed complex neurites and were resistant against 1 μ M MeHg toxicity. The same was observed when retinal neurons were plated on a higher density and developed on a glial carpet. Retinal neurons cultured in low density and exposed to 10 μ M MeHg released 5-fold more LDH than control cultures. On the other hand, incubation of MeHg 10 μ M or kainate 200 μ M in the presence of AMG (1:1) had a minor toxic effect on retinal neurons.

Conclusion: Contact with Müller glia or their soluble factors is neuroprotective for peripheral and central neurons exposed to the neurotoxicants MeHg and kainate.

Palavras-Chave: Glia; Trophic Factors ; Retina; Excitotoxicity

Apoio Financeiro: Financial Support: CNPq, FAPERJ and PRONEX

Effect of neurons on the differentiation of functional dopaminergic phenotype in purified cultured Müller cells

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Müller cells constitute the main glial cell type in the retina and span the tissue from the inner to the outer limiting membranes. Several functions have been attributed to these cells including structural and nutritional roles and removal of ions and neurotransmitters from the extracellular space. The potential of Müller cells to actively participate in cellular communication within the nervous system has been recently uncovered. Moreover, it has been suggested that this type of cell can generate neurons under appropriate conditions. The knowledge on controlling this particular differentiation could be extremely useful as potential therapy against some neuro disorders. In the present work, we evaluate the capability of Müller cells to be used as a source of dopaminergic cells and the factors that influence this process, more particularly the role of neurons, using its conditioned medium. We have shown that Müller cells in culture not only express dopaminergic markers as the dopamine transporter (DAT), tyrosine hydroxylase (TH), dopa decarboxylase (DDC) and the transcription factor for dopaminergic differentiation Nurr1. Here, we show that Müller glia is able to produce dopamine from tyrosine, measured by HPLC assay (70 ± 2 pmoles/mg of protein). Furthermore, cAMP- increasing agents like forskolin (FK) and the pituitary adenylate cyclase-activating peptide (PACAP) increase both TH expression and also the dopamine production from tyrosine (176 ± 3 and 224 ± 5 , respectively). However, neuron conditioned medium prevents both the increase in dopamine production (74 ± 4) and the increase in Nurr1 immunolabeling induced by those agents. Taken together these results show that this potential dopaminergic pool of Müller cells can be manipulated to generate a higher number of committed cells. This process seems to be under the control of neuron-mediated signaling. **Palavras-Chave:** Glia, Müller cell, dopamine

Apoio Financeiro: CNPq, FAPERJ and PRONEX.

[³H]GABA AND [³H]D-ASPARTATE MOBILIZATION IN RAT CORTICAL ASTROCYTES IN CULTURE

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Objective: Glial cells interact in a bidirectional way with neurons in order to maintain the extracellular environment. Astrocytes, the major macroglial cells found in the vertebrate cortex are key players of recently recognized tripartite excitatory/inhibitory synapses. We studied how cultured prosencephalic astrocytes modulate the levels of the major excitatory and inhibitory neurotransmitters.

Methods: Cerebral hemispheres derived from postnatal Wistar rat pups were dissected, dissociated and cells were plated in a density of 10⁶ cells. Neurons were removed by extensive washing until growing astrocytes reached confluence. Uptake of [³H]D-Aspartate or [³H]GABA in these cultures was initiated by adding pre-warmed Hank's solution containing 0.25 µCi of [³H]D aspartate or [³H]GABA per well for 90 min. Cell cultures were scraped, centrifuged and the radioactivity was measured.

Results: [³H]GABA was taken up in a Na⁺, Cl⁻ and temperature dependent manner in astrocyte cultures suggesting involvement of a specific transporter. No [³H]GABA release was detected in response to 2mM glutamate, 80mM K⁺ or GABA itself. [³H]D-Aspartate was taken up after 90min incubation. Nevertheless, as opposed to [³H]GABA, [³H]D-Aspartate release was induced by glutamate, in a dose-dependent manner (EC₅₀ approx 50µM). Moreover, [³H]D-Aspartate release was Na⁺-, but not Ca²⁺-dependent. Addition of 200 µM THA, a competitive inhibitor of glutamate uptake and a substrate for the transport system, had no effect on [³H]D-Aspartate release. Addition of 2 µM MK-801 (noncompetitive blocker of the NMDA receptor) and 100 µM DNQX (competitive blocker of the non-NMDA receptor) inhibited [³H]D-Aspartate release induced by glutamate on prosencephalic astrocytes suggesting the participation of ionotropic glutamate receptors on the release of excitatory aminoacids.

Conclusion: While [³H]GABA and [³H]D-Aspartate were taken up into cultured cerebral hemispheres astrocytes, stimulation by depolarizing agents (glutamate and K⁺) induce [³H]D-Aspartate, but not [³H]GABA release, which could have important consequences in an excitotoxic scenario. Also, glutamate receptors modulate [³H]D-Aspartate release.

Palavras-Chave: glia, glutamate, Gaba

Apoio Financeiro: CNPq and FAPERJ.

ONTOGENESIS AND REGULATION OF THE GABAERGIC GAT-3 GLIAL TRANSPORTER IN THE EMBRYONIC AVIAN RETINA.

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Objective: γ -aminobutyric acid (GABA) is the main inhibitory amino acid in the central nervous system. GABA effect is terminated through the action of specific membrane transporters (GAT) found in neurons and glial cells. Although, in the vertebrate retina, GAT-3 is found mainly in Müller glial cells GABA is not taken up by them in intact chicken retina. But we recently shown that cultured Müller cells take up GABA (Neurotox. Res. 2007, 12, 145-153). We decided to evaluate and characterize the mediators involved in the GAT-3 activity. We also studied the ontogenesis of GAT-3 expression during avian embryonic retina.

Methods: Mixed retinal cultures were prepared from embryonic day 8 (E8) retinas (30×10^6 cells) and treated with ascorbic acid, seven days later, to purify glial cells. For neuron enriched cultures, cells were plated at a density of 2×10^6 cells/plate in minimum essential medium plus 1% fetal calf serum to avoid glia proliferation. The conditioned medium was collected after 3 days and used in purified Müller cultures. In order to identify possible neuronal candidates that could modulate [^{3} H]-GABA uptake in Müller glial cells, this was performed in the presence of several modulators (neurotransmitters, activators of signaling pathways, etc). Immunohistochemistry was also done with an antibody anti-GAT-3 in the embryonic avian retina from E7 through post-hatch stages.

Results: [^{3} H]-GABA uptake in purified Müller glia cells in culture were decreased in 20% when preincubated with glutamate 2 mM or kainate 100 μ M. PMA, a phorbol ester that activates protein kinase C reduced in $40 \pm 6\%$ [^{3} H]-GABA uptake in Müller glia cells. Finally, immunoassay revealed that GAT-3 expression is present since E11 in the avian retina, mainly labeling cells in the inner nuclear layer and plexiform layer.

Conclusion: Our data identified glutamate as a possible candidate secreted from neurons that modulates the activity of the GAT-3 transporter in Müller glia cells. Since GAT is the main target of GABA removal, this represents an important step understanding excitotoxicity in the avian retina.

Palavras-Chave: Muller glia, Gaba, neuro-glial signaling , PKC

Apoio Financeiro: CNPq, FAPERJ and PRONEX

B.06.020

EL TRANSPORTADOR DE MONOCARBOXILATO MCT2 SE LOCALIZA EN POBLACIONES NEURONALES OREXÍGENAS Y ANOREXÍGENAS DEL HIPOTÁLAMO.

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Objetivos: La función neuronal está estrechamente apoyada y regulada por elementos gliales. Se ha propuesto que las células gliales que están en contacto con las neuronas del núcleo arqueado del hipotálamo pueden incorporar glucosa y liberar lactato, molécula que activaría a las neuronas involucradas en el mecanismo de respuesta a la glucosa. Estas neuronas sintetizan y secretan neuropéptidos anorexígenos (POMC y CART) y orexígenos (NPY y AgRP). De esta forma, el sistema sensor de glucosa cerebral sería un mecanismo que involucraría la interacción funcional entre la glia y la neurona. Para validar este mecanismo es necesario demostrar que ambos tipos celulares expresan transportadores de monocarboxilatos (MCTs).

Métodos: En este estudio analizamos la expresión del mRNA de MCT2 en hipotálamo mediante hibridación in situ no isotópica. Para definir la distribución de MCT2 en el núcleo arqueado, colocalizamos MCT2 con los neuropéptidos POMC, CART, AgRP y NPY, a través de microscopia confocal, utilizando cortes del hipotálamo con doble y triple marcaje.

Resultados: Previamente, hemos demostrado que MCT1, MCT2 y MCT4 se expresan en el hipotálamo, en la región del núcleo arqueado, MCT1 y MCT4 se localizan en la glia hipotalámica. En este estudio, los resultados de la localización del mRNA indican que MCT2 se expresa en las neuronas del núcleo arqueado proximal y distal. Los análisis de inmunolocalización, a través de microscopia confocal, permiten definir que MCT2 se localiza en poblaciones neuronales orexígenas y anorexígenas.

Conclusiones: Dado que MCT2 es un transportador que realiza principalmente influjo de lactato y MCT1 y MCT4 eflujo de lactato, la distribución de los MCTs podría mediar una interacción metabólica entre glia y neurona en estas regiones cerebrales.

Palabras-Chave: Hipotálamo, MCTs, Sensing de Glucosa.

Apoio Financeiro: Financiado por Proyecto de tesis doctoral CONICYT 23070095 y Anillo-PBCT-Banco Mundial ACT-02.

B.06.021

Efecto de glutamato en la acumulación de ácido ascórbico en astrocitos. (Glutamate effect in astrocytes vitamin C accumulation).

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Introducción y Objetivos. La forma oxidada de la vitamina C, ácido deshidroascórbico (DHA), es captada por los astrocitos, proceso que puede ser modificado por glutamato. De esta forma analizamos el efecto de glutamato en la captación de DHA, utilizando como modelo astrocitos mantenidos en cultivo por 15 y 30 días. El DHA sería captado por transportadores de glucosa (GLUTs) y atrapado intracelularmente por medio de su reducción a ácido ascórbico (AA). **Metodología.** Se utilizaron técnicas inmunohistoquímicas asociadas a microscopia confocal para determinar la expresión de GLUT1 en cerebro de rata adulta y en cultivos primarios de astrocitos. Además se analizó mediante estudios de transporte y análisis de HPLC el efecto de glutamato en la captación y reducción de DHA en cultivos de astrocitos de 15 y 30 DIV. **Resultados.** Con la metodología utilizada determinamos que los astrocitos *in vivo* expresan el transportador GLUT1 y que este transportador se localiza preferentemente en los pies vasculares de los astrocitos. En los cultivos primarios de 30 DIV determinamos que los astrocitos incrementan la expresión del transportador GLUT1 incorporando una mayor cantidad de DHA y glucosa. Determinamos que glutamato incrementa la acumulación intracelular de vitamina C (captación de DHA) a los 15 DIV. Sin embargo, a los 30 DIV, el efecto fue inverso. Paralelamente, glutamato incrementa la reducción intracelular de DHA a los 15 y 30 DIV. **Conclusiones.** Con este estudio demostramos que DHA es reciclado a través de GLUT1, y además, glutamato incrementa la acumulación de AA a través de la reducción del DHA en astrocitos.

Palavras-Chave: ácido deshidroascórbico (DHA); GLUTs, glutamato

Apoio Financeiro: Financiado por Anillo ACT-02.

B.06.022

Spontaneous physical activity stimulates spinal cord neuroplasticity in rats.

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Objectives: Many biological events can be stimulated by physical activity, like neuroplasticity. The spinal cord changes its morphology, biochemistry and functionality when stimulated by exercise. However, many molecular and cellular events have not been elucidated. The aim of this work was identify some neuroplastic events mediated by neurotrophic factors, neuromodulators and by growth associated protein 43 (GAP-43).

Methods: Male adult Wistar rats were obtained and kept in regular light/dark cycle with free access to food and water according to Brazilian Committee of Animal Experimentation. After an accommodation period, the animals were submitted to physical activity in a running wheel (trained group) in their active phase (dark period) and in the rest phase (light period) the rats were maintained in cages without running wheel. The rats of sedentary group were maintained in individual cages without running wheel. The running wheel was 12 cm of ratio and the cages received air flux of 1.8 liter/minute (Columbus, Oxymax Equal Flow System, USA). The training protocol extended for five weeks, seven days a week. The animals were euthanized and their spinal cord collected and processed for *in situ* hybridization. The results were statistically analyzed by student's T-test.

Results: The mRNA of GAP-43 of anterior horn was more expressed in trained group than sedentary group at cervical level ($p < 0.05$). In posterior horn of lumbar intumescence, the mRNA of substance P was 150% higher in the trained group than in sedentary one ($p < 0.01$). However, the analysis of mRNA signal of brain derived neurotrophic factor (BDNF) revealed a diminution in anterior horn of cervical and lumbar levels and in lateral funiculus of cervical level after physical activity ($p < 0.01$). The analysis of the expression of glial derived neurotrophic factor (GDNF) mRNA reveals diminution in the trained group in lateral funiculus of lumbar intumescence ($p < 0.05$).

Conclusion: The spontaneous physical activity promotes modifications in spinal cord tissue, triggering neuroplastic events. This approach can be used to promote central nervous system health, e.g. promoting protection and increasing the functionality.

Palavras-Chave: neuroplasticity, physical activity, spinal cord, neurotrophic factors

Apoio Financeiro: FAPESP, CNPq

GLUTAMATE MODULATION OF MELATONIN SYNTHESIS INVOLVES PARACRINE INTERACTIONS BETWEEN PINEALOCYTES AND ASTROCYTES THROUGH ACTIVATION OF NF- κ B.

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Objectives: The mammalian pineal gland is composed mostly by two types of cells: pinealocytes and glial cells. The pinealocytes are secretory cells responsible for melatonin synthesis and secretion. Glutamate is known as a modulator of the melatonin synthesis, acting by reducing serotonin N-acetyltransferase enzyme activity and, as a consequence, melatonin synthesis. The objective of this work was to investigate the role of glial cells and pinealocytes in the glutamate modulatory role on melatonin synthesis.

Methods: Isolated pinealocytes, astrocytes and astrocytes/pinealocytes cultures obtained from rat pineal glands were used. Glutamate receptors were characterized in astrocytes and pinealocytes by RT-PCR and intracellular calcium was analyzed by confocal microscopy. Pinealocytes in culture and in co-culture were stimulated by noradrenaline 1 μ M plus glutamate (100 to 600 μ M). Melatonin was quantified by HPLC with electrochemical detection. NF- κ B was analyzed by electrophoretic mobility shift assay.

Results: In isolated pinealocytes glutamate had no effect on melatonin synthesis (nor=71.13 $\pm$ 3.74ng/ml; nor+glu=66.88 $\pm$ 4.42ng/ml) and only when pinealocytes were cultivated together with astrocytes glutamate reduced melatonin synthesis stimulated by noradrenaline (nor=74.54 $\pm$ 3.29ng/ml; nor+glu=51.66 $\pm$ 3.51; p<0,01). AMPA, NMDA, mGluR1, mGluR5, mGluR2 and mGluR3 mRNAs were found in both cellular types. Glutamate, NMDA, AMPA e DHPG (Group I metabotropic agonist) elevates intracellular calcium in astrocytes. NF- κ B was activated by glutamate in astrocytes, but not in pinealocytes.

Discussion: Melatonin synthesis inhibitory effect of glutamate seems to be mediated by the interaction between astrocytes and pinealocytes. The effect of glutamate on astrocytes involves NF- κ B activation.

Palavras-Chave: Pineal gland, melatonin, astrocytes, pinealocytes, glutamate, NF- κ B

Apoio Financeiro: Fapesp e CNPq

C.01.001

The GRP75 Chaperone (Mortalin) and its role in Alzheimer's Disease

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OBJECTIVES: Our research is dedicated to the discovery of molecular pathways in neurological diseases. Here we have two main goals: 1) To determine whether mortalin interacts with apoE in animal models of Alzheimer's disease (AD) and in human tissues from AD patients; and 2) to determine changes in mortalin regulation as a function of apoE and Alzheimer's disease (AD). GRP75 (also known as Mortalin) is a chaperone protein associated with cell survival, stress response, intracellular trafficking, control of cell proliferation, mitochondrial biogenesis, and cell fate determination. We use human apoE-targeted replacement mice and hippocampus of patients with AD to study the role of mortalin in AD using 2-D DIGE-based proteomics.

METHODS: Proteins were isolated from human and mice tissues, and then fractionated by 2-D DIGE followed by identification by mass spectrometry. Protein-protein interaction was determined by immunoprecipitation and western blotting.

RESULTS: We observed that mortalin is differentially regulated (including changes in protein expression and changes in protein phosphorylation) in mice brains. We also have observed that in human brains this protein is differentially regulated as a function of both, the disease and the genotype. Furthermore, we have observed that the two proteins have direct interactions in cell cultures, and isolated tissues. With the current knowledge about the role of apoE in AD, and the participation of mortalin in mitochondrial function we propose that the Grp75-associated chaperone system is used by apoE as a shuttle mechanism that leads to mitochondrial dysfunction and oxidative stress in Alzheimer's disease.

CONCLUSIONS: Mortalin interacts with apoE in a differential manner that is determined by both, the apoE genotype (apoE3 vs. apoE4) and by the disease state (disease vs. non-disease). It is plausible that this interaction leads to mitochondrial dysfunction and changes in the mitochondrial response to oxidative stress, major determinants of AD.

Palavras-Chave: Mortalin, apoE, Mitochondria, Alzheimer

Apoio Financeiro: Duke University Medical Center

C.01.002

XBP-1 deficiency protects against Amyotrophic Lateral Sclerosis due to increased autophagy.

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Over 100 mutations in the gene encoding Superoxide Dismutase-1 (SOD1) are known to cause familial Amyotrophic lateral sclerosis (fALS), which triggers the misfolding and aggregation of SOD1. The primary mechanism responsible for the progressive motoneuron loss in ALS remains unknown, yet recent evidence implicates the participation of adaptive responses to endoplasmic reticulum (ER) stress in the disease process, a pathway known as the Unfolded Protein Response (UPR). **Objective:** In order to study the contribution of the ER stress to fALS, we have targeted the expression of the transcription factor X-Box binding protein-1 (XBP-1) by creating a conditional knockout mouse. XBP-1 is the master regulator of the UPR and controls the expression of multiple proteins involved in protein folding and protein quality control. **Results:** Unexpectedly, despite predictions that XBP-1 deficiency would enhance the severity of experimental ALS, we observed that these mice were more resistant to developing the disease. These effects were associated with increased autophagy in motoneurons, a cellular pathway involved in lysosome-mediated degradation of proteins. Similarly, in vitro experiments demonstrated that targeting XBP-1 with small interfering RNA drastically decreased the generation of toxic mutant SOD1 protein aggregates due to enhanced autophagy, suggesting possible therapeutic benefits of targeting this pathway in ALS. **Conclusions:** Our results are indicative of a close homeostatic connection between the UPR and autophagy in the nervous system.

Palavras-Chave: Superoxide Dismutase-1, Amyotrophic lateral sclerosis endoplasmic reticulum stress, autophagy

Apoio Financeiro: This work was supported by FONCECYT #1070444 and #3085017 and FONDAP #15010006

Participation of TGF- β Smad3 signaling pathway on microglial amyloid uptake

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Alzheimer disease (AD) is the most common cause of dementia in the elderly. In AD, A β and pro-inflammatory cytokines can induce several activation processes in glial cells, such as phagocytosis, secretion of cytokines and the activation of different signal transduction pathways. TGF- β levels are increased in cerebral spinal fluids in AD. However, the levels of Smad3, its principal effector, are decreased in hippocampus. We have shown that astrocytes can modulate microglial cytotoxic activation through secretion of TGF- β ; previous reports have also shown that TGF- β can increase phagocytosis of A β . **Objective:** To determine the participation of TGF- β Smad3 signaling pathway on microglial amyloid uptake. **Methods:** Microglial cells obtained from neonatal mice and rat glial cultures were exposed to TGF- β and TGF- β pathways inhibitors. We evaluated the scavenger receptor expression by western blot and immune fluorescence. Phagocytosis was evaluated by A β -Fluo III uptake assays. We also evaluated the expression of Smad3 in the hippocampus of mice exposed to acute and chronic inflammatory conditions. **Results:** TGF- β induced a 2.4 ± 1 (mean $\pm$ SD) fold increase on SR-A, decreased by $74 \pm 14\%$ (mean $\pm$ SD) SR-BI and did not change SR-MARCO expression after 48h. TGF- β increased A β -Fluo III uptake by $81 \pm 4\%$ and $89 \pm 20\%$ (mean $\pm$ SD) in mouse and rat microglia. Inhibition of Smad3 resulted in a decrease of the inducing effect of TGF- β on SR-A expression and A β -Fluo III uptake. Young mice exposed to pro-inflammatory stimulus increased Smad3 expression by $58 \pm 20\%$ (mean $\pm$ SD) whereas adult mice exposed to chronic stimulus did not change Smad3 expression. **Conclusions:** TGF- β could increase A β uptake through its modulatory effect on scavenger receptor expression. TGF- β effect appeared to be at least partially mediated by the Smad pathway and modulated by age and pro inflammatory condition. Conditions that could be relevant for the pathogenesis of AD.

Palavras-Chave: Alzheimer, Microglia, TGF-beta, Smad3, Scavenger Receptors

Apoio Financeiro: Grant support from DIPUC is acknowledged

C.01.004

Physical exercise prevents cognitive deficits induced by intracerebroventricular administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in C57BL/6 mice, an animal model of Parkinson's disease.

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Objectives: An increasing amount of evidence suggests that subtle cognitive deficits can be observed at the early stages of Parkinson's disease (PD), even before the development of its classical motor symptoms. The majority of research conducted with animal models of PD has focused upon the effects on the nigrostriatal dopaminergic system and motor behavior. In the present study, we investigated the neuroprotective effects of exercise in the cognitive deficits induced by the intracerebroventricular (i.c.v.) injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice through the water maze paradigm. **Methods:** Thirty-two C57BL/6 male mice (6 months old) were assigned to four groups (n=8 per group): (1) untrained-vehicle; (2) trained-vehicle; (3) untrained-MPTP; and (4) trained-MPTP. Trained mice ran on a nightly running wheel for 28 days, before drug treatment. MPTP (100 µg/mouse) or vehicle (saline) were administered by i.c.v. route (Brain Res. 706:181, 1996). The cognitive performance of the animals was evaluated using a working memory version of the water maze task (Nature 297:681, 1982). **Results:** Mice ran 182.3±7.3 m at mean velocity 8.4±0.4 m/min, with daily increases in the performance. In the last night they improve fourfold the distance (836.9±61.9m) and twofold the running velocity (17.8±1.3 m/min). Two weeks after MPTP administration, ANOVA revealed a significant effect for the treatment factor [$F(3,124)=6.67$, $P\leq 0.05$] in the escape latency for finding the platform. Subsequent Newman-Keuls test indicated significant differences ($P<0.05$) between the untrained-MPTP (40.6±3.9s) and all the other groups (untrained-vehicle: 17.4±2.8s; trained-vehicle: 23.1±3.6s; and trained-MPTP: 28.2±3.5s). In contrast, MPTP-treated mice don't display any motor dysfunction at this period, as evaluated in the activity chamber task. **Conclusion:** Our findings demonstrate the potential of voluntary physical exercise as cognition-enhancing procedure to protect against cognitive decline in a mouse model of PD. These results reinforce and extend the notion of the beneficial effects of exercise in PD.

Palavras-Chave: Parkinson disease; MPTP; memory; exercise.

Apoio Financeiro: CNPq, CAPES, FAPESC.

Long-term environmental impoverishment and aging alter episodic-like memory, spatial learning and astrocytes of hippocampal dentate gyrus

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Environmental and age-related effects on memory were analyzed and compared to changes observed in astrocyte morphology and distribution in the hippocampus and dentate gyrus. Aged (A) (20 months) and young (Y) (6 months) adult female albino Swiss mice, were housed from weaning either in impoverished (I) or in enriched (E) conditions, as follow: enriched young adults, (EY, n=12), impoverished young adults (IY, n=13), enriched aged adults (EA, n=30), and impoverished aged adults (IA, n=16). All subjects were tested for episodic-like memory by one-trial object recognition. Young subjects (EY, n=12; IY, n=13) and a different set of aged (EA, n=15; IA, n=11) mice groups, were trained in the water maze paradigm for five consecutive days. All tests were video recorded and analyzed with a computer program (ANYMAZE tracking system, Stöelting). Time spent to explore “former”, “recent”, “displaced” and “stationary” objects during the episodic-like memory test was expressed as a proportion (percentage) of the total time of exploration. The learning rate for the water maze was assessed by measuring average escape latencies, total distance traveled, distance traveled in opposite quadrant, and swimming trajectory tracks. In all statistical tests the threshold for significance was set at $p < 0.05$. After these behavioral tests, brain sections were immunolabeled for glial fibrillary acid protein (GFAP) to identify astrocytes. Only young adult and aged mice from enriched environments (EY, EA) were able to integrate object recognition in a spatio-temporal context. The subjects from impoverished environments were unable to make the appropriate distinctions. IY, EY and EA groups were able to learn and remember the position of the hidden platform, but the IA group was not. Regional hippocampal astrocyte morphologies were different in young adult and aged mice. The enriched environment mainly affected astrocytes morphology in the granular and molecular layers of the dentate gyrus. Taken together, the results provide a better understanding of how environment influence memory and related astrocytic plasticity throughout the lifespan of female albino Swiss mice.

Palavras-Chave: enriched and impoverished environments, aging; memory; astrocytes, hippocampus, dentate gyrus, albino Swiss mice.

Apoio Financeiro: FINEP, CNPq, PROPESP-UFPA, IBN net

Proanthocyanidin-rich fraction obtained from *Croton celtidifolius* (Euphorbiaceae) improves olfactory, cognitive and motor impairments following intranasal administration of MPTP in rats.

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Objective: Intranasal (i.n.) administration of MPTP induces progressive impairments in olfactory, cognitive and motor functions in rats, mimicking the symptomatology of Parkinson's disease (PD). *Croton celtidifolius* is a tree found in the Atlantic Forest, and chemically characterized by phenolic composites such as proanthocyanidins. We have previously demonstrated anti-oxidative, anti-inflammatory and dopaminergic properties of the proanthocyanidin-rich fraction (PRF) of *C. celtidifolius*. In the present study we investigated whether the treatment with PRF can counteract the effects of i.n. administration of MPTP.

Methods: Male Wistar rats were assigned to four groups (n=10 animals per group): G1 and G2 groups were pre-treated with saline (control, i.p.) during five consecutive days whereas G3 and G4 groups were pre-treated with PRF 10mg/kg/i.p. for the same period. In the third day of the pre-treatment, the animals were submitted to i.n. administration of saline (G1 and G3) or MPTP 1mg/nosril (G2 and G4). The rats were submitted to a battery of behavioral paradigms including olfactory discrimination, social recognition, forced swim and activity chamber tasks.

Results: PRF pre-treatment prevented the disruption in the olfactory discrimination ability [%time in the familiar compartment (s): G1=53,19±1,17; G2=37,33±2,79; G3=49,52±1,26; G4=53,25±2,58] and in the short-term social recognition memory [Investigation time (s): G1=42,00±6,45; G2=69,00±16,11; G3=37,00±16,44; G4=21,20±6,19] observed in MPTP-treated rats. Moreover, PRF pre-treatment improved the MPTP-induced depressive-like behavior [Immobility time (s): G1=48,60±7,92; G2=73,50±6,33; G3=14,60±2,97; G4=20,40±3,52] and motor impairments [total crossings: G1=270,60±40,8; G2=127,25±17,02; G3=269,80±19,23; G4=163,75±14,75] observed in the forced swim and the activity chamber tasks, respectively.

Conclusion: These findings demonstrate that the prior treatment with the PRF is able to prevent the progressive impairments on the olfactory, cognitive and motor functions following administration of MPTP. Taken together our results suggest the potential of PRF as a useful tool in the attenuation of symptomatology of PD.

Palavras-Chave: *Croton celtidifolius*, proanthocyanidin, Parkinson's disease, MPTP

Apoio Financeiro: CNPq, CAPES, FAPESC

Viral infection and murine model of prion disease: behavioral and neuropathological findings

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Objective: The present work investigated possible interactions between an arbovirus infection and the ME7-induced prion disease, assessing the temporal evolution of the disease in a murine model. **Methods:** 84 mice C57BL/6J, female, 6 weeks old, were submitted to a bilateral intra-hippocampal injection of ME7 prion strain or normal brain homogenate. At 15th week after injection, the animals were challenged intra-nasally with a viral suspension of Piry arbovirus (0.001%). In order to assess the progression of prion disease and its interaction with arbovirus infection, the animals were divided into 6 groups, according the time of perfusion: 7, 11, 15, 18, 20 and 22 weeks. The following behavioral tests were applied: burrowing, open field (OF), elevated plus maze (EPM), inverted screen and rod bridge. The animals were deeply anesthetized, perfused and 70µm vibratome brain sections were immunolabeled for GFAP and virus Piry antigens, Tomato lectin histochemistry and Nissl. **Results:** Behavioural changes in animals with prion disease were observed after 11 weeks pos-injection, with hyperactivity detected by OF, followed by the inversion of the natural behavior of avoiding open arms of the EPM. As expected, burrowing activity decreased early in the disease, and reduced even more after associated viral infection. Inverted screen and rod bridge, showed that strength and motor coordination were decreased only at terminal stages of the disease. The inflammatory response of the central nervous system revealed an increased number of astrocytes and microglial activation with the disease progression, and a reduction of the number of cells of the granular layer of dentate gyrus estimated by optical fractionator at terminal stages of the disease. The viral infection with Piry seems to exacerbate the glial inflammatory response, increasing the number of microglial cells, without additive astrogliosis and cellular death. **Conclusion:** the results demonstrated that the arbovirus infection exacerbate the behavioural impairments and inflammatory response already induced by chronic neurodegeneration of prion disease.

Palavras-Chave: Hippocampus, Prion disease, viral infection, neurodegeneration, behaviour, murine model

Apoio Financeiro: CNPq

C.01.008

Quantitative MRI of the corpus callosum in Alzheimer's disease and Mild Cognitive Impairment

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Background: The presence of cerebral atrophy is a hallmark in Alzheimer's disease (AD) correlating with neuronal loss and cognitive impairment. The atrophy of the corpus callosum (CC) seems to be implicated in the interhemispheric disconnection, mainly for loss of the associative parietal cortex neurons. Identifying tecdual alterations that follow this volumetric decrease might improve the understanding of the fisiopathological process of the progressive disability in this disease.

Objectives: Quantify the volumetric changes of CC and the tecidual changes associated with AD and Mild Cognitive Impairment (MCI) patients, using quantitative techniques of magnetic resonance imaging (MRI).

Methods: 44 individuals were studied with MRI, being 16 normal elderly (NE), 18 patient with AD and 10 patient with MCI. Manual segmentation was accomplished to extract volumetric values and data from maps of magnetization transfer (MT) and quantification of T2 relaxation time (relaxometry).

Results: significant difference was found between NE and DA in volume ($p = 0,041$), MTR ($p = 0,023$), and relaxometry ($p = 0,001$). Similar results were found between MCI and DA but not between MCI and NE.

Conclusions: In our groups, the CC changes correlates with the cognitive impairment in DA, but not in MCI.

Palavras-Chave: Alzheimer, Corpus Callosum, MRI

Apoio Financeiro: FAPESP, FAEPA

C.01.009

A double-blind, randomized, placebo-controlled pilot study of omega-3 fatty-acids supplementation in depressed Parkinson's patients

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Objectives: This study aimed investigate the possible antidepressant effect of fish oil supplementation, rich in Docosahexaenoic acid and Eicosapentaenoic acid, fatty acids from omega-3 family, in Parkinson's patients with depression measured by Montgomery-Asberg Rating Scale (MADRS), the Clinical Global Impressions Scale (CGI) and Beck Depression Inventory (BECK).

Method: Double-blind, placebo-controlled study analyzed depression in 31 patients with Parkinson's Disease and Major Depression (DSM-IV). The patients were double-blind separated in 2 groups that received fish oil or mineral oil capsules for 3 months; each group was separated in 2 new groups: one taking antidepressant medication and another not taking it. The results in the scales utilized before and after supplementation were analyzed by t-Student test.

Results: The group supplemented with fish oil showed a significant improvement in depression's symptom, comparing beginning and end of supplementation. Patients supplemented with fish oil showed a significant decrease in MADRS ($F_{3,25} = 5.65$; $*P=0.01$) and CGI-Depression scores ($F_{3,25} = 3.67$; $*P=0.02$), and there was no difference among groups in BDI ($F_{3,25} = 1.42$; $P=0.20$). 14 patients (42%) met criteria for $\geq 50\%$ reduction in MADRS score, 7 patients (22%) met criteria for remission (final MADRS total score ≤ 12), and 2 patients (6%) discontinued supplementation of fish oil. HPLC analysis of fatty acid profile showed increase of omega-3 fatty acid in erythrocyte membrane of patients taking fish oil (DHA $t=3.75$; $P= *0.019$).

Conclusion: These results reveal that, regardless of antidepressant medication, Parkinson's patients taking fish oil presented improvement in depressive symptoms suggesting that the intake of these fatty acids can be used for an antidepressant effect or as adjuvant therapy with other medication. This is a first pilot study with Parkinson's patients and fish oil supplementation and requires replication in a larger sample.

Palavras-Chave: Depression, parkinson disease, omega 3

C.01.010

Evaluation of Estrogen Neuroprotective Effect on Nigrostriatal Dopaminergic Neurons Following 6-Hydroxydopamine Injection into the Substantia Nigra Pars Compacta or the Medial Forebrain Bundle

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Objectives: Studies involving estrogen treatment of ovariectomized rats have attributed to this hormone a neuroprotective effect on substantia nigra pars compacta (SNpc) neurons. We investigated the effect of estradiol replacement in ovariectomized rats on the survival of dopaminergic cells and the integrity of their projections to the striatum following 6-hydroxydopamine (6-OHDA) lesion.

Methods: After bilateral ovariectomy, a pellet containing estradiol or corn oil was implanted subcutaneously in female rats. After 5 days, either 1mg/1ml of 6-OHDA (6-OHDA group) or 1ml of vehicle (Sham group) was injected into the right SNpc (Experiment 1) or medial forebrain bundle (MFB) (Experiment 2). Control groups (C) were not submitted to stereotaxic surgery. Ten days after ovariectomy, rats were killed by decapitation. Trunk blood was collected for estradiol measurement and the striatum was assayed for dopamine (DA) and its metabolites. Some rats were perfused and the tyrosine hydroxylase (TH) immunostaining was processed in the SNpc. Data were analyzed by two-way ANOVA.

Results: The estrogen groups presented higher plasma estradiol concentration compared to oil groups ($F_{1,32}=33.16$; $P=0.0001$). Estradiol replacement did not prevent the reduction of striatal DA ($F_{1,32}=0.53$; $P=0.47$), DOPAC ($F_{1,32}=0.007$; $P=0.93$) and HVA ($F_{1,32}=1.25$; $P=0.27$) concentrations caused by 6-OHDA injection in the SNpc. The optical density ($F_{1,28}=0.28$; $P=0.60$) and the number of TH-immunoreactive neurons in the SNpc ($F_{1,28}=0.32$; $P=0.57$) were not affected by estradiol either. On the other hand, estradiol treatment was effective in reducing the decrease in striatal DA ($F_{1,45}=5.27$; $P=0.026$) and DOPAC concentrations ($F_{1,45}=4.39$; $P=0.042$) following 6-OHDA injection into the MFB.

Conclusions: Results suggest therefore that estrogen protect nigrostriatal dopaminergic neurons against a 6-OHDA injury to the MFB but not the SNpc. This may be due to the severity of lesions promoted in these models of Parkinson's disease.

Palavras-Chave: Neuroprotection, Parkinson disease, estrogen, 6 OHDA

Apoio Financeiro: CNPq, CAPES, FAPESP

C.01.011

Astrocytes neuroprotection against microglia-mediated apoptosis—induced by A β in hippocampal cell cultures

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Neuronal death in Alzheimer's disease can be due to A β toxicity or be mediated by glial cell-dependent inflammation. We have previously shown that astrocytes have a protective effect for both direct and indirect A β neurotoxicity *in vitro*. **Objective:** Evaluate if the protective effect depends on the modulation of microglial activation or on the production of neuroprotective factors. **Methods:** To assess that question, we exposed hippocampal cell cultures to various combinations of conditioned media from glial cultures exposed to 2 μ M A β . Apoptosis was evaluated through TUNEL assay. Specific cell type was identified with identity markers (β -Tubulin III for neurons, GFAP for astrocytes and *Griffonia simplicifolia*-lectin for microglia). The activation of glial cells was evaluated by the Griess assay, which quantifies nitrites, a stable metabolite of nitric oxide. We generated conditioned media from astrocyte's cultures stimulated with pro-inflammatory molecules, 1 μ g/ml LPS+10 ng/ml IFN γ (LI), (CM-AS LI) which was used for culturing microglia exposed to A β , recovering the conditioned media of the stimulated microglia (CM-MG (A β +AS LI)) to treat the hippocampal cultures. **Results:** Hippocampal cultures exposed to microglial conditional media (CM-MG A β) showed a 2-fold increase in the number of apoptotic cells. In contrast, when hippocampal cultures were exposed to conditioned media of mixed glia (astrocytes+microglia) stimulated with 2 mM A β (CM-MX A β), apoptosis was not induced. CM-MG(A β +AS LI) failed in inducing apoptosis as was previously observed with CM-MG A β , and apoptotic cell numbers were similar to those observed in control cultures. In contrast when hippocampal cells were exposed to a 1:1 mixture CM-AS LI+CM-MG A β , a 4-fold increase in apoptosis was observed compared to apoptosis in control cultures. **Conclusion:** Those results strongly suggest that activated astrocytes modulate the activation of microglia by A β , decreasing their cytotoxicity. However, astrocytes appear also to secrete soluble factor(s) that potentiate the toxic factors secreted by activated microglia or perhaps sensitize hippocampal cells. Further studies will be needed to identify those factors.

Palavras-Chave: neuroprotection, Glia, Amyloid beta

Apoio Financeiro: grant FONDECYT 1040831 and VRAID-Puente08 is acknowledged.

C.01.012

Synaptic dysfunction induced by Ab aggregates in rat hippocampal neurons is driven by calcium-permeant Ab pores

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Objectives: Alzheimer's disease (AD) is associated with the accumulation of proteinaceous aggregates in the brain, formed mainly by amyloid- β peptides (Ab_{1-40} and Ab_{1-42}). It is believed that these aggregates are one of the principal causes of the neurodegeneration observed in AD. On the other hand, recent findings suggest that intracellular calcium plays a critical role in the pathogenesis of AD. Therefore, in the present study, we examined the role of calcium in hippocampal neurons exposed for short (acute, 30 min) and long (chronic, 24 h) time periods to Ab aggregates.

Methods: Hippocampal neurons were obtained from 18 day pregnant mouse embryos (C57BL/6) as previously described according with NIH recommendations. Ab aggregates were used in different concentrations (0.05 - 10 mM). Subsequently, we performed Patch Clamp, Immunocytochemistry, Electron Microscopy and Western blot techniques to characterize the effects of Ab.

Results: We found that $\text{A}\beta$ aggregates localized rapidly (30 min) in synaptic regions, which was correlated with a rise in intracellular Ca^{2+} . Furthermore, acute treatment with $\text{A}\beta$ aggregates induced an increase in synaptic transmission and vesicle release monitored by patch clamp and FM1-43 destaining, respectively ($\Delta\text{F}/\text{Fi}$, control = 0.22 ± 0.03 vs $\text{A}\beta$ = 0.41 ± 0.05 , $*p < 0.05$). On the other hand, chronic treatment with Ab aggregates (24 h) altered synaptic function, which was characterized by a decrease in vesicular release ($\Delta\text{F}/\text{Fi}$, control = 0.68 ± 0.05 vs $\text{A}\beta$ = 0.4 ± 0.05 , $*p < 0.05$), a reduction in number of synaptic vesicles and also in a decrease in presynaptic protein levels such as SV2 and synaptophysin. Interestingly, the application of a peptide (NA7 or NA4a) designed to block membrane conductance enhancements associated with the amyloid pore caused a strong blockade of the Ab aggregate effects on synaptic parameters in both acute and chronic applications (control = 1.6 ± 0.1 Hz; Ab = 0.1 ± 0.04 Hz; Ab+NA7 = 1.7 ± 0.1 Hz). The mechanisms involved in the action of NA7 or NA4a did not involve changes in the distribution of Ab aggregates in hippocampal neurons.

Conclusion: The present results indicate that calcium is critical for the early and late effects of Ab aggregates on synaptic transmission in hippocampal neurons. Furthermore, these results strongly suggest that amyloid pores could be involved in synaptic dysfunction mediated by Ab aggregates.

Palavras-Chave: Alzheimer Disease, Amyloid Beta Peptide, Toxicity, Depletion, Amyloid Pore,

Apoio Financeiro: Grant ACT-04, Fondecyt 1060368

C.01.013

Effects of chronic sleep restriction on the apoptosis in the hippocampus of aged rats

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Paradoxical sleep plays a vital role in the survival of animals. Its deprivation causes alterations in brain functions and behaviors including activities of enzymes, neurotransmitter levels, impairment of neural excitability and memory consolidation. Paradoxical sleep deprivation (PSD) has been implicated in learning and memory impairment. However, the neurobiological mechanisms of this effect remain unclear. To date, few studies have focused on the aging processes on cognition that is known to be reduced during life-span. The present study sought to evaluate the effects of chronic sleep restriction (CSR) on the apoptosis in hippocampus in aged rats. 11 male old Wistar rats (22 months) were used in this experiment. The sleep restriction procedure (15 days) was based in submitting the rats of the platform during 18h (4h pm – 10h am), and the rats were allowed to sleep during 6 h (10h am – 4h pm). The multiple platform technique was used for deprivation of sleep. The control group (CTRL) was maintained in the home-cages. Formaldehyde-fixed, paraffin-embedded blocks of brain were analyzed on the basis of TdT-mediated dUTP nick end labeling (TUNEL) assay. Two slices of each (unilateral hippocampus) rats were counted and the percentage of TUNEL positive over total number of cells was performed. The ANOVA one-way showed that no difference between groups [CTRL=2,2 ±0,6 (n=5); CSR=4,0±2,1 (n=6)]. The relationship between deprivation of sleep and cell death has been little studied and still keeps controversial, but in spite of differences having not found between the groups, it is important to emphasize the high variability observed only in CSR. More over, more apoptosis was observed in CA2/CA3 areas of three rats restricted of sleep (6,1±0,3). Another proposal an ongoing investigation in our lab to explain the impairment in memory after deprivation of sleep, and despite the preliminary results, we observed reduced glutamate-stimulated cytosolic calcium in hippocampus of old rat restricted of sleep. This reduction in the receptor glutamate sensitivity is related to the impairment of long-term potentiation. More experiments are necessary to elucidate this important question.

Palavras-Chave: aging, sleep restriction, apoptosis, hippocampus

Apoio Financeiro: AFIP, CEPID/FAPESP

CELLULAR PRION PROTEIN AND STRESS-INDUCIBLE PROTEIN 1- INTRACELLULAR TRAFFICKING AND SIGNALING

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The secreted co-chaperone stress inducible protein 1 (STI1) interacts with the cellular prion protein (PrP^C) at the cell surface promoting neuroprotection and neuritogenesis. We investigated the STI1 and PrP^C trafficking and the role of endocytosis in PrP^C-dependent signaling. In order to evaluate whether STI1 could affect PrP^C trafficking, SN56 cells expressing GFP-PrP^C were analyzed by confocal microscopy before and after STI1 treatment. Wild type STI1, but not a mutant unable to bind PrP^C, STI1_{D230-245}, induced GFP-PrP^C internalization. To determine the role of PrP^C endocytosis in signaling, we used a CF-10 cells expressing a mutant PrP^C whose internalization is impaired (N-PrP3F4). STI1 evoked PKA-activation in cells expressing, PrP3F4 or N-PrP3F4. In contrast, transient activation of ERK1/2 by STI1 did not occur in N-PrP3F4. Fluorescent recombinant STI1 and STI1_{D230-245} were effectively internalized by SN56 cells. These observations were confirmed in PrP null CF-10 cells indicating that STI1 endocytosis is PrP^C independent. To investigate the intracellular localization of STI1, several endocytic markers were used. After 10-20 minutes of cell contact, STI1 accumulates in endocytic organelles that are positive to raft markers flotillin-1 and caveolina-1. Clathrin-mediated endocytosis appears to be important for the initial entry of at least part of internalized STI1, but early endosomes did not seem to participate in STI1 localization. After internalization STI1 is found in Rab7 positive vesicles suggesting the STI1 is found in late endosomes/lysosomes. Interestingly, STI1 and PrP^C colocalized predominantly at the cell surface and shortly after internalization, but downstream trafficking was different for these two proteins. The results illuminate STI1 trafficking and suggest that PrP^C endocytosis mediated by STI1 is a critical process for ERK1/2 signaling.

Palavras-Chave: Prion, Trafficking, Signaling

Apoio Financeiro: CNPq, FINEP, Millennium Institute, PRONEX-MG, Howard Hughes Medical Institute and FAPESP

COMPARAÇÃO ENTRE RECONSTRUÇÕES 3D COMPUTADORIZADAS E ANÁLISE QUANTITATIVA DA SUBSTÂNCIA NEGRA HUMANA DURANTE O ENVELHECIMENTO

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A doença de Parkinson (DP) é um freqüente de transtorno do movimento em indivíduos com mais de sessenta anos de idade. O início dos sintomas ocorre quando há grande perda neurônios dopaminérgicos da substância negra (SN). Como a SN sofre alterações durante o envelhecimento normal, é importante caracterizar esse fenômeno para fins comparativos com a DP.

Foram realizadas reconstruções computadorizadas 3D da SN em três casos controle e um caso com DP. Houve grande variabilidade individual de tamanho e forma.

Para determinar as razões dessa variabilidade, os casos foram submetidos à análise estereológica, pela técnica *disector* em cortes seriados rostro-caudais da SN de 400 a 440 µm de espessura, corados com galocianina. A porção posterior da *Pars Compacta (PsC)* apresentou maior complexidade para reconstrução em 3D.

O volume da *PsC* na SN variou de 26 a 64.8 mm³, sendo menor volume do lado direito(D) de uma paciente do sexo feminino acometida por DP, e o maior volume um caso controle do sexo masculino, com 49 anos. Além disso, a densidade as células nervosas variou consideravelmente, entre 3,219 neurônios/mm³ - em caso controle com 49 anos; até 5,607 neurônios/mm³ - em caso controle do sexo feminino com idade de 50 anos. O número total de neurônios variou de 115.502 (*PsC* - D caso feminino com DP) a 253.414 neurônios (*PsC* E - caso controle feminino de 50 anos).

Obviamente não há uma correlação simples entre tamanho e forma da SN de um lado e volume, neurodensidade e número de neurônios de outro. Para elucidar a função e a integridade da SN no envelhecimento e em doenças neurodegenerativas, um grande número de SNs devem ser estudadas de forma abrangente e hierárquica, incluindo a combinação de diferentes métodos. **Palavras-Chave:** envelhecimento, substância negra, humano, quantitativo, reconstrução, doença de Parkinson

Apoio Financeiro: CAPES, IIEP-AE, FAPESP

Effect of chronic supplementation with fish oil in animal model of Parkinson's disease

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OBJECTIVES: We investigated the neuroprotector effect of n-3 polyunsaturated fatty acids present in fish oil (FO) in an animal model of Parkinson's disease.

METHODS: Male Wistar rats aged 21-days-old were separated in two groups: (1)FO (fish oil daily supplemented until 90-days-old) and (2)C (control not supplemented). Then, each group was subdivided in three: (a)lesioned with 4µg of 6-hydroxidopamine (OHDA) into the median forebrain bundle (FO/OHDA or C/OHDA); (b)Sham group (FO/Sham or C/Sham) and (c)control group, not lesioned (FO/C or C/C). All animals were submitted to behavioral tests. Endogenous levels of dopamine (DA) and its metabolites were also assayed.

RESULTS: The lesioned rats exhibited increase of rotation in the apomorphine-induced rotation test (C/OHDA 42,15±0,84; C/Sham 4,57±0,98), ANOVA $F(1,79)=1084,30$; $p=0,0001$. However, the number of rotations in the FO animals showed to be significantly lower when compared to control group (FO/OHDA 36,28±2,14; C/OHDA 42,15±0,84), ANOVA $F(1,79)=10,43$; $p<0,05$. In the open field test, the number of crossing (C/OHDA 57,85±4,59) and rearing (C/OHDA 13,75±2,56) were reduced in the lesioned group, but FO had no significant effect in either crossing (FO/OHDA 60,23), ANOVA $F(1,71)=0,52$; $p=0,47$ or rearing (FO/OHDA 13,22±3,55), ANOVA $F(1,42)=0,14$; $p=0,71$. The tremulous jaw movement (C/OHDA 95,0±14,20) was not reversed by the FO (FO/OHDA 91,37±9,70), ANOVA $F(1,40)=0,07$; $p=0,79$. The OHDA injection caused a significant decrease in striatal DA (C/OHDA 46,83±10,06; C/C 103,80±3,46), ANOVA $F(1,43)=106,63$; $p=0,0001$, although FO treatment had no effect on these concentrations (FO/OHDA 34,69), ANOVA $F(1,43)=2,00$; $p=0,16$.

CONCLUSION: FO supplementation proved to be efficient in decreasing the number of apomorphine-induced rotation, but it had no significant effect in preventing the loss of dopaminergic neurons, contrary to expectations. Further studies are necessary to investigate protection caused by FO in parkinsonian symptoms.

Palavras-Chave: Parkinson disease, polyunsaturated fatty acids

β -AMYLOID (1-42) OLIGOMERS INDUCE DIFFUSE AMYLOID DEPOSITION ASSOCIATED WITH REACTIVE ASTROGLIOSIS IN THE RAT BRAIN

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It has been proposed that accumulation of β -amyloid peptide (A β) and the deposition of diffuse plaques in the AD brain result in glial cell activation. Activation of glial cells may mediate A β toxicity by releasing interleukins, and other cytotoxic molecules that can self-propagate the neuroinflammatory process (Akiyama et al., 2000; Koistinaho and Koistinaho, 2002). In addition, evidence exists that glia cells are also involved in the clearance of A β from the CNS (Pihlaja et al., 2007; Knoch et al., 2008). The aim of this study was to investigate the interaction between glia cells, particularly astrocytes, and A β peptide *in vivo*. In Wistar rats, anaesthetised with equitiesina (0.4ml/100gr), injections of A β (1-42) oligomers (BACHEM, 2 μ g in 5 μ l) were placed into the left posterior cingulate cortex. Control animals were injected with scrambled A β 1-42 (BACHEM, 2 μ g in 5 μ l). After 24h and 72h, animals were deeply anaesthetised and perfused with fixative. Series of sections were immunoreacted for the localization of A β peptide (CLONE 6E10; Signet), and glial fibrillary acid protein (GFAP, Sigma). Immunocytochemical analysis with the 6E10 marker revealed a dark staining at the level of A β 1-42 injection site. Many A β -immunoreactive structures-like diffuse amyloid forms were also observed adjacent to the injection of A β , and extending to the hippocampus and to several cortical regions at an early stage (24h), increasing significantly at later stages (72h). Injections of A β 1-42 oligomers into the retrosplenial cortex also induce reactive astrocytes at both 24h and 72h after injection. In contrast to control animals in which astrogliosis was almost found restricted to the injection site, likely caused by mechanical damage, in A β oligomers-treated animals astrocyte proliferation was also observed extending further apart from the amyloid injection site. A β -immunoreactive material was also found within reactive astrocytes at the injection site at an early (24h) stage, and significantly increases at later (72h) time points. Confocal images analyze showed that GFAP expressed in the cytoplasm of astrocytes, in many cases co-localized with A β -immunoreactivity in structures-like diffuse amyloid deposition in hippocampus and in several cortical regions, particularly in the frontal and entorhinal cortex, ipsilateral and contralateral to the injection of A β . The expression of GFAP in A β -immunoreactive structures-like diffuse forms showed an age-dependent progressive evolution. The injection of scrambled peptide showed background staining surrounding the injection site, and no structures-like diffuse amyloid deposition were observed in such control animals. These data provide valuable support *in vivo* for a potential relationship between activate astrocytes and diffuse amyloid forms at an early stage of A β -toxicity.

Palavras-Chave: Alzheimer's disease, Neurotoxicity, Neuroinflammation, In vivo animal models, Limbic system

Apoio Financeiro: Grant from Junta de Castilla y León, Ref. VA030A07 to A.G-R

C.01.018

Neuropathological characterization of a Down syndrome patient with a long course of severe dementia

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Objectives

To characterize morphological and immunohistochemically a clinical and genetically confirmed Down's syndrome (DS) in an unusual elderly patient having severe dementia of Alzheimer-type. To compare the burden and distribution of these changes with the ones found on sporadic Alzheimer's disease (AD) cases.

Methods:

A 67-years old female, was admitted at the Geriatrics clinics of the Clinicas hospital of the University of São Paulo Medical School (USPMS). She had DS, and dementia for seven years. After her death due pneumonia, the brain was donated to the Brain Bank of the Brain Aging Study Group of USPMS and processed according with its protocol. On the sampled bilateral brain areas, the following immunoperoxidase reactions were performed: PHF-1 (anti-tau protein), 4G8 (anti- β -amyloid protein) and EQV-1 (anti- α -sinuclein protein). The reactions were developed using DAB (diaminobenzidine tetrahydrochloride) and analyzed by semi-quantitative methods. The results were compared to the neuropathological criteria to AD.

Results:

The brain was severely atrophic (760g). There were no evident injuries or vascular disease. No α -sinuclein related changes were highlighted. Tau and β -amyloid changes were compatible with AD, however in a higher burden in all the examined areas, including those considered to be spared in AD. The changes were bilateral.

Conclusion:

DS is a congenital syndrome that can be caused by various chromosome abnormalities, especially by trisomy 21. Patients usually develop early signs of dementia. The reported case had an unusual life-span and a late age of dementia onset. However, the progression of AD changes in this case was much faster than in sporadic ones and, even brain areas usually spared were affected. Studies on DS patients can result in a better understanding of AD pathogenesis and progression.

Palavras-Chave: Down's syndrome, Alzheimer's disease

Inhibition of mitochondria-bound hexokinase by beta-amyloid triggers neuronal oxidative stress and cell death.

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Background: Recent studies have demonstrated that energy metabolism is impaired in the brains of Alzheimer's disease (AD) patients. However, the cellular mechanisms underlying the changes in neuronal metabolism have not been fully elucidated. Because glucose is the main energy source for the mammalian brain, we have investigated the role of hexokinase (HK), a key glycolytic enzyme, in the limited metabolic capacity of neurons exposed to beta-amyloid peptide (Ab). Ab accumulates in AD brain and has been implicated as the primary agent in the initiation of neuronal dysfunction, including the marked neuronal oxidative stress that is characteristic of AD.

Objectives: To investigate the role of hexokinase in the mechanisms of neurotoxicity instigated by Ab and the role of hexokinase, a key glycolytic enzyme, in the limited metabolic capacity of neurons exposed to Ab.

Methods: Neuronal viability assay, determination of intracellular glucose-6P content, determination of hexokinase activity and western blot analysis with neuronal cultures.

Results: Here we show that treatment of cortical neurons in culture with Ab inhibited mitochondria-bound HK (m-HK) by causing its release from mitochondria, and led to excessive ROS production and neuronal oxidative stress. Ab had no direct effect on m-HK activity in isolated mitochondria. Interestingly, the cell permeant glucose analog 2-deoxyglucose (2-DOG), which serves as an alternative substrate of m-HK, prevented Ab-instigated ROS formation and blocked Ab toxicity to cultured cortical neurons.

Conclusions: These results suggest that inhibition of neuronal m-HK activity by Ab plays an important role in AD pathology by impairing brain energy metabolism and favoring neuronal oxidative stress. Moreover, decreased neuronal glucose utilization and its accumulation in the central nervous system (CNS) may be an additional contributing factor in the establishment of the recently recognized CNS insulin resistance in AD.

Palavras-Chave: beta-amyloid peptide; hexokinase; neurotoxicity ; oxidative stress; Alzheimer's disease

Apoio Financeiro: CNPq; Faperj; HHMI

C.01.020

Correlação do grau de aterosclerose das artérias carótidas comum e interna com alterações cognitivas no envelhecimento

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Objetivo: O envelhecimento populacional e o aumento das alterações cognitivas são emergentes na população brasileira. Acredita-se que lesões vasculares levem ao desenvolvimento de demências. Como as artérias carótidas são responsáveis pela irrigação cerebral, o comprometimento aterosclerótico desses vasos sanguíneos poderá afetar o encéfalo, prejudicando as funções cognitivas. Desta forma, objetivamos correlacionar o grau de obstrução das artérias carótidas comum e interna com alterações cognitivas.

Desenho: Estudo transversal. *Materiais e Métodos:* Foram selecionados 219 casos aleatórios do Banco de Encéfalos Humanos do Grupo de Estudos em Envelhecimento Cerebral da Faculdade de Medicina da USP (BEHGEEC), dos quais foram coletadas as carótidas. O estudo utilizou-se da análise macroscópica, quanto à porcentagem de obstrução da luz, classificando-se as artérias em: sem obstrução, obstrução de 50%, obstrução de 50% a 75%, obstrução maior que 75% e oclusão total. Demais dados foram coletados das entrevistas feitas com os cuidadores dos casos com idade superior a 50 anos, classificando-os de acordo com a escala clínica semi-estruturada denominada CDR (*Clinical Dementia Rating*), segundo os protocolos do BEHGEEC.

Aplicou-se o teste exato de Fischer e Chi-Square com confiabilidade de 95%. *Resultados e Discussão:* A partir dos 219 casos avaliados, 184 eram não-dementados e 35 eram dementados. Observou-se que tanto os indivíduos dementados e os indivíduos não dementados, em sua maioria, apresentaram aterosclerose carotídea, sendo o primeiro acometido por 94% do grupo e o segundo por 94,4% ($p = 1,00$). *Conclusão:* Não há relação entre aterosclerose de artérias carótidas e demência.

Palavras-Chave: Demência - Aterosclerose - Artérias carótidas.

Apoio Financeiro: FAPESP- LIM-HCFMUSP

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Which is the most toxic species in the aggregation pathway of transthyretin?

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Wild type transthyretin (TTR) has been involved in senile systemic amyloidosis (SSA) while more than 100 variants have been associated with familial amyloidotic polyneuropathy (FAP). There is a debate in the literature concerning the identification of the toxic species present in the pathway to fibril formation (native β -protofibril $\rightarrow$ fibril). In the current study, we are investigating the cytotoxicity of the different aggregates of the wild-type TTR, and its variants using neuroblastoma cells. Aggregates are being produced either by acidification or after a cycle of compression-decompression. The MTT assay shows that after centrifugation the supernatant of the samples affect the redox potential in a higher way than the pellet to these cells. The Viability assays shows that the supernatant of the samples is most toxic than the pellet fraction. These results suggest that the smaller aggregates present in the supernatant would be responsible for the dysfunction observed in the patients with FAP and SSA. Interestingly, aggregates present in the supernatant of the aggressive variant L55P showed to be the most cytotoxic, and this mutant showed to be toxic even at neutral pH. The species present in the pellet has a strong capacity to bind Congo Red, an amyloid marker, in contrast with the supernatant, confirming the absence of mature amyloid fibrils in the supernatant of these solutions. The characterization of these toxic species by size exclusion chromatography shows the formation of oligomers during the aggregation pathway of TTR. The characterization of the morphology and the size of these toxic species by AFM and transmission electron microscopy shows the presence of oligomers and not mature amyloid fibrils.

Palavras-Chave: Keywords: MTT, transthyretin, amyloidosis

Apoio Financeiro: Supported by: CNPq, FAPERJ and CAPES

Activation of Microglia Cells by the Aggregates Formed from the A25T variant of Transthyretin: implication in central nervous system amyloidosis

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Transthyretin (TTR) is a 127-residue homotetrameric β -sheet-rich protein that transports thyroxine in the blood and cerebral spinal fluid (CSF). Among all TTR variants, A25T is the most unstable tetramer. Its great instability induces TTR degradation by hepatocytes. However, the presence of thyroxine (T4) in the choroid plexus leads to TTR stabilization and secretion in the CSF. In the present study we aim to investigate the mechanisms of A25T dissociation and aggregation as well as its role in leptomeningeal amyloidosis. Our data show that A25T is less stable than the wt and L55P, the most aggressive variant of TTR. We also investigated the aggregation of these variants in a CSF-like environment and in human plasma by using acrylodan-labeled proteins. The aggregation of A25T was much more pronounced than that displayed by the wt or L55P. The morphology of the aggregates formed under different conditions was analyzed by atomic force microscopy and Congo Red binding and the aggregates exhibited a significant amyloid structure. In order to have insights on the lower stability of A25T, we solved its structure by x-ray crystallography. The analysis of the structure of A25T revealed a more expanded tetramers in accordance with its lower stability. Since leptomeningeal amyloidosis symptoms suggest local inflammation, we argued whether A25T aggregates would induce an inflammatory response. By using microglial primary cultures, we observed that addition of A25T aggregates changed the morphology of these cells, suggesting their activation. Besides, the levels of IL-1 β and NO were higher and comparable to that induced by LPS, suggesting an inflammatory response promoted by these aggregates. The aggregates of A25T were detected inside the microglia suggesting they were being phagocytosed. Indeed, the addition of cytochalasin D blocked their entrance into these cells. These results suggest that amyloid aggregates may have a role in the pathogenesis of central nervous system amyloidosis exacerbating tissue damage mediated by microglia. Support: CNPq and FAPERJ.

Palavras-Chave: amyloidosis, Transthyretin, Microglia, inflammatory response

Apoio Financeiro: CNPq and FAPERJ.

Running wheel and treadmill prevents cognitive and motor deficits in the reserpine-treated rat model of Parkinson's disease.

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Objectives: Although Parkinson's disease (PD) is classically considered to be a motor system disease, subtle cognitive impairments can be observed even during the early phases of PD. The most of the drugs currently available for the treatment of PD (such as levodopa) are more efficient in alleviating motor than cognitive impairments. Here, we evaluated the potential of physical exercise to prevent cognitive and motor deficits in the reserpine-treated rat model of PD. **Methods.** Forty-eight Wistar adult/male rats were assigned to six groups (n=8 per group): (1) untrained-saline; (2) untrained-reserpine; (3) running wheel (RW)-saline; (4) RW-reserpine; (5) treadmill-saline; and (6) treadmill-reserpine. Trained rats had free access to a RW or to run in a treadmill for 28 days. The animals were injected subcutaneously (s.c.) with saline (control) or reserpine (1 or 5 mg/kg) 24 h before the experiments. The rats were submitted to a battery of behavioral paradigms including olfactory discrimination, social recognition, water-maze, open-field and accelerated rota-rod tasks. **Results:** No significant differences regarding the locomotor function and olfactory discrimination ability were observed in animals treated with the low reserpine dose (1 mg/kg). However, untrained-reserpinized rats demonstrated an impairment of short-term social memory in comparison to controls [investigation time (s): untrained-saline 67.3±19.5; untrained-reserpine (147.4±12.7)]. This cognitive deficit was prevented by RW (58.4±15.3) and treadmill (71.4±10.9). On the other hand, the highest reserpine dose (5 mg/kg) induced motor impairments in the rota-rod [latency to fall (s): untrained-saline 156.9±12.2; untrained-reserpine (68.1±9.3)] and open-field [crossings: untrained-saline (69.1±5.4); untrained-reserpine (43.1±8.0)] tasks. Both exercise groups plus reserpine performed better on the latency to fall (RW: 122±12.9 s; treadmill: 122.9±14 s) and open-field' crossings (RW: 76.9±6.4; treadmill: 80.6±8.2) than untrained-reserpine group. **Conclusion:** Our results reinforce the potential of light to moderate physical exercise as an useful tool in the attenuation of motor and cognitive symptomatology of PD.

Palavras-Chave: Parkinson disease; memory; exercise

Apoio Financeiro: CNPq, CAPES, FAPESC.

**OLIGOMERS AND FIBRILS OF THE BETA-AMYLOID PEPTIDE INCREASE
EXTRACELLULAR EXCITATORY AMINO ACIDS IN HIPPOCAMPAL CULTURES**

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Objectives: Alzheimer's disease (AD) is characterized by progressive accumulation of neurotoxic beta-amyloid peptide (A β) aggregates in the brains of affected individuals. The neurotoxicity of A β aggregates has been related to the over activation of NMDA-type glutamate receptors (NMDARs) and excitotoxicity by mechanisms that are not yet fully elucidated. In this study, we investigated the effects of soluble A β oligomers (also known as ADDLs) and amyloid fibrils on the extracellular accumulation of glutamate and d-serine, a co-agonist of NMDARs, in hippocampal neuronal cultures.

Methods and results: Extracellular glutamate and d-serine concentrations were determined in the media of neuron-enriched cultures of rat hippocampus after four hours of incubation in the absence or in the presence of ADDLs (500 nM) or A β fibrils (5 μ M). No cell death was observed in these conditions. ADDLs increased extracellular glutamate levels by 48 ± 14 % and extracellular d-serine levels by 12 ± 4 %. A β fibrils increased extracellular glutamate by 55 ± 12 % and extracellular d-serine by 41 ± 16 %. Blockade of NMDARs (with 10 μ M MK-801) and inhibition of the propagation of action potentials (by 0.3 μ M tetrodotoxin) both prevented the increase in extracellular glutamate and D-serine instigated by ADDLs and Ab fibrils. Interestingly, taurine (a GABA_A receptor agonist) also blocked ADDL- and fibril-induced accumulation of excitatory amino acids (EAAs) in the medium. Fibrillar A β also increased the expression of neuronal serine racemase, the enzyme responsible for d-serine synthesis, as determined by immunocytochemistry.

Conclusions: ADDLs and A β fibrils induce extracellular accumulation of glutamate and d-serine in hippocampal cultures by mechanisms that appear to involve neuronal activity. The increase in d-serine is possibly related to A β -induced elevation of neuronal serine racemase expression. A β -induced extracellular accumulation of the EAAs glutamate and d-serine may be related to NMDAR-dependent neurotoxicity in AD.

Palavras-Chave: Alzheimer's disease, beta-amyloid peptide, EAA, hippocampal neurons

Apoio Financeiro: CNPq, FAPERJ, HHMI

C.01.025

Atividade de ectonucleotidases em modelo animal de hemiparkinsonismo induzido por 6-OHDA

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Objetivos A doença de Parkinson caracteriza-se por uma perda de neurônios dopaminérgicos da substância negra e conseqüente depleção de dopamina no corpo estriado (NeuroRx 1(1): 139, 2004). A adenosina extracelular age no estriado como um antagonista fisiológico da dopamina via receptores adenosinérgicos A_{2A} (Neuroscience 141: 19, 2006), enquanto a guanosina tem um papel protetor no encéfalo (Cell Mol Neurobiol 22: 353, 2002). A inativação da sinalização do ATP e a produção de adenosina e guanosina pode ser mediada por uma variedade de ectonucleotidases (Neurochem Res 16: 1303, 1991; Brain Res 950: 74, 2002). O objetivo desse estudo foi avaliar a hidrólise dos nucleotídeos extracelulares da adenosina e guanosina em fatias de corpo estriado de ratos com lesão da via nigroestriatal induzida pela infusão unilateral de 6-hidroxidopamina (6-OHDA).

Métodos Ratos Wistar machos (290 – 350 g, 3-4 meses de idade) receberam infusão unilateral de 6-OHDA ou apenas veículo no feixe prosencefálico medial. Duas semanas após, os ratos receberam anfetamina (i.p.). Apenas os animais que apresentaram rotações ipsilaterais no grupo 6-OHDA foram considerados lesionados. Uma semana após, avaliamos a atividade das ectonucleotidases em fatias do estriado.

Resultados A infusão de 6-OHDA provocou um aumento na hidrólise de ADP, AMP e GTP em 15, 25 e 41% ($21,42 \pm 2,34$, $5,13 \pm 1,37$ e $16,59 \pm 5,57$ nmol Pi/min/mg), respectivamente, quando comparados com animais não operados (CONTROLE: $18,55 \pm 1,21$, $4,10 \pm 1,38$ e $11,72 \pm 4,0$ nmol Pi/min/mg) e SHAM ($17,68 \pm 2,56$, $4,01 \pm 0,58$ e $9,11 \pm 3,63$ nmol Pi/min/mg). Já a hidrólise de GDP foi diminuída em 60% em relação aos animais CONTROLE e SHAM ($0,83 \pm 0,2$, $2,13 \pm 0,47$ e $2,12 \pm 0,81$ nmol Pi/min/mg, respectivamente) (ANOVA seguida do teste de Dunnett, $p < 0,05$).

Conclusão As alterações enzimáticas encontradas podem estar favorecendo o processo neurodegenerativo causado pela infusão de 6-OHDA, pois geram alterações de concentração de adenosina e guanosina prejudiciais à sobrevivência e funcionamento do corpo estriado.

Palavras-Chave: ectonucleotidases, adenosina, guanosina, hemiparkinsonismo, 6-OHDA.

Apoio Financeiro: CNPq

EMOTIONAL, COGNITIVE AND NEUROCHEMICAL ALTERATIONS IN A PREMOTOR STAGE MODEL OF PARKINSON'S DISEASE

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OBJECTIVES:

In addition to classic motor symptoms, Parkinson's disease (PD) is characterized by cognitive and emotional deficits, which have been demonstrated to precede motor impairments. The present study addresses the question of whether a partial degeneration of dopaminergic neurons using 6-hydroxydopamine (6-OHDA) in rats is able to induce pre-motor behavioral signs.

METHODS:

The levels of dopamine, noradrenaline, and serotonin in various brain regions were analyzed by HPLC-ED. Behavioral tests that assessed a variety of psychological functions, including locomotor activity (open field test), emotional reactivity and depression (sucrose consumption and forced swimming tests), anxiety (elevated plus maze test) and memory (Morris water maze and social recognition tests) were conducted on 6-OHDA-lesioned rats.

RESULTS:

Bilateral infusion of 6-OHDA in the striatum of rats caused early (one week) (Ctrl: 6204±588; 6-OHDA: 2522±341.3) and late (three weeks) (Ctrl: 4684±620.8; 6-OHDA: 2278±449.6) loss of dopamine in the striatum, and a late (three weeks) loss of dopamine in the prefrontal cortex (Ctrl: 97.4±18.8; 6-OHDA: 36.2±14.1), but not in the hippocampus (Ctrl: 35.8±2.8; 6-OHDA: 23.4±7.8). Additional noradrenergic and serotonergic alterations were observed after 6-OHDA administration. The results indicated that 6-OHDA - lesioned rats show decreased sucrose consumption (Ctrl: 80.5±5.9; 6-OHDA: 56.2±7.2) and an increased immobility time in the forced swimming test (Ctrl: 58.4±5.6; 6-OHDA: 125.9±11.2), an anhedonic-depressive-like effect. Also, an anxiogenic-like activity in the elevated plus maze test (% open arms entries: Ctrl: 38.1±2.7; 6-OHDA: 18.8±6.2) and cognitive impairments were observed on the cued version of Morris water maze (Latency to find the platform: Ctrl: 13.6± 8.2; 6-OHDA: 23.4±9.3) and social recognition (Investigation time on the 2nd presentation: Ctrl: 56.9± 7.0; 6-OHDA: 103±15.1) tests.

CONCLUSIONS:

These findings suggest that partial striatal dopaminergic degeneration and associated dopaminergic, noradrenergic and serotonergic alterations in striatum and prefrontal cortex may have caused the emotional and cognitive deficits observed in this rat model of early phase Parkinson's disease. **Palavras-Chave:** 6-OHDA, striatum, prefrontal cortex, depression, anxiety, memory

Apoio Financeiro: CNPq, FAPESC

Insulin prevents neuronal damage induced by Abeta oligomers

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The major clinical hallmark of early Alzheimer's disease (AD) is an inability to form new memories. For many years, this form of dementia was attributed to neuronal cell death induced by deposits of fibrillar amyloid- β (A β). However, the current view of AD is that early memory loss is due to a synapse failure caused by soluble and diffusible A β oligomers, also known as ADDLs. These molecules have been shown to build up in the brain and cerebrospinal fluid of AD patients, and they are known to act as specific synaptic ligands producing changes in dendritic spine morphology and loss of plasticity-linked receptors. Our group investigated whether there are mechanisms that can protect synapses against ADDLs and, thus, help to preserve brain cognitive functions. In the central nervous system, insulin has an important role in cognition and learning. We have recently reported that ADDLs induce loss of dendritic insulin receptors (IRs), suggesting that defective insulin signaling may be an early step in AD pathogenesis. We now aimed to evaluate whether insulin could protect neurons against ADDL-induced damage in hippocampal neurons and slices in culture. Using mature hippocampal cultures, we found that ADDL-induced IR loss was completely blocked by insulin. Furthermore, insulin significantly prevented ADDL-induced neuronal oxidative stress. Recent results indicated downregulation of brain-derived neurotrophic factor (BDNF) mRNA induced by ADDLs. BDNF plays a pivotal role in synaptic plasticity and cognition. Interestingly, insulin treatment prevented ADDL-induced downregulation of BDNF. These results indicate that insulin protects neurons from the damage induced by ADDLs and suggest that boosting brain insulin signaling may be an effective approach to prevent ADDL-induced insulin resistance and memory impairment in AD. **Palavras-Chave:** Insulin signaling, neuronal oxidative stress, Abeta oligomers, synaptic dysfunction.

Apoio Financeiro: CNPq, FAPERJ, The Human Frontier Science Program Organization.

EFFECTOS DEL EJERCICIO FÍSICO SOBRE CAMBIOS MICROVASCULARES EN SUBSTANCIA NEGRA Y ESTRIADO DURANTE EL ENVEJECIMIENTO.

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En el proceso de envejecimiento hay cambios degenerativos en el sistema dopaminérgico. Parte de estos cambios podrían estar asociados a una disminución de la microvascularización en el estriado y sustancia negra (SN). Estudios previos sugieren que el ejercicio físico puede reducir cambios degenerativos en el sistema dopaminérgico. **Objetivos:** Hemos estudiado posibles cambios con el envejecimiento en la microvascularización del estriado y SN, así como en los niveles de factor de crecimiento endotelial vascular (VEGF). El VEGF no es un solo importante factor angiogénico, sino que también poseen efectos tróficos sobre las neuronas dopaminérgicas. Además estudiamos si esos posibles cambios degenerativos se pueden reducir con la actividad física. **Materiales y Métodos:** Se utilizaron 6 grupos (n=60) de ratas machos de 4, 14 y 22-27 meses respectivamente, que fueron sometidas o no sometidas (control) al ejercicio físico en un Treadmill durante 28 días consecutivos. Los animales fueron posteriormente sacrificados y procesados para análisis de expresión de VEGF por PCR a tiempo real, así como inmunohistoquímica (antiRECA-1) para marcar vasos sanguíneos en el estriado y SN. **Resultados:** en el estudio se observó una disminución del número de vasos sanguíneos en el estriado y SN con el envejecimiento. Por otra parte también observó en estas estructuras una reducción de expresión de mRNA de VEGF con el envejecimiento. Los grupos sometidos al ejercicio presentaron una mayor densidad de microvascularización y mayores niveles de expresión de VEGF que sus respectivos controles. **Conclusión:** los resultados indican que hay una disminución en el grado de vascularización y expresión de VEGF durante el envejecimiento, que podría contribuir a cambios degenerativos en el sistema dopaminérgico. Interesantemente estos cambios se podrían reducir significativamente con un régimen adecuado de ejercicio físico.

Palabras-Chave: ENVEJECIMIENTO, VEGF , EJERCICIO Y VASCULARIZACIÓN

Apoio Financeiro: Ministerio da Educación y Ciencia (MEC) y Ministerio de Sanidad de España, Xunta de Galicia.

Neuroprotective effect of choline administration after global brain ischemia in rats

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Introduction and Objective: Cerebral vascular accident (CVA), besides being one of the most important causes of mortality and morbidity, demands expensive costs with patients care. The citicoline (cytidine-5'-diphosphate choline or CDP-choline) is the only drug whose neuroprotection against ischemic lesions have been proved in a several numbers of experimental and clinical studies (randomized and double-blind). However its neuroprotective mechanism has not been fully explained. As the link between CDP and choline is broken after administration and its beneficial effects we not reproducible with the CDP administration alone, it is probably that choline is the only responsible for the neuroprotection of citicoline. This work aimed at verifying the effects of choline administration on CA1 pyramidal cells of rat hippocampus after global brain ischemia.

Method: Male wistar rats were submitted to the 2-vessel occlusion model of 10-min forebrain ischemia associated with hypotension. Animals were saline or choline supplemented twice a day by oral gavage for 7 days: Group A (saline, N=5); Group B (400mg choline/Kg/day, N=6). Brains were perfused-fixed, sectioned and stained for neuronal counting.

Results: 7 days choline treatment after global brain ischemia in rats significantly reduced neuronal damage in CA1 region of dorsal hippocampus ($p < 0,01$; Mann-Whitney U-test).

Conclusion: The present result is therefore consistent with the hypothesis that choline may be the responsible for the neuroprotective effect of citicoline, having the advantage of being a low-cost treatment of ischemic injury. We hope that this protection of compromised neurons in the disease location not only will reduce the number of neurons that need to be regenerated but might also result in enhanced neurotransmitter input and electrical stimulation of neural stem cells and their progeny that migrate to the local producing a more propitious environment to neurogenesis.

Palavras-Chave: choline, global brain ischemia, neuroprotection, neurogenesis

Apoio Financeiro: CAPES

LARGE NUMBERS OF NEURONS ARE LOST IN RAT CEREBELLUM, OLFACTORY BULB AND HIPPOCAMPUS WITH AGING

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Objectives: Several studies have shown that tissue loss in normal aging entails widespread white matter and localized neuronal loss, which are associated with the deterioration of mental functions ((Neurobiol Aging 8:521,1987; Neurology 50:972,1998) A simple method to quantify alterations in the cellular composition of the brain would be helpful in the evaluation of treatments that slow down the effects of aging. Here we use the Isotropic Fractionator, a rapid, simple nonstereological method, to estimate the number of neuronal and non-neuronal cells in the rat brain.

Methods: Seventeen Wistar rats of 3 months (n=9), 12 months (n=6) and 22 months (n=3) of age were euthanized and perfused with 4% paraformaldehyde. Their brains were dissected into five regions of interest (cerebral cortex, hippocampus, cerebellum, olfactory bulb and remaining areas), which were weighed and analyzed separately with the Isotropic Fractionator.

Results: Although no brain structure loses mass significantly between 3 and 12 months of age, the number of neurons is significantly smaller at 12 months in the hippocampus (32% reduction, p=0.0104), in the cerebellum (16% reduction, p=0.0451) and in the olfactory bulb (23% reduction, p=0.0142) than in 3-month-old rats. The number of neurons in the cerebral cortex and remaining areas is not significantly different between these ages. The reduction is even more pronounced in the 22-month-old cerebellum (36% reduction relative to 3 months, p=0.0339), but not in the hippocampus and olfactory bulb.

The number of non-neuronal cells is significantly reduced between 3 and 12 months in the olfactory bulb (p=0.0045) and in the remaining areas (p=0.0045). In contrast, no changes in numbers of non-neuronal cells are found in the cerebral cortex, hippocampus and in the cerebellum with aging.

Conclusions: Our data indicate that aging entails a marked neuronal loss in the hippocampus, cerebellum and in the olfactory bulb that is masked by the maintenance of the mass of these structures. This loss is already apparent at 12 months of age, when rats are not yet considered old in the literature.

Palavras-Chave: Aging, neuronal loss, Isotropic Fractionator

Apoio Financeiro: CNPq e Faperj

Aminoguanidina Atenua Dano Cognitivo em Modelo Experimental de Demência Induzida por Estreptozotocina.

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Introdução e Objetivos: A estreptozotocina (STZ) vem sendo utilizada via intracerebroventricular (ICV) para induzir demência do tipo Alzheimer em roedores. Dentre as diversas características fisiopatológicas da doença, está o estresse oxidativo (EO) resultante da neuroinflamação. Nesse sentido, investigamos o efeito da aminoguanidina (AG), um inibidor da enzima óxido nítrico (NO) sintase induzível e antioxidante em potencial, sobre a memória de longa duração e parâmetros de EO em ratos.

Métodos: Ratos Wistar machos adultos foram anestesiados com xilazina/cetamina e infundidos com STZ (3mg/kg) ICV (5 mL/lado) (grupo STZ) ou veículo (grupo SHAM). No 3º e 5º dias após a cirurgia estereotáxica para acessar os ventrículos laterais, os animais STZ receberam uma injeção de AG intraperitoneal na dose de 100 mg/kg ou veículo. Duas semanas após a cirurgia, os ratos foram treinados em esQUIVA inibitória (EI) e testados 24 horas depois. Após o teste, os animais foram mortos por decapitação e separados hipocampo (HC) e córtex frontal (CF). Estes foram homogeneizados e submetidos a ensaios colorimétrico para dosagem de nitritos e fluorimétrico para dosagem de glutatona (GSH).

Resultados: O teste em EI mostrou que os animais STZ que receberam veículo não aprenderam (teste de Wilcoxon), enquanto o grupo STZ que recebeu AG aprendeu a tarefa (teste de Wilcoxon, $p < 0,05$). Os níveis de nitritos foram elevados no grupo STZ ($27,5 \pm 1,56$) e reduziram com a AG ($17,34 \pm 0,52$; $p < 0,01$) em HC e CF (STZ= $20,13 \pm 0,44$; AG= $12,31 \pm 0,57$; $p < 0,01$). Já a GSH, diminuiu no grupo STZ em HC ($11,4 \pm 2,23$), o que foi revertido no grupo AG ($43,3 \pm 3,92$; $p < 0,05$).

Conclusões: O déficit cognitivo observado nos animais STZ foi revertido pela AG, o que pode ter acontecido, entre outros fatores, devido à ação antioxidante da mesma, pois tivemos diminuição dos níveis de NO e aumento de GSH, um importante antioxidante do sistema nervoso central.

Palavras-Chave: estreptozotocina, intracerebroventricular, demência, aminoguanidina, esQUIVA inibitória, óxido nítrico, glutatona.

Apoio Financeiro: CNPq, CAPES, FINEP/ Rede IBN 01.06.0842-00, FAPERGS.

Searching for auto-antibodies in Amyotrophic Lateral Sclerosis

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Background: Amyotrophic Lateral Sclerosis (ALS) is an invariably fatal neurodegenerative disease characterized by a gradual loss of superior and inferior motoneurons. Approximately 90% of ALS cases belongs to a sporadic form of the disease, whose etiology is still unknown.

Objectives: Previous results from our laboratory (J. Neurosci. 26(10):2661,2006) favor an autoimmunity hypothesis as a potential cause for sporadic ALS and for that reason we decided to study more profoundly this possibility.

Methods: We purified Immunoglobulins G (IgGs) from serum of sporadic ALS patients (ALS-IgGs) and characterized them immunoreactively (by immunofluorescence and Western Blotting) and electrophysiologically (by intracellular recordings).

Results: In approximately 50% of the cases, ALS-IgGs presented a strong immunoreactivity against neuromuscular junctions of mouse diaphragm colocalizing with Acetylcholine Receptor, a post-synaptic membrane marker of that structure (representative fluorescence intensity: 3.97 ± 0.76 for ALS and 1.76 ± 0.54 for control). In addition, a large proportion of patient's sera showed immunoreactivity against rat motoneurons in the spinal cord as well as Purkinje cells in the cerebellum (motoneurons representative fluorescence intensity: 44.01 ± 3.52 for ALS and 3.87 ± 0.29 for control; Purkinje cells representative fluorescence intensity: 12.34 ± 0.88 for ALS and 2.58 ± 0.71 for control). Several of these sera also incremented the miniature end-plate potential (MEPP) frequency of preparations from mouse diaphragm (representative fold increase: 3.56 ± 0.37). Furthermore, the sera of these patients were characterized by the selective presence of antibodies that recognize different synaptosomal membrane proteins from rat cerebellum as well as from cerebral cortex (range of intensities: 0.45-1.85 for ALS and 0.03-0.20 for control), with approximate molecular weights of 50, 70 and 88 KDa.

Conclusions: The results presented in this work add evidences in favor of the autoimmunity hypothesis as one of the possible causes of ALS and suggest the presence of auto-antibodies in patients' serum that would be relevant in the development of the disease. **Palavras-Chave:** Amyotrophic Lateral Sclerosis, Autoimmunity

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C.01.033

Astroglial changes in rats submitted to chronic cerebral hypoperfusion

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Introduction: The permanent occlusion of common carotid arteries (2VO), resulting in a significant reduction of cerebral blood flow (hypoperfusion) in rats, constitutes a well established experimental model to investigate neuronal damage and cognitive impairment that occurs in human ageing and Alzheimer's disease.

Objectives: In the present study, we evaluated two astroglial proteins - S100B and glial fibrillary acidic protein (GFAP) - in cerebral cortex and hippocampus, glutamate uptake in hippocampus, as well as S100B in cerebrospinal fluid (CSF). Cognition, as assessed by reference and working spatial memory protocols, was also investigated.

Methods: Adult male Wistar rats were submitted to 10 weeks of chronic cerebral hypoperfusion after the 2VO method. The contents (ng/ μ g prot) of GFAP and S100B were measured by ELISA. Astrogliosis was revealed by immunohistochemistry. L-[2,3-³H] glutamate uptake (nmol/mg prot/min) was also analyzed. Cognitive impairment was evaluated by spatial water maze tasks. Statistical analysis: ANOVA and Student's t test.

Results: It was observed in hippocampus, a significant increase in immunocontent of S100B (from 0.44 ± 0.01 to 0.61 ± 0.03 , $P=0.008$) and GFAP (from 5.6 ± 0.4 to 8.1 ± 1.0 , $P=0.028$) and a significant decrease in glutamate uptake (from 0.64 ± 0.07 to 0.40 ± 0.07 , $P=0.03$). Interestingly, we observed a decrease in S100B (from 1.58 ± 0.14 to 0.87 ± 0.22 , $P=0.018$) in CSF. As for the cognitive outcome, there was an impairment of both reference and working spatial memory in the water maze. The latency to find the platform position was shorter in sham, as compared to the hypoperfusion group in the probe trial ($t=-2.011$, $P=0.05$) and 2VO rats spent less time in the target quadrant, as compared to controls ($t=2.291$, $P=0.03$). In the working memory task, from trial 2 on, there was a decline in the mean time to find the platform in the sham rat, this effect did not appear in 2VO rats ($P=0.01$).

Discussion: Our data support the hypothesis that astrocytes play a crucial role in the mechanisms of experimental neurodegeneration and that hippocampal pathology arising after chronic hypoperfusion gives rise to memory deficits.

Palavras-Chave: astrocytes; GFAP; hippocampus; hypoperfusion; S100B; 2-VO method

Apoio Financeiro: CNPq, CAPES, FINEP/Rede IBN 01.06.0842-00, FAPERGS.

Efeito do Exercício Físico sobre a Memória Espacial e Parâmetros Gliais em Ratos Submetidos à Infusão Intracerebroventricular de Estreptozotocina.

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Introdução e Objetivos: A estreptozotocina (STZ) administrada via intracerebroventricular (ICV) causa alterações bioquímicas e morfológicas semelhantes às observadas na demência do tipo Alzheimer. Sabe-se que o exercício físico traz diversos benefícios para o sistema nervoso, incluindo a melhora cognitiva. A proteína S100B, produzida e secretada por astrócitos, tem ação neurotrófica. Entretanto, o aumento crônico do seu conteúdo extracelular tem sido associado a doenças neurodegenerativas e, juntamente à proteína fibrilar glial ácida (GFAP), é um excelente marcador de alteração neuroglial.

Métodos: Ratos Wistar machos adultos foram anestesiados com xilazina/cetamina e infundidos com STZ (3mg/kg) ICV (5 mL/lado) ou veículo. Uma semana após a cirurgia, os ratos foram adaptados e treinados durante cinco semanas em esteira ergométrica. Os animais formaram os grupos: STZ com exercício (STZ-exe; n=6), STZ no sedentário (STZ-sed; n=6), veículo com exercício (SHAM-exe; n=6) e veículo no sedentário (SHAM-sed; n=4). Após o treinamento na esteira, a memória espacial foi avaliada no Labirinto Aquático de Morris (LAM). Ao final, os animais foram anestesiados e deles foi retirada uma fração de líquido e soro para dosagem de S100B (método ELISA) e realizada perfusão intracardíaca com paraformaldeído 4% para imunohistoquímica (GFAP).

Resultados: No quinto dia de treinamento no LAM as latências (s) foram: SHAM-sed 18,00±5,39; SHAM-exe 10,00±1,73; STZ-sed 30,00±8,76; STZ-exe 11,50±2,23. O grupo STZ-sed foi diferente dos demais (P<0,05). No soro, a concentração da proteína não sofreu alteração e no líquido os resultados são (ng/mL): SHAM-sed 12,06±3,1; SHAM-exe 13,12±2,4; STZ-sed 8,19±1,1; STZ-exe 11,96±0,9, com tendência à queda no grupo STZ-sed. Uma análise qualitativa da imunohistoquímica para GFAP mostra uma alteração astrocítica no grupo STZ-sed a qual foi amenizada pelo exercício.

Conclusões: o exercício físico claramente melhorou a performance dos animais no LAM. Isso pode ter ocorrido através da liberação de diversas neurotrofinas, como descrito na literatura, e também pela modulação da atividade astrocítica, como mostrado através do imunoc conteúdo extracelular de S100B e alterações na proteína GFAP.

Palavras-Chave: estreptozotocina, intracerebroventricular, demência, exercício físico, labirinto aquático de Morris, S100B, GFAP.

Apoio Financeiro: CNPq, CAPES, FINEP/ Rede IBN 01.06.0842-00, FAPERGS.

C.01.035

Caracterización de los efectos comportamentales del agonista nicotínico 3-Bromocitisina.

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Por su localización presináptica, los receptores nicotínicos de acetilcolina (nAChR) neuronales modulan la neurotransmisión, que en el caso del sistema dopaminérgico nigroestriatal, ha sido propuesto como un posible mecanismo terapéutico para el tratamiento de la Enfermedad de Parkinson (1).

Recientemente, derivados semisintéticos bromados del agonista nicotínico citisina, como 5 y 3-Bromocitisina (3-Br) han sido evaluados en modelos *in vitro e in vivo*, demostrando una alta eficacia para la liberación de dopamina en el cuerpo estriado (CE) (2). En estudios comportamentales en ratas, hemos observado que la administración aguda y sistémica de 3-Br induce un aumento significativo en la actividad locomotora a diferencia de otros agonistas como nicotina. Además 3-Br induce una concomitante disminución en la conducta de exploración vertical (*rearings*) en un ambiente nuevo.

Para avanzar en la comprensión de los mecanismos involucrados en los efectos conductuales de 3-Br, se bloquearon los nAChR expresados en el CE y el Núcleo Accumbens (Nacc) mediante la administración local del antagonista nicotínico, clorisondamina (Clo). Tres días después se evaluaron los efectos inducidos por 3-Br (0.1mg/Kg) en el paradigma de campo abierto.

La inyección de Clo en el CE y NAcc bloqueó significativamente el aumento de la locomoción (592 ± 305 vs 204 ± 147 ; 416 ± 197 vs 207 ± 81 ; $p < 0.01$) y la disminución de los *rearings* (7 ± 8 vs 26 ± 7 ; 5 ± 5 vs 19 ± 9 ; $p < 0.01$) inducidos por 3-Br. Estos resultados muestran que tanto el CE como el NAcc participan los efectos de 3-Br, sugiriendo una interacción entre el sistema dopaminérgico y nicotínico colinérgico en ambas regiones. A los efectos de comprobar la participación dopaminérgica se administró el antagonista dopaminérgico haloperidol (Hal, 0.025mg/Kg) y se evaluaron los efectos inducidos por 3-Br. Los resultados mostraron que el aumento en la actividad motora fue bloqueado por Hal (575 ± 275 vs 363 ± 141 $p < 0.05$) comprobando una interacción entre el sistema dopaminérgico y colinérgico. Sin embargo Hal no afectó los *rearings* sugiriendo una interacción con otros sistemas.

1. *Biochem. Pharmacol.* 74:24, 2007

2. *Eur. J of Pharmacol.* 536:1, 2006

Palavras-Chave: Actividad motora, agonistas nicotínicos, 3-Bromocitisina, Enfermedad de Parkinson

Apoio Financeiro: PEDECIBA

C.01.036

Infusão intracerebral de Epicatequina em ratos lesionados com 6-hidroxi-dopamina: efeitos comportamentais

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Estudos recentes têm indicado que a epicatequina (EPC) pode produzir efeitos preventivos em patologias neurodegenerativas como na doença de Parkinson. Pesquisas usando modelos *in vitro* de parkinsonismo têm gerado evidências que sugerem a capacidade da EPC em prevenir a morte celular induzida por 6-OHDA. Objetivo: investigar a ação da EPC sobre o comportamento de ratos em um modelo de parkinsonismo. Métodos: trinta ratos Wistar machos receberam infusões de 6-OHDA em duas porções da via nigroestriatal do hemisfério direito através de cirurgia estereotáxica. Vinte e quatro horas após a lesão, os animais foram alojados em três grupos e infundidos com dose única de 1,5µl de EPC em duas concentrações: 1mg/10ml (EPC1) e 1mg/ml (EPC2) ou solução veículo através de cânula posicionada sobre o núcleo estriado. Após 15 dias, os animais foram submetidos ao teste de rotação induzida por metilfenidato (40mg/kg) e tiveram os giros sobre o próprio eixo contados durante 30 minutos. Também foi realizado o teste da fita adesiva para avaliar a capacidade sensório-motora das patas dianteiras durante 2 minutos. Resultados: no teste das rotações induzidas por metilfenidato, os resultados indicaram diferenças entre os grupos apontando para uma tendência do grupo EPC1 a produzir menos rotações (ANOVA, F(2,66), p= 0,09). Já no teste da fita adesiva, os resultados não indicaram diferenças significativas na sensibilidade das patas do grupo que recebeu a dose menos concentrada (EPC1, F(1,118), p>0,05) em relação ao grupo controle. Testes t com dados log-transformados indicam diferença significativa (p= 0,01) para a preferência pela pata ipsilateral à lesão nos animais controle e igual preferência no grupo EPC2 (p=0,002). Conclusões : o número reduzido de rotações realizadas pelos animais do grupo EPC1, sugere tendência para melhora da lesão provocada pela 6-OHDA. Os resultados do teste da fita adesiva reforçam a idéia tendência, ao apontar a não preferência para pata direita nesse mesmo grupo, denotando a preservação da sensibilidade do animal em ambas as patas. Os dados sugerem que em doses menos concentradas a EPC pode estar atuando no atraso ou na recuperação da lesão nigroestriatal.

Palavras-Chave: 6-OHDA, Epicatequina, Parkinsonismo

Apoio Financeiro: ProDoc/CAPES

Alterações Gliais em Modelo Animal de Demência Induzido pela Infusão de Ibotenato no Núcleo Basal Magnocelular.

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Introdução e Objetivos: Caracteriza-se como demência, a disfunção cortical e subcortical crônica e progressiva que resulta em declínio cognitivo. O ibotenato, toxina do fungo *Amanita muscaria*, tem sido utilizado para induzir a demência através da morte de neurônios colinérgicos do núcleo basal magnocelular (NBM) de roedores. A S100B, uma proteína sintetizada e secretada por astrócitos, tem importante papel na sobrevivência neuronal por estimular o crescimento de neuritos. A GFAP (proteína glial fibrilar ácida) é usada como marcador para diversas lesões encefálicas e tem como função dar suporte à célula por ser parte do citoesqueleto. Com este trabalho, avaliamos o desempenho cognitivo na tarefa de esquiwa inibitória (EI) e as possíveis consequências sobre os parâmetros gliais (S100B e GFAP) em um modelo de demência pela infusão de ibotenato no NBM.

Métodos: Ratos Wistar machos adultos foram anestesiados com xilazina/cetamina e através de coordenadas estereotáxicas para atingir o NBM foram realizadas infusões bilaterais de ibotenato (8µg/ul) e/ou veículo (NaCl 0,9%) 0,5ul/lado. Após 28 dias, os ratos foram treinados em EI e testados 24 horas depois. Após o teste, os animais foram mortos por decapitação, separados e homogeneizados os hipocampus para medir o imunoconteúdo de GFAP e S100B pelo método Elisa ou perfundidos com paraformaldeído 4% para realização de imunohistoquímica para GFAP.

Resultados: No teste da EI, houve diferença significativa entre os grupos ibotênico e salina ($p < 0,05$), comprovando o declínio cognitivo e a validade do modelo de demência. Em relação aos parâmetros gliais, houve diferenças significativas tanto no imunoconteúdo de GFAP (IBO: $0,93 \pm 0,17$; SAL: $5,29 \pm 0,19$; $P < 0,05$) quanto no de S100B (IBO: $1,15 \pm 0,07$; SAL: $1,42 \pm 0,09$; $P < 0,05$) entre os grupos.

Conclusões: O conteúdo de GFAP e S100B diminuiu no grupo ibotênico em relação ao grupo controle, sugerindo que ocorrem modificações astrocíticas em resposta ao dano gerado pelo modelo, o que contribuiu para o déficit cognitivo observado nestes animais.

Palavras-Chave: ibotenato, núcleo basal magnocelular, demência, esquiwa inibitória, S100B, GFAP.

Apoio Financeiro: CNPq, CAPES, FINEP/ Rede IBN 01.06.0842-00, FAPERGS.

C.01.038

Early neuronal alterations in an experimental model of sleep apnea: The S100B-RAGE link.

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Obstructive sleep apnea (OSA) is a very common silent chronic pathology characterized by intermittent hypoxia (IH). Patients usually reach health care with large complications and cognitive impairment. Receptor for Advanced Glycated End Products (RAGE) is expressed by neurons in hypoxic stress and binds glial factor S100B, increased by IH, to induce apoptosis or survival depending on the concentration.

Objectives: Study early neuronal alterations induced by IH and expression of RAGE and S100B.

Methods: Adult male Wistar rats were exposed during the sleep phase (8hs/day) to cycles of 6 min of hypoxia (10% O₂) and 6 min of normoxia for 40 (1 day, IH40) or 110 cycles (3 days, IH110). Brain sections were processed for immunohistochemistry with antibodies anti microtubule associated protein 2 (MAP2), Neurofilament-200KDa (Nf-200), neuronal nuclear protein NeuN, RAGE, S100B. Sections were also subjected to Toluidine blue (TB), Hoechst staining (HS) and a commercial kit to recognize Active Caspases (Caspitag). **Results:** IH reduced number of normal NeuN staining in cortex (Cx) layers IV-V from 93.6±5.6% to 87±18.1% (IH40) and 51.4±22.5% (IH110). CA-1 reduction was from 94.7±5.3% to 56.7±27.3% (IH40) and 38±14.8% (IH110). Neurons with normal morphology by TB were also reduced in CA-1 from 94.9±4.1% to 77.9±8.5% (IH110) and CA2/3 from 96.7±2.3% to 75.1±6.1% (IH110). Neuronal death was confirmed by Caspatag and HS that showed apoptotic characteristics in CA-1 and Cx. MAP-2 staining showed CA-1 neurons 33.3±17.5% shorter in IH110. Nf-200 staining showed shorter filaments without statistical significance. RAGE expression was induced by IH in isolated Hippocampus (Hip) and Cx neurons surrounded by astrocytes with increased S100B expression. These RAGE+ neurons did not show degeneration signs. **Conclusions:** IH induces early severe alterations in Hip and brain Cx neurons going from neurite length alterations to apoptosis. Since RAGE is expressed in neurons from these areas and S100B is available, they might be involved in the determination of neuronal death or survival after IH.

Palavras-Chave: hypoxia, neuronal degeneration, glia, astrocytes

Apoio Financeiro: ANPCYT PICT 33735, CONICET PIP 6063, IBRO RHF

C.01.039

Function and expression of ryanodine receptors in hippocampal neurons exposed to beta-amyloid peptide aggregates

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Brain accumulation and aggregation of the beta amyloid peptide (A β) play a central role in Alzheimer's disease, which is characterized by progressive memory loss. Soluble A β oligomers (also known as ADDLs) act on hippocampal postsynaptic spines and block long-term potentiation (LTP). Some types of LTP are known to depend on Ca²⁺-induced Ca²⁺ release (CICR) mediated by ryanodine receptors (RyR); yet little is known about RyR function on neuronal Ca²⁺ signal generation and related dysfunctions elicited by ADDLs. **Objectives:** Here, we investigated the contribution of RyR to Ca²⁺ signals and neurotoxicity induced by ADDLs, as well as, the effects of ADDLs on RyR expression in neuronal cells. **Methods:** Primary rat hippocampal neurons were incubated with ADDLs [500 nM-1 μ M] or A β fibrils [1 μ M] and viability was evaluated by MTT 24 h later. Ca²⁺ signals were analyzed by time series confocal microscopy in neurons preloaded with Fluo-4 AM and exposed to ADDLs [0.5-1 μ M] or to A β fibrils [1 μ M]. Levels of expression of RyR2 and RyR3 were determined by RT-PCR, and neuronal RyR distribution was studied by immunocytochemistry after exposing neurons to ADDLs (0.5 μ M) for 3 min to 24 h. All data are expressed as Mean $\pm$ SE. **Results:** In mature hippocampal cultures, RyR2 and RyR3 were present in neurons and glial cells, and were clustered in small aggregates in the soma and dendrites of pyramidal neurons. Incubation with ADDLs [0.5 μ M] reduced mRNA expression of RyR2 to 0.61 ± 0.25 of control, and of RyR3 to 0.74 ± 0.41 of control. Both ADDLs and A β fibrils induced long lasting Ca²⁺ signals, with maximum values of F/Fo = 1.20 ± 0.08 and F/Fo = 1.40 ± 0.01 , respectively. Pre-incubation with ryanodine [50 μ M] for at least 1 h to inhibit RyR activity, prevented ADDLs-induced intraneuronal Ca²⁺ rises. Yet, RyR inhibition did not prevent ADDLs or fibril-induced inhibition of MTT reduction, at least with the protocols used here. **Conclusions:** These results indicate that RyR, presumably via CICR, contribute to ADDLs-induced Ca²⁺ signals and that ADDLs downregulate neuronal RyR expression, suggesting that RyR expression and function may be altered in Alzheimer's disease. **Palavras-Chave:** Alzheimer's disease; ryanodine receptors; calcium

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C.01.040

Homocysteine alters phosphorylation of intermediate filament proteins from C6-glioma-cells

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Objectives: Homocysteine (Hcy), a thiol-containing amino acid derived from the metabolism of methionine, markedly enhances the vulnerability of neuronal cells to excitotoxic and oxidative insults. Evidences show that high Hcy levels contribute to neuronal cell injury in various pathological conditions, however the cellular and molecular mechanisms by which hyperhomocysteinemia contributes to neurodegeneration are poorly understood. In the present study we investigated the effects of Hcy on the *in vitro* phosphorylation of glial fibrillary acidic protein (GFAP) and vimentin (Vim) in C6-glioma cells, since these cells have a greater rate of oxidative metabolism than astrocytes and have been considered a model to study the *in vitro* effects of neurotoxicants.

Methods: Cells were incubated with ³²P orthophosphate in the presence or absence of 100 and 500 mM Hcy. The intermediate filament enriched cytoskeletal fraction was isolated and the radioactivity incorporated into the GFAP and Vim was measured. We also investigated whether the effect of Hcy could also be due to alterations in the oxidative stress, energy homeostasis and glutamate system. Therefore, cells were supplemented with folic acid, trolox, ascorbic acid, creatine, ionotropic and metabotropic glutamate antagonists (DL-AP5, CNQX and MCPG) in the presence or absence of Hcy.

Results: We verified that exposure of C6 cells to 100 and 500 mM Hcy decreased the incorporation of ³²P into GFAP and Vim. We observed that folic acid, trolox, ascorbic acid and creatine did not prevent the effect of Hcy on *in vitro* phosphorylation of these proteins. However, when cells were exposed to Hcy in the presence of ionotropic or metabotropic glutamate antagonists, we observed the prevention of the effect of Hcy on the incorporation of ³²P into GFAP and Vim.

Conclusions: Considering that abnormal phosphorylation levels of cytoskeletal proteins are associated with neurodegeneration, our results support the involvement of the cytoskeleton in the neural damage induced by Hcy.

Palavras-Chave: homocysteine, cytoskeleton, phosphorylation, intermediate filaments, C6-glioma cells.

Apoio Financeiro: CAPES, CNPQ, PRONEX, PROPESQ-UFRGS, FAPERGS

Lead exposure stimulates vascular endothelial growth factor expression, attenuates astrogliosis and extends survival of mice expressing Amyotrophic Lateral Sclerosis-linked mutated superoxide dismutase-1

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by motor neuron loss and astrogliosis. Exposure to lead (Pb) was proposed as risk factor for ALS; however, it was also recently associated to increased survival¹. Pb can elicit inflammatory and trophic responses in astrocytes, including induction of vascular endothelial growth factor (VEGF)², which is trophic for motor neurons.

OBJECTIVES: To investigate the effects of Pb exposure on disease course and on astrocyte-motor neuron trophic interactions in ALS models.

METHODS: Disease course was analyzed in transgenic mice expressing the ALS-linked G93A superoxide dismutase-1 (SOD1^{G93A}) and non-transgenic (nonTg) littermates exposed to Pb acetate (0.2 g/l) or vehicle in drinking water. Motor neuron survival was assessed in co-cultures of nonTg or SOD1^{G93A} bearing astrocytes previously exposed to Pb or vehicle. Glial fibrillary acidic protein (GFAP) and VEGF immunoreactivity were determined in spinal cord tissues and astrocyte cultures.

RESULTS: Pb exposure prolonged SOD1^{G93A} mice survival compared to vehicle treatment (136 ± 5.6 vs 126 ± 3.2 days), and induced a striking astrogliosis reduction in the spinal cord. Moreover, Pb augmented VEGF expression in SOD1^{G93A} mice spinal cord and the percentage of VEGF immunoreactive glial-like cells ($77.1 \pm 3.5\%$ vs $51.7 \pm 3.2\%$). Accordingly, VEGF expression was also increased in cultured SOD1^{G93A} astrocytes by Pb, and the treatment diminished their previously shown³ toxicity for motor neurons ($84.7 \pm 12.5\%$ vs $56.4 \pm 7.2\%$ of motor neuron survival), an effect that was reverted by VEGF blocking antibodies.

CONCLUSIONS: Our data show a protective role of Pb in the ALS mouse disease course. The differential trophic response of SOD1^{G93A} astrocytes to Pb, evidenced by decreased astrogliosis and VEGF-increased expression may underlie this observation. Analysis of the mechanisms regulating Pb-induced VEGF expression in astrocytes may lead to new approaches to delay ALS progression.

REFERENCES: ¹Environ Health Perspect. in press; ²J Biol Chem. 275:27874, 2000; ³J Neurochem. 97:687, 2006. **Palavras-Chave:** astrocytes, lead, VEGF, Amyotrophic Lateral Sclerosis

Apoio Financeiro: Comisión Sectorial de Investigación Científica, Universidad de la República, Uruguay

C.01.042

Cytisinoids and Parkinson's disease

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The motor symptoms that are at the core of Parkinson's disease (PD) are linked to the loss of nigrostriatal dopaminergic neurons. L-DOPA or dopamine receptor agonists, the key therapeutic strategies in use today, are unable to stop the neurodegenerative process and are not devoid of secondary effects. Acetylcholine modulates nigrostriatal dopamine system function through nicotinic acetylcholine receptors (nAChR) and nAChR agonists have long been considered as potential therapeutic agents (1). In addition, epidemiological studies have shown that smokers have a lower incidence of PD, and nicotine, has been postulated to be responsible for this neuroprotective effect.

We found that cytisine a nAChR agonist and its brominated derivatives can elicit striatal dopamine release *in vitro* (2) and *in vivo*.

The aim of the present study was explore cytisine and two brominated derivatives as potential pharmacological tools for PD treatment.

We used a model of experimental PD, consisting in an unilateral 6-hydroxydopamine (6-HODA) lesion of the Substantia Nigra (SN), and the following evaluation of the dopamine striatal levels, as a measure of remaining dopaminergic terminals (3). nAChR agonist: cytisine, 5-Bromocytisine (5-Br) and 3-Bromocytisine (3-Br), as nicotine, were administered 4h before 6ug 6-OHDA injection and 20, 44 and 68 after. Furthermore we evaluate agonists capacity to modify horizontal locomotor activity in an Open Field paradigm as the number of intersections of the infrared beams. For locomotor activity we used control and bilateral 6-OHDA SN lesioned animals.

The results show that cytisine and 5-Br effectively prevent the striatal decrease of dopamine elicited 6-OHDA, while 3-Br was unable. However, 3-Br was the only one able to increase locomotor activity at the open field after a single dose. Even more, this effect was preserved in bilateral 6-OHDA nigrostriatal lesioned animals.

Others nAChRs agonist different from nicotine could prevent striatal dopamine loss after 6-OHDA lesions. 3-Br increases horizontal locomotor activity, even in 6-OHDA bilateral lesioned animals.

1. *Biochem. Pharmacol.* 74:24, 2007

2. *Eur. J of Pharmacol.* 536:1, 2006

3. *Brain Res.* 888:336, 2001

Palavras-Chave: Parkinson, cytisinoids, 6-hydroxydopamine, Corpus Striatum

Apoio Financeiro: Wellcome Trust Collaborative Research Initiative Grant 073295Z03Z

Aging and a model of Alzheimer's disease cause an alteration in the transport of insulin growth factor I (IGF1) in blood brain barrier and in the hippocampus in mice: a possible effect to neurodegenerative disease.

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Objective: The neurodegenerative diseases are a major concern in our society due to the alteration in life style and longevity. These factors alter the production and action of many hormones. Serum and brain IGF-I levels change in several neurodegenerative conditions both in humans and in animal models. Defects in IGF-1 receptor (IGF1r) and signaling are associated with an increase in amyloid plaque and pathology of Alzheimer's disease (AD). **Materials and methods:** In this study we evaluated cognition (recognition task), the transport of IGF1 through the blood brain barrier (BBB) and the immunocontent of IGF1r and Vglut1 in hippocampus of aged mice (CF1, 24 months). Moreover, cognition and the transport of IGF1 through the blood brain barrier were also, evaluated in genetically modified mice (APP/PS1, 8 months) which is a well characterized model of Alzheimer's disease. **Results:** Old mice and APP/PS1 showed an increased of IGF1 transport through blood brain barrier compared to its control groups ($413 \pm 96 > 379 \pm 33$ ng/ml; $461 \pm 115 > 161 \pm 29$ ng/ml respectively; $p < 0.05$). Also aging and APP/PS1 mice showed a trend to impairment in the performance of recognition memory task. Western analysis detected increased IGF-1r (50%; $p < 0.05$) levels and decreased levels of VGlut1 (25% $p < 0.05$) in hippocampus.

Conclusion: IGF1 is a hormone proposed to be neuroprotective and a putative treatment to AD. Our results showed that transport of IGF1 in BBB and its receptor in hippocampus were increased with aging and in a model of AD mice; this could be a tentative to compensate an altered signaling and/or function of the IGF1 in brain. Decreased of levels of VGlut1 could be associated with decreased IGF1 signaling in aging brain. Additional investigations could help to better characterize the relationship between aging, IGF1 signaling and glutamate system and possible role in neurodegenerative disease.

Palavras-Chave: Aging, Alzheimer Disease, IGF1, glutamatergic system

Apoio Financeiro: CNPq- CAPES

Astroцитos corticales de la enfermedad Niemann-Pick tipo C manifiestan cambios en sus canales formados por conexina43 similares a los presentes en respuestas neuroinflamatorias

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Objetivos: La enfermedad Niemann-Pick tipo C (NPC) es ocasionada en un 95% de los casos por mutaciones en la proteína transportadora de colesterol NPC1 y se caracteriza por desórdenes en la reserva intracelular de lípidos. El sistema nervioso central (SNC) es afectado y manifiesta neurodegeneración asociada a una respuesta inflamatoria. Se sabe que agentes proinflamatorios aumentan la actividad de los hemicanales (HCs) y disminuyen la comunicación intercelular mediada por canales de uniones en hendidura (CUH). En este estudio evaluamos si los HCs y CUH formados por conexina43 (Cx43) de astroцитos corticales de ratones deficientes de NPC1 se encuentran alterados.

Metodología: Se usaron astroцитos corticales de ratones neonatos silvestres (AC-NPC1^{+/+}), heterocigotos (AC-NPC1^{+/-}) y “knock out” (AC-NPC1^{-/-}). El estado funcional de los HCs y de los CUH se evaluó utilizando las técnicas de captación de colorante desde el medio extracelular y transferencia de colorante entre células vecinas, respectivamente. Los niveles relativos de la Cx43 en la membrana plasmática se midieron por biotilación de proteínas de superficie celular y los niveles totales de Cx43 por análisis de Western blot.

Resultados: Los AC-NPC1^{-/-} presentaron menor actividad de CUH (43,7±7,9%; media ± EE) que los AC-NPC1^{+/+} (53,8±3,6%) y que los AC-NPC1^{+/-} (80,5±5,3%). En AC-NPC1^{-/-} la permeabilidad celular de la membrana sensible a La³⁺, bloqueador de HCs, fue mayor (1,1±0,17 U.A.) que en AC-NPC1^{+/+} (0,56±0,06) y que en AC-NPC1^{+/-} (0,15±0,03 U.A.). Los niveles totales de Cx43 fueron similares en los tres grupos, mientras que los niveles de Cx43 de superficie fueron más bajos en el grupo AC-NPC1^{-/-} e intermedios en el grupo AC-NPC1^{+/-}.

Conclusiones: Los AC-NPC1^{-/-} presentan cambios en la actividad de los HCs y CUH similares a los cambios inducidos por agentes proinflamatorios. Esto sugiere que diversas funciones astrogiales que favorecen el normal funcionamiento neuronal se encuentran impedidas en NPC, lo que posiblemente aumenta la susceptibilidad neuronal a agentes estresantes presentes en la enfermedad como el péptido β-amiloide, contribuyendo a la neurodegeneración. **Palabras-Chave:** Neuroinflamación, conexina43, hemicanales.

Apoio Financeiro: FONDECYT grant 1070591

INTRACEREBROVENTRICULAR INJECTION OF STREPTOZOTOCIN MAY PRODUCE A RAT MODEL OF ALZHEIMER'S DISEASE

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Objectives: Alzheimer's disease (AD) is the most common cause of dementia in aged humans; it is clinically characterized by a progressive loss of memory and deterioration of higher cognitive functions. More recent data have suggested that the onset of AD may be related to an association between insulin deficiency and an insulin-resistant state. Consistent with this notion, there are reports showing that the AD brain exhibits alterations of insulin and insulin receptors (IRs) in several regions and that the glucose metabolism is also abnormal. In this context, intracerebroventricular (icv) injection of streptozotocin (STZ) into the rat brain has been proposed as an animal model of AD because it induces a dysfunction of the insulin system.

Methods: Four month-old adult male *Wistar* rats subjected to bilateral stereotaxic icv injections of either STZ (3 mg/Kg of body weight) or vehicle were tested in both reference and working memory versions of the Morris' water maze task, 7 days after surgery during 35 days. After the 35 days the animals had been sacrificed and specific brain regions submitted to the immunoblotting method to evaluate the expression of the choline acetyltransferase (ChAT) and phosphorylated microtubule associated protein tau.

Results: Rats injected with STZ, relative to the controls, exhibited a marked disruption of performance in the reference and the working memory versions of the water maze. On the other hand, it can also be said that the animals are capable to acquire relevant information about the task, particularly in the reference memory version. In addition, rats injected with STZ showed a reduction in the ChAT content in the basal ganglia, amigdala, and cortex, although there was a significant enhance of ChAT levels in cerebellum. Analysis of tau indicates an increase of the phosphorylated protein levels in the basal ganglia, amigdala and cortex, and a reduction in the cerebellum. **Palavras-Chave:** Alzheimer, streptozotocin, memory, ChAT and tau

Apoio Financeiro: FAPESP

Minocycline Administration Reversed the Hypofunction observed in the Open Field Test in MPTP-lesioned rats.

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OBJECTIVE: this schedule evaluated the effect of the administration of minocycline in the model of Parkinsonism induced by MPTP in rats evaluated in the Open Field test. **METHODS:** male Wistar rats were divided in the groups (n=8/group): Sham+saline, Sham+minocycline, MPTP+saline and MPTP+minocycline. The MPTP groups were arranged in a stereotaxic equipment (David Kopf, 957L model) and were infused bilaterally with 1µmol of MPTP dissolved into 2.1µmol of saline (0.35µL/min) within the substantia nigra pars compacta. Minocycline (50mg/kg) or saline (1ml/kg) was administered 1 h before the surgery. Sham groups were also operated; however, these animals did not receive MPTP infusion. Twenty-four hours after the surgery, rats were evaluated in the Open Field test during 5 minutes; parameters observed were locomotion and rearing frequencies, the latency and immobility times. Data were analyzed by ANOVA followed by Tukey test; values were expressed as mean±S.E.M. **RESULTS:** 24 h after the surgery, MPTP+saline rats reduced the locomotion (22.38±1.19) and rearing (7.5±0.89) frequencies in comparison to Sham+saline (locomotion 98.63±1.74; rearing 25.38±1.15) group. Besides, MPTP+saline rats increased the latency (24.00±1.31) and the immobility (145.88±3.51) times when compared to Sham+saline (latency 2.25±0.45; immobility 3.00±2.10) animals. In contrast, the MPTP+minocycline rats increased the locomotion (98.88±1.34) and rearing (26.75±1.16) frequencies, and decreased the latency (2.5±0.60) and immobility (7.12±2.76) times in comparison to MPTP+saline animals. There was no difference between MPTP+minocycline rats and sham+minocycline groups. **CONCLUSIONS:** the MPTP-lesioned rats reduced motor function 24 h after the surgery in comparison to sham animals when evaluated in the open field test; this result was reversed by previous administration of minocycline (50mg/kg). Thus, our data demonstrated that minocycline, a tetracycline derivate, was able to prevent the substantia nigra pars compacta neurotoxicity induced by MPTP intranigral infusion. **Palavras-Chave:** Parkinson disease, MPTP, minocycline, open field test

Apoio Financeiro: CAPES, CNPq

c-Abl kinase and Fe65 adaptor protein modulate AICD cellular activity

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c-Abl kinase and Fe65 adaptor protein modulate AICD cellular activity

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After APP proteolytic processing by alpha- beta- and gamma-secretases, several fragments are released, including APP intracellular domain (AICD), which has been proposed as a transcriptional inductor. AICD, moves to the nucleus with the adaptor protein Fe65 and regulates transcription. It has also been described that APP as well as Fe65 can be phosphorylated by c-Abl kinase. **HYPOTHESIS:** Taking into account all of the above we have proposed that c-Abl kinase activity could modulate AICD transcriptional induction, in the presence of Fe65. **RESULTS:** In Neuro2A clones for human APP Swedish (APPsw) variant we observed higher levels of AICD and increased expression of Neprilysin, a proposed target gene for AICD. The Neprilysin expression was decreased by treatment with imatinib mesylate (STI-571 or Glivec), a c-Abl inhibitor, suggesting a modulation of AICD dependent transcription by c-Abl kinase. We observed the interaction between c-Abl kinase, AICD fragment and Fe65 adaptor protein. AICD expression has also been linked with apoptosis induction. APPSw cells show diminished viability and higher levels of apoptosis than control cells. Imatinib decreased the apoptosis in APPsw and the apoptotic response induced by Fe65 over-expression was inhibited by cotransfection with a kinase death c-Abl plasmid and potentiate by wt c-Abl plasmid. However in the APPsw cells, the ability of kinase death c-Abl to protect against Fe65 was reduced. Finally, we detected by luciferase reporter assay higher transactivation of the pro-apoptotic p73 isoform, TAp73 promotor, and an increase in the p73 levels in APPSw cells. **CONCLUSIONS:** Our results show that c-Abl modulates AICD dependent cellular responses, transcriptional induction as well as apoptotic response, which could participate in generation and progression of the neurodegenerative pathology, observed in Alzheimer's Disease.

Palavras-Chave: c-Abl, APP, AICD, Alzheimer

Apoio Financeiro: FONDECYT N° 1080221, FONDAP N° 13980001

C.01.048

Potential of adult neural stem cells for the treatment of Parkinson's disease

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Parkinson's disease is a neurodegenerative disorder characterized by a progressive loss of dopaminergic neurons of the substantia nigra pars compacta. In the search of new sources of cells for regenerative therapies, the adult neural stem cells (aNSC) are an alternative because these cells are able to differentiate into neurons, astrocytes and oligodendrocytes in vitro and in vivo. Our group observed that the transforming growth factor beta (TGF-beta) is pro-neurogenic on NSC in vivo and in vitro.

The objective of our work is to study the use in regenerative and protective therapies of aNSC obtained from one of main areas in the brain where neurons are generated during adulthood, the subventricular layer in the lateral ventricles (SVZ).

We performed primary cultures dissecting the SVZ of 8 to 10 month old Wistar rats. These cultures were characterized by Nestin, astrocyte marker (GFAP), microglia (GSA) and beta III tubulin (Tuj) immunocytochemistry. Besides, the effect of TGF-beta on aNSC was analyzed by adding the cytokine in the culture medium. Survival and inflammatory response after striatum transplantation of undifferentiated aNSC were determined one, three and six weeks after the grafting. At that time points animals were perfused intracardially, brains were dissected out and Nissl staining and GFAP or GSA immunohistochemistry were performed to analyze inflammatory response to the transplant and transplanted cells survival.

Preliminary results showed that in primary cultures 7.4% of the cells were Tuj, 1.1% GFAP and 2.5% GSA-positive, respectively. TGF-beta improved the percentage of Tuj positive cells by one fold. On the other hand, transplanted aNSC were identifiable 1, 3 and 6 weeks after transplantation. Although a large portion of grafted cells underwent cell death 14.5% were identified after 6 weeks and the inflammation was only evident in the graft area.

These data show that aNSC could become an important source for cell based therapies for neurodegenerative disease and TGF-beta might be a promising tool for aNSC neuronal differentiation.

Palavras-Chave: stem cells - Parkinson - transforming growth factor beta

Apoio Financeiro: ANPCYT - CONICET

High glucose enhances the effects of oxygen-glucose deprivation on connexin based channels and mortality of cortical astrocytes

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Introduction: Human studies indicate that hyperglycemia during brain ischemia increases the extent of injury and causes extensive cell death. In *in vitro* ischemia models, activity of astroglial hemichannels (HCs) and gap junction channels (GJCs) are enhanced and depressed, respectively. However, the effects of high glucose concentrations during hypoxia on astrocytic HCs and GJCs remain unknown.

Objectives: To evaluate if high glucose levels during hypoxia increase HC opening and decrease GJC mediated coupling during reoxygenation. We also studied whether hypoxia in high glucose promotes connexin channel-mediated astroglial death.

Methods: In cultured rat cortical astrocytes, dye uptake and dye coupling were used to evaluate HC and GJC activity, respectively, right after reoxygenation following 3 or 6h of hypoxia with different glucose concentrations. Astrocytes from Cx43^{-/-} and wild type mice were used. At similar reoxygenation periods astroglial death was evaluated with rhodamine-dextran (10kD) incorporation.

Results: After 3h of hypoxia in high glucose (12-37mM) the rate of dye uptake increased and dye coupling decreased transiently in a glucose concentration-dependent manner reaching a maximal value with 27mM glucose. Six hours of hypoxia in 27mM glucose caused a higher rate of dye uptake (to 400% $\pm$ 23 of control at ~1h reoxygenation) and a greater reduction in dye coupling (to 10% $\pm$ 2 of control at ~1h reoxygenation) that remained constant over 3.5h of reoxygenation. These changes were absent in Cx43^{-/-} astrocytes. Moreover, hypoxia in 27mM glucose induced 43% $\pm$ 4 astroglial death after 6h of reoxygenation in wild type (but not Cx43^{-/-}) mice that was prevented with a mimetic peptide of the second extracellular loop of Cx43 that blocked HCs and did not affect GJCs.

Conclusions: High glucose levels enhance the increase in HC opening and reduction in cell-cell communication via GJC induced by hypoxia-reoxygenation. The increase in HC opening enhances astroglial susceptibility to hypoxia-reoxygenation. Thus, treatment with HC blockers may prove to be therapeutic during reperfusion after stroke.

Palavras-Chave: connexin43, stroke, astrocytes, diabetes

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AVALIAÇÃO COMPORTAMENTAL DO MODELO ANIMAL DE PARKINSONISMO INDUZIDO POR INFUSÃO BILATERAL DE ROTENONA NA SUBSTÂNCIA NEGRA DE RATOS

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Objetivos: Avaliar parâmetros comportamentais no modelo animal da rotenona 12µg/µl (PEG) quando infundida bilateralmente na substância negra (SN) de ratos Wistar machos.

Métodos e resultados: Os experimentos foram divididos em duas etapas após a cirurgia estereotáxica: campo aberto e esquiwa ativa de duas vias. Resultados analisados por ANOVA de duas vias seguido do teste de Newman-Keuls; $P < 0,05$ foi considerado significativo; dados expressos pela média $\pm$ EPM. Na primeira etapa, 35 animais foram divididos em quatro grupos: Controle (N=10), Sham (N=10), rotenona 12µg/µl (ROT12) (N=8) e rotenona 24µg/µl (ROT24) (N= 7). Os quatro grupos passaram pelo teste do campo aberto e quatro parâmetros foram analisados (frequência de locomoção-LOC e de levantar-LEV; tempo de latência-LAT e imobilidade-IMOB), em quatro tempos diferentes (24hs, dia 10, 20 e 30 após a cirurgia estereotáxica). Os resultados mostraram que houve uma diminuição da LOC do grupo ROT12 nas 24hs ($59,9 \pm 3,8$) comparado aos grupos controle, Sham e ROT24; ROT12 no dia 10 ($81,7 \pm 4,5$) comparado ao controle e ROT24 nas 24hs e dia 20 ($86,7 \pm 4,9$ e $98,9 \pm 3,1$) quando comparado ao controle. No LEV, houve diminuição para o grupo ROT12 24hs ($11,4 \pm 1,9$) comparado ao controle e Sham; ROT12 dia 20 ($30,0 \pm 3,2$) comparado ao controle e ROT24 24hs e dia 20 ($18,3 \pm 2,7$ e $27,3 \pm 2,6$) comparado ao controle. No IMOB, houve um aumento apenas para ROT12 24hs ($2,9 \pm 1,5$) comparada aos outros grupos. E em LAT, ROT12 apresentou aumento apenas em 24hs ($6,0 \pm 1,9$) comparado aos grupos controle e Sham. Na segunda etapa, outros animais foram avaliados no teste de esquiwa ativa de duas vias nos dias 7 (treino), 8, 15 e 30 após a cirurgia estereotáxica. Os animais foram separados nos grupos controle (N=10), veículo (N=10) e ROT12 (N=9). O grupo ROT12 no dia 30 apresentou diminuição do número de esquivas ($8,1 \pm 2,8$) quando comparado aos grupos controle e veículo.

Conclusão: Nossos dados sugerem que o modelo animal da rotenona foi capaz de mimetizar alguns sinais da doença de Parkinson após avaliações motora e de aprendizado/memória.

Palavras-Chave: Doença de parkinson; Rotenona; aprendizado e memória; comportamento motor

Apoio Financeiro: CNPq

UTILIZAÇÃO DA SENSIBILIDADE AO CONTRASTE PARA AVALIAR A RESPOSTA DAS VIAS PARVO E MAGNOCELULAR EM PORTADORES DE ESQUIZOFRENIA

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Objetivos: O objetivo do presente estudo foi utilizar a Função de Sensibilidade ao Contraste (FSC) para avaliar o desempenho das vias magnocelulares e parvocelulares em portadores de esquizofrenia.

Métodos: Participaram dos experimentos cinco voluntários, sendo todos portadores de esquizofrenia, com idade entre 20-47 anos ($M = 33$ anos e $DP = 9,87$) e com a acuidade visual normal ou corrigida. Utilizou-se um delineamento experimental com medidas repetidas para medir 20 curvas de sensibilidade, sendo 10 em condições de luminância fotópica e 10 em escotópica. A FSC foi determinada pelo método psicofísico da escolha forçada. Foram utilizados estímulos de grade senoidal com frequências de 0,25, 2,0, 4,0, 8,0 cpg (grau de ângulo visual). Todas as medidas foram realizadas a distância de 150 cm do monitor, binocularmente, em um monitor LG de 19 polegadas. Durante cada sessão experimental foi apresentado, sucessiva e aleatoriamente, pares de estímulos contendo uma frequência senoidal e um círculo cinza homogêneo de luminância média ($42,6 \text{ cd/m}^2$ ou $0,04 \text{ cd/m}^2$). A tarefa do voluntário era escolher sempre o estímulo que continha a frequência senoidal.

Resultados: A análise dos resultados preliminares (ANOVA para medidas repetidas) demonstrou diferença significativa na percepção visual dos portadores de esquizofrenia nas condições de luminância fotópica e escotópica ($F_{1, 198} = 33, 2617$; $p < 0,05$). A análise com teste post-hoc Tukey HSD mostrou que houve diferença significativa nas frequências de 2,0 cpg ($p < 0,001$) e 4,0 cpg ($p < 0,001$), sendo que na condição fotópica os portadores de esquizofrenia foram da ordem de 3,95 e 3,38 vezes mais sensível do que na luminância escotópica para as frequências de 4,0 e 8,0 cpg, respectivamente.

Conclusão: Os dados preliminares indicam que o sistema visual dos portadores de esquizofrenia apresenta sensibilidade ao contraste diferente para grade senoidal em condições escotópica e fotópica, isto é, a via magno é mais sensível do que a via parvocelular, conforme o esperado.

Palavras-Chave: Sensibilidade ao contraste; Esquizofrenia; Sistema visual; Parvocelular; Magnocelular

Apoio Financeiro: CNPq

AVALIAÇÃO DA SENSIBILIDADE AO CONTRASTE EM PORTADORES DE ESQUIZOFRENIA PARA GRADES SENOIDAIS EM CONDIÇÕES ESCOTÓPICAS

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Objetivos: A esquizofrenia é um transtorno neuropsiquiátrico caracterizado por disfunções cognitivas e perceptuais. Neste sentido, o presente trabalho teve como objetivo utilizar a função de sensibilidade ao contraste (FSC) para caracterizar a percepção visual de contraste em portadores de esquizofrenia.

Métodos: Participaram dos experimentos nove voluntários adultos, com idade entre 20-48 anos, sendo cinco portadores de esquizofrenia (Grupo Experimental, GE com M = 33 anos, DP = 9,87) e quatro isento de patologia neuropsiquiátrica (Grupo Controle, GC com M = 23 anos, DP = 3,46), todos apresentavam acuidade visual normal ou corrigida. Foi realizado um delineamento experimental com medidas repetidas, para medir, até então, 18 curvas de sensibilidade (10 para o GE e 08 para o GC). Para mensurar a FSC utilizou-se o método psicofísico da escolha forçada. Foram utilizados estímulos de grade senoidal com frequências de 0,25, 2,0 4,0, 8,0 cpg (grau de ângulo visual), em condições de luminância escotópica. Todas as medidas foram realizadas a uma distância de 150 m, de forma binocular, em um monitor LG de 19 polegadas. Em cada sessão experimental era apresentado pares de estímulos, aleatórios e sucessivos, que continham uma frequência senoidal e um círculo cinza homogênea de luminância média (0,04 cd/m²). A tarefa do participante era escolher sempre o estímulo de frequência senoidal.

Resultados: A análise dos resultados (ANOVA para medidas repetidas) revelou diferença significativa entre o GC e o GE ($F_{1,218} = 28,4021$; $p < 0,05$). A análise com teste post-hoc Tukey HSD demonstrou diferença nas frequências de 4,0 e 8,0 cpg ($p < 0,05$), onde o GC foi da ordem de 1,59 e 1,54 vezes mais sensível do que o GE nas frequências de 4,0 e 8,0 cpg respectivamente.

Conclusão: Os resultados indicaram que os portadores de esquizofrenia necessitam de maior contraste para perceber as frequências espaciais utilizadas quando comparado com os participantes isentos de patologia. No entanto este prejuízo foi maior na percepção de frequências espaciais altas, sugerindo alteração nas vias parvocelulares. **Palavras-Chave:** Sensibilidade ao Contraste; Esquizofrenia; Grades Senoidais; Escotópica; Percepção Visual;

Apoio Financeiro: CNPq

AVALIAÇÃO COMPORTAMENTAL EM MODELO DE DEMÊNCIA INDUZIDO POR HIPOPERFUSÃO ENCEFÁLICA CRÔNICA

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Objetivos: Muitos trabalhos demonstram que a redução do fluxo sanguíneo cerebral precede os sintomas de diversos tipos de demência. O modelo de Hipoperfusão Encefálica Crônica (HCE) com oclusão das carótidas comuns se enquadra neste contexto devido às alterações que provoca, contudo tem apresentado altos índices de mortalidade dos animais (90%). Os objetivos deste trabalho foram avaliar as alterações comportamentais induzidas pela oclusão das carótidas comuns, e comparar os resultados obtidos com o procedimento realizado concomitante em ambas artérias com a modificação do método, onde as carótidas são ocluídas com 1 semana de intervalo.

Métodos: Ratos Wistar adultos machos foram anestesiados e através de uma incisão na linha média do pescoço, ambas as carótidas comuns foram expostas e ocluídas permanentemente. O segundo grupo realizou o mesmo procedimento, mas com o intervalo de 1 semana entre a oclusão das duas carótidas. O grupo controle recebeu a mesma manipulação, mas sem a oclusão. 90 dias pós-cirurgia, a memória de referência e trabalho foi avaliada no labirinto aquático de Morris.

Resultados: Na memória de trabalho, ANOVA seguida pelo teste de Duncan, revela que a latência (seg) para encontrar a plataforma foi maior nos grupos isquêmicos em ambos modelos, indicando um prejuízo na memória no 2º, 3º e 4º *trials* ($p < 0,05$; carótidas ocluídas juntas, controle e isquêmicos respectivamente: 32 ± 5 - 50 ± 3 ; 27 ± 4 - 48 ± 4 ; 25 ± 4 - 48 ± 4 ; Carótidas ocluídas separadamente: 40 ± 4 - 52 ± 3 ; 26 ± 3 - 50 ± 3 ; 22 ± 1 - 47 ± 3). O mesmo ocorreu com a memória de referência, onde os grupos isquêmicos tiveram uma maior latência no 2º, 3º, 4º e 5º dias quando comparados ao grupo controle ($p < 0,05$ Carótidas ocluídas juntas: 27 ± 3 - 50 ± 3 ; 27 ± 3 - 45 ± 3 ; 23 ± 2 - 51 ± 4 ; 24 ± 3 - 46 ± 4 ; Carótidas ocluídas separadamente: 41 ± 4 - 51 ± 2 ; 36 ± 3 - 54 ± 2 ; 33 ± 3 - 53 ± 3 ; 22 ± 2 - 52 ± 3).

Conclusão: A HCE causa déficit de memória espacial em ratos Wistar, porém o procedimento cuja oclusão é realizada com 1 semana de intervalo parece ser vantajoso, uma vez que induz menor mortalidade dos animais (10%) podendo ser considerado um bom modelo experimental de demência. **Palavras-Chave:** fluxo sanguíneo cerebral, demência, comportamento, memória

Apoio Financeiro: CNPq, PRONEX/Fapergs

ANÁLISE DO VOLUME HIPOCAMPAL EM MODELO DE DEMÊNCIA INDUZIDO POR HIPOPERFUSÃO ENCEFÁLICA CRÔNICA

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Objetivos: Estudos sobre envelhecimento e desordens neurodegenerativas relacionadas à idade são cada vez mais frequentes, devido ao aumento destas enfermidades relacionadas ao fenômeno de envelhecimento cerebral. Atualmente existem vários modelos animais que são empregados para desvendar este processo, e o modelo de Hipoperfusão Encefálica Crônica (HCE) vem sendo utilizado, pois a diminuição do fluxo cerebral, e o conseqüente dano hipocampal são um importante achado em humanos acometidos por este mal.

Por isso, o objetivo deste trabalho foi analisar o volume hipocampal total e do Corno de Ammon (CA) de ratos machos submetidos a HCE.

Metodologia: Ratos Wistar adultos machos foram anestesiados e através de uma incisão na linha média do pescoço, a carótida comum esquerda foi exposta, separada do nervo vago e ligada com fio de sutura. Uma semana após, o mesmo procedimento foi realizado com a carótida comum direita. O grupo controle recebeu a mesma manipulação, mas sem a oclusão. Noventa dias pós-cirurgia, os animais foram sacrificados, seus encéfalos foram fixados via perfusão transcadiaca, congelados e após foram feitas secções coronais (50µm) em criostato. Em seguida, as fatias foram coradas com Hematoxilina e Eosina; as áreas hipocampal total e do Corno de Ammon (CA) foram mensurados (programa Image J) e o volume destas estruturas foi calculado (Método Cavalieri), para análise de dano e possível atrofia.

Resultados: ANOVA de 1 via demonstra que não houve atrofia hipocampal total (tanto hemisfério direito quanto esquerdo) em ratos submetidos à isquemia quando comparados ao seu grupo controle (Hemisfério Direito: 40,59±1; 40,62±0,8 / Esquerdo: 39,07±0,99; 39,7±0,82). Da mesma forma, no Corno de Ammon não houve significância entre grupo isquêmico e grupo controle em ambos os lados (26,95±0,66; 27,36±0,61 / Esquerdo: 26,23±0,68; 26,73±0,47).

Conclusão: A Hipoperfusão Encefálica Crônica não causa atrofia hipocampal total, nem da área do Corno de Ammon neste modelo experimental.

Palavras-Chave: demência, Hipocampo, Corno de Ammon, HCE

Apoio Financeiro: CNPq, PRONEX/Fapergs

MEDIDAS DE SENSIBILIDADE AO CONTRASTE VISUAL PARA FREQUÊNCIAS ANGULARES EM PORTADORES DE ESQUIZOFRENIA

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Objetivos: O Laboratório de Percepção Visual, Neurociências e Comportamento (LPVNeC-UFPB) tem desenvolvido diversos trabalhos relacionando processamento visual de contraste e patologias como depressão, câncer, epilepsia dentre outras. O presente trabalho tem como objetivo avaliar a função de sensibilidade ao contraste (FSC) em portadores de esquizofrenia utilizando estímulos angulares em condições fotópicas.

Métodos: Participaram dos experimentos dez voluntários adultos, sendo cinco participantes portadores de esquizofrenia (Grupo Experimental, GE) e cinco isentos de patologias neuropsiquiátricas (Grupo Controle, GC), com idade entre 20-47 anos (GE com $M = 33$ anos e $DP = 9,87$ e GC com $M = 28$ anos e $DP = 8,26$) e com a acuidade visual normal ou corrigida. Utilizamos o método psicofísico da escolha forçada e um delineamento experimental com medidas repetidas para medir, até o momento, 20 curvas de sensibilidade (10 curvas para o GE e 10 curvas para o GC). Foram utilizados estímulos angulares com frequências de 3,0, 24, 48, 96 ciclos/360° em condições de luminância fotópica. Os experimentos foram realizados binocularmente e os estímulos calibrados para uma distância de 150 cm. Durante cada sessão experimental foi apresentado sucessiva e aleatoriamente pares de estímulos contendo uma frequência angular e um círculo cinza homogêneo de luminância média (42,6 cd/m²). A tarefa do voluntário era escolher sempre o estímulo que continha a frequência angular.

Resultados: A análise dos resultados parciais (ANOVA para medidas repetidas) demonstrou diferença significativa entre o GC e o GE ($F_{1,198} = 35,4407$; $p < 0,001$). As análises com teste post-hoc Tukey HSD mostraram diferenças estatísticas apenas nas frequências de 3,0 e 96 ciclos/360° ($p < 0,001$), onde o GE precisou da ordem de 1,62 vezes mais contraste para perceber a frequência de 3,0 ciclos/360° e 2,35 vezes mais contraste para perceber a frequência de 96 ciclos/360°.

Conclusão: Os resultados preliminares sugerem que a esquizofrenia altera os mecanismos visuais que processam frequências angulares baixas e altas em condições fotópicas de luminância.

Palavras-Chave: Sensibilidade ao contraste; Esquizofrenia; Sistema visual; frequências angulares

Apoio Financeiro: CNPq

PERCEPÇÃO VISUAL EM PORTADORES DE ESQUIZOFRENIA: AVALIAÇÃO DA SENSIBILIDADE DE GRADES SENOIDAS EM CONDIÇÕES FOTÓPICAS

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Objetivos: O presente trabalho teve como objetivo utilizar a função de sensibilidade ao contraste (FSC) para caracterizar a percepção visual da forma em portadores de esquizofrenia. A FSC é um instrumento que avalia os fatores ópticos e neurais do Sistema Visual Humano (SVH) e estuda os processos visuais básicos.

Métodos: Participaram dos experimentos dez voluntários adultos, sendo cinco participantes portadores de esquizofrenia (Grupo Experimental, GE) e cinco isentos de patologias neuropsiquiátricas (Grupo Controle, GC), com idades equivalentes variando entre 20-47 anos (GE com M = 33 anos e DP = 9,87 e GC com M = 28 anos e DP = 8,26) e com a acuidade visual normal ou corrigida. Utilizamos um delineamento experimental com medidas repetidas para medir, até o momento, 20 curvas de sensibilidade (10 curvas para o GE e 10 curvas para o GC). A FSC foi determinada pelo método psicofísico da escolha forçada. Foram utilizados estímulos de grade senoidal com frequências de 0,25, 2,0 4,0, 8,0 cpg (ciclos por grau de ângulo visual) em condições de luminância fotópica (42,6 cd/m²). Todas as medidas foram realizadas com visão binocular e a uma distância de 150 cm de um monitor LG de 19 polegadas. Durante cada sessão experimental foi apresentado sucessiva e aleatoriamente pares de estímulos contendo uma frequência senoidal e um círculo cinza homogêneo de luminância média. A tarefa do voluntário foi escolher sempre o estímulo que continha a frequência senoidal.

Resultados: A análise dos resultados parciais com ANOVA para medidas repetidas mostrou diferença significativa entre o GC e o GE ($F_{1, 198} = 40,3208$; $p < 0,001$). A análise com teste post-hoc Tukey HSD demonstrou diferença apenas na frequência de 8 cpg ($p < 0,001$), onde o GC foi da ordem de 2, 30 vezes mais sensível do que o GE.

Conclusão: Os resultados preliminares sugerem que a esquizofrenia altera os mecanismos visuais que processam frequências espaciais altas em condições fotópicas de luminância.

Palavras-Chave: Sensibilidade ao contraste; Esquizofrenia; Sistema visual; Grades senoidas

Apoio Financeiro: CNPq

Glial hemichannels: Good pathways in peace but dangerous weapons during neuroinflammation.

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Introduction: During the last decade, the existence of hemichannels (HCs: half of gap junction channels) has been recognized. HCs are located at the cell surface (unapposed membrane) and serve as membrane pathway for diffusional transport between intra and extracellular compartments. They are permeable to ions and small molecules, including autocrine and paracrine signals (e.g., ATP, glutamate and PGE₂). The protein subunits of HCs can be connexins (Cxs: 21 family members) or pannexins (Pxs: 3 family members). A subthreshold stimulation by acute pathological threatening conditions enhances glial Cx43 HC activity, favoring ATP release that induces preconditioning. **Objective:** To demonstrate the existence of HCs in microglia and astroglia under resting conditions and after treatment with deleterious proinflammatory conditions. **Methods:** The functional state of HCs was studied by measuring the uptake of extracellular ethidium using fluorescence microscopy and time lapse recording. Cells were treated with metabolic inhibitors, hypoxia reoxygenation or pro-inflammatory cytokines. The sensitivity to extracellular Ca²⁺-free solution and to Cx or Px HC blockers were used to demonstrate the involvement of each HC type. **Results:** Under resting conditions, cultured microglia expressed Cxs 43 and 45 and Px1. The activity of the latter was enhanced by the activation of P2X receptors but functional Cx HCs were absent. Astroglial cells also expressed Cxs (26 and 43) and Px1 but forms only functional Cx HCs. Microglia became overactivated upon treatment with deleterious threatening conditions and released bioactive molecules that increased the HC activity in astroglial cells. In the absence of microglia, deleterious stimuli also enhanced the activity of astrocytic Cx43 HCs. The increase in astrocytic Cx HC activity occurred at least via two mechanisms: 1) increase in surface HC levels and/or 2) a gating mechanism activated by oxidation of Cx43. The enhanced glial HC activity reduced the viability of astroglial cells. **Conclusions:** Reduction of microglia activation or inhibition of HCs should reduce neurodegeneration.

Palavras-Chave: Connexins; pannexins; microglia; astroglia; neurodegeneration

Apoio Financeiro: Fondecyt 1070591.

Specific neuronal binding of A β oligomers chronically infused into the ventricular system of the rat.

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Our working hypothesis is that early memory loss in Alzheimer's disease is a synaptic failure caused by soluble A β oligomers, which have been shown to act as pathogenic ligands in cultured neurons (Mol. Cell Biol. 8:101, 2007; IUBMB Life 59:332, 2007). Because little is known about oligomer toxicity *in vivo*, we here investigated whether A β oligomers infused chronically through an intracerebroventricular canula into adult Wistar rats may diffuse and target neurons *in vivo*. Animals were administered 1 μ g of A β oligomers, 3 days a week for 5 weeks. Brains were fixed and frozen-cut into 40 μ m sections processed immunohistochemically for detection of A β oligomers, PSD95 (post synaptic sites) or GFAP (astrocytes). We found that A β oligomers can diffuse from the ventricle to cerebral parenchyma and bind to neurons *in vivo*. In neurons, the pattern of oligomer labeling matches the distribution of the post synaptic protein PSD95. Numerous neurons with bound oligomers were found in the hippocampus, frontal cortex, basal ganglia and amygdala. These areas are known to be essential for memory, and operant behavior. Some labeling was found in primary somesthetic cortex, but not in other brain regions (visual cortex, thalamus and cerebellum). Oligomers were not found to bind to GFAP positive astrocytes. Our results demonstrate for the first time that A β oligomers might selectively target neurons in cognitive-related regions *in vivo*. The regional specificity of neuronal targeting by A β oligomers may explain why Alzheimer in early stages is a disease of profound memory loss **Palavras-Chave:** Alzheimer, A β oligomers, stereotaxic, selectivity binding

Apoio Financeiro: CNPQ, FAPERJ, PRONEX

Stimulation of cholinergic neural transmission reverses iron-induced recognition memory deficits.

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Objectives: Increasing evidence has indicated that excessive iron in selective regions of the brain may generate cytotoxic free radical formation, thereby possessing implications for the etiology of neurodegenerative disorders. Increased levels of iron have been reported in neurodegenerative disorders, such as Parkinson's and Alzheimer's diseases, as well as in normal brain aging. We have previously demonstrated that rats given iron during the neonatal period presented severe recognition memory impairment in the adulthood. We also showed that this memory impairment could be attenuated by selegiline (a MAO inhibitor) and desferoxamine (an iron chelator). The aim of the present study was to evaluate the acute effect of the cholinergic agents galanthamine (an acetylcholinesterase inhibitor) and oxotremorine (a muscarinic receptor agonist), on iron-induced memory deficits. **Methods and Results:** Male Wistar rats received vehicle or iron (10.0mg/kg) orally at postnatal days 12-14. When the animals reached 3 months old, they were trained in a novel object recognition task. A recognition index, expressed by the ratio $T_N/(T_F+T_N)$ (T_F =time spent exploring the familiar object; T_N =time spent exploring the novel object), was calculated for each animal. Iron-treated rats showed long-term recognition memory impairments (median [interquartile ranges] = 0.75 [0.68/0.78], and 0.55 [0.47/0.60], for control and iron-treated groups, respectively; n=10). Iron-treated rats that received galanthamine (1mg/Kg ip) immediately after the training session, showed normal recognition memory (0.74 [0.71/0.79]; n=10), when compared to control group. Conversely, iron-treated rats that received oxotremorine (0.5mg/Kg ip) immediately after the training session, showed an improvement but not a complete reversion of memory impairment (0.66 [0.60/ 0.69]; n=10). **Conclusions:** The results show that pharmacological manipulations of the cholinergic system reverse the neonatal iron-induced recognition memory deficits. The present findings are in agreement with the hypothesis that, at least in part, iron-induced cognitive deficits are related to a dysfunction of cholinergic neural transmission in the brain.

Palavras-Chave: iron, neurodegeneration, memory, galanthamine, oxotremorine, rats

Apoio Financeiro: CNPq, FAPERGS

Vesicular Acetylcholine Transporter Expression and Neuromuscular Junction Innervation During Amyotrophic Lateral Sclerosis.

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Amyotrophic Lateral Sclerosis (ALS) is neurodegenerative disease characterized by progressive motoneuron death and consequent muscle weakness and atrophy. Although the onset of symptoms occurs ~100 days of age, followed by critical motoneuron loss from the lumbar spinal cord in the SOD1^{G93A} mouse model of ALS, recent data suggest that muscle denervation and axonal retraction occur as early as 30 days. Therefore, characterization of muscle denervation before the clinical onset is of critical relevance for the diagnosis and treatment of ALS. To characterize muscle denervation outcome during ALS, we performed Positron Emission Tomography (PET) scans using the radiotracer [¹⁸F]FBT, an antagonist of the vesicular acetylcholine transporter (VACHT), and carried out analysis of VACHT expression at Neuromuscular Junction (NMJ). MicroPET scans were recorded for 90 minutes after administration of [¹⁸F]FBT (100µCi) using a Concord micro PET-P4. Four SOD1^{G93A} mutants and WT mice between 104 to 111 days of age and three unilateral sciatic nerve-transected mice were scanned. PET scans were dose and muscle volume corrected. Muscle volume was determined by MRI. Immunohistochemistry and immunoblots were performed in hindlimb muscles using a anti-VACHT antibody. PET scans showed a consistent increase of [¹⁸F]FBT uptake in SOD1^{G93A} mutants and nerve transected side 7 days after surgery. In agreement with PET scans, immunoblots showed a significant increased VACHT expression in muscle extracts of SOD1^{G93A} mice. However, immunostaining of mutant and experimentally denervated muscles showed a marked decrease in NMJ innervation. These results suggest a role for axonal retraction from the NMJ as revealed by loss of colocalization between pre (VACHT Ab) and postsynaptic (labeled alpha-bungarotoxin) markers. Furthermore, increased signal in immunoblots and PET scans suggest nerve sprouting and VACHT upregulation with progression of ALS.

Palavras-Chave: Amyotrophic Lateral Sclerosis, Neuromuscular Junction, VACHT, PET.

Apoio Financeiro: CAPES-FIPSE exchange program for biomedical research and NIH

Aging, cognitive decline and reduction of the number of astrocytes in the molecular and granular layers of the dentate gyrus

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Objective: To investigate if there is possible correlation between the number of astrocytes in DG and behavioral changes in aged C57Bl6 mice, since stereological estimation of glial cell numbers in dentate gyrus (DG) was not correlated with aged behavioral changes. Possible correlation between the number of astrocytes in DG and behavioral changes are investigated in aged C57Bl6 mice. **Methods:** Adult (A- 6 months old, n=6) and senile (S- 24 months old, n=5) female C57Bl6 mice were submitted to Y maze, non-match-to place spatial memory, and open field (OF) tests. Video recorded was analyzed with ANYMAZE tracking system (Stöelting Science). Threshold for statistical significance was set at $p < 0.05$. After behavioral tests, subjects were sacrificed and brain sections were immunolabeled for glial fibrillary acid protein (GFAP). Regions of interest and cell counting on each layer were stored and analyzed by optical fractionator (Stereoinvestigator® MicroBrightField, Willston, VT, USA). **Results:** It was detected a significant reduction of the number of astrocytes in the granular (S-12052,77 $\pm$ 1360,42; A-16523,93 $\pm$ 649,28), molecular layers (S-32673,54 $\pm$ 3473,41; A-43552,10 $\pm$ 1506,65) and in DG total counts (S-66017,45 $\pm$ 4998,86; A-85097,77 $\pm$ 3162,73) in S subjects, when compared with A (t-Student test, $p < 0,05$). Familiar from new arms of Y maze, and displaced from stationary objects in the spatial memory tests, could not be distinguished by S subjects ($p > 0.05$). Locomotor and exploratory activity were reduced in OF measured as distance traveled (S-1.44m $\pm$ 0.36; A-4.23m $\pm$ 0.69), mean speed (S-0.48cm/s $\pm$ 0.12; A-1.43 cm/s $\pm$ 0.22) and immobile time (S-273.85s $\pm$ 5.76; A-219.98 $\pm$ 13.20) in S subjects in comparison with A ones (ANOVA, Bonferroni *a priori*, $p < 0,05$). The number of entries in the new arm of Y maze and the number of astrocytes of granular layer were correlated (Pearson, $R^2 = 0.5785$, $p = 0.0284$), molecular ($R^2 = 0.7698$, $p = 0.0042$) and with total DG counts ($R^2 = 0.7373$, $p = 0.0063$). Traveled distance in the new arm of Y maze and the number of astrocytes of granular ($R^2 = 0.7039$, $p = 0.0092$), molecular ($R^2 = 0.6588$, $p = 0.0144$), and of the DG total counts ($R^2 = 0.7219$, $p = 0.0075$) shown good correlation. **Conclusion:** The reduction of the number of astrocytes in DG and cognitive decline in senile C57Bl6 mice seems to be interdependent.

Palavras-Chave: Aging; astrocytes; cognitive decline; dentate gyrus; mice

Effects of probiotic bacterial quorum sensing peptides on lifespan of cerebellum neuron cells

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Quorum sensing (QS) provides bacteria with the ability to communicate and change behaviors across kingdoms. While many studies have focused on QS of pathogenic bacteria, the effects of QS from beneficial probiotic bacteria on eukaryotic cells have received poor attention. In our laboratory we are interested on QS of a particular probiotic intestinal bacterium (*Bacillus subtilis* Natto) that produces several beneficial effects on human health. *B. subtilis* produces up to eight different pentapeptides (Phr) with QS activity that would be involved in the healthy interaction with human cells. In this respect, we and others reported a stimulatory effect of *B. subtilis* on innate immunity, galectin expression and a Phr-mediated activation of cytoprotective proteins in intestinal epithelial cells. Because of the known effects of probiotic bacteria on the immune system, and the connection of this system with the nerve system, we were intrigued to explore the effect of QS on neural cells. In this communication we report the effects of *B. subtilis* QS peptides on lifespan and resistance to oxidative stress of cultures of neurons isolated from rat cerebellum. Physiological concentrations of PhrA and PhrC QS peptides produced a dose-dependent increase on the expression (measured by RT-PCR) of the anti-aging gene *sirt7* in neuron cultures. Increased levels of Sirtuin 7 would result in prolonged neural survival and enhanced stress-resistance, a hypothesis that we confirmed by immunocytochemistry studies that showed that, in the presence of Phr peptides, the levels of proteasome-related ubiquitin (a small 76 residue protein involved in intracellular degradation of miss-folded proteins) were decreased. Furthermore, rat neurons showed increased survival and decreased oxidative damage, evidenced by labeling with the fluorescent markers propidium iodide and SYTO 9 dye, when cultured in the presence of either PhrA or PhrC. These results suggest a key and previously unknown role of QS molecules on nerve system oxidative protection and lifespan, effects that would be the target for future developments of therapeutic molecules to treat aging-related neurodegenerative diseases. **Palavras-Chave:** quorum sensing, probiotic bacteria, neuron lifespan

Apoio Financeiro: CONICET, FONCyT and Cyllene Biotechnology

Efeito da administração aguda do extrato metanólico de *Rosmarinus officinalis* sobre parâmetros de estado oxidativo celular em estruturas cerebrais de ratos

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Espécies da família Lamiaceae, como *Salvia* spp, têm sido usadas no tratamento da Doença de Alzheimer. *Rosmarinus officinalis* L. (Lamiaceae) é conhecida como “alecrim” e amplamente utilizada como condimento. Nosso objetivo foi avaliar o efeito da administração aguda do extrato metanólico (EM) de *R. officinalis* sobre parâmetros de estado oxidativo celular em estruturas cerebrais. Métodos: O material vegetal, obtido no Mercado Público Central de Porto Alegre (RS, Brasil), foi submetido à maceração com metanol, sendo o extrato conduzido à secura em evaporador rotatório. Foram utilizados 25 ratos Wistar adultos machos. Os animais receberam (v.o.) EM de *R. officinalis* (30 e 100 mg/kg), salina ou etanol. Após 60 minutos, os animais foram decapitados, as estruturas cerebrais, córtex frontal, hipotálamo e estriado, congeladas em N₂ líquido. As estruturas foram homogeneizadas em tampão fosfato e centrifugadas. O sobrenadante foi usado nos ensaios. O *probe* 2'-7'-diclorofluoresceína diacetato foi usado para estimar o conteúdo de radicais livres. A determinação de substâncias reativas ao ácido tiobarbitúrico foi usada como índice de lipoperoxidação (LPO). Os tióis totais foram determinados pelo método de Ellman. As atividades das enzimas antioxidantes, superóxido dismutase (SOD) e glutathione peroxidase (GPx), foram determinadas utilizando os kits, respectivamente, RANSOD e RANSEL (Randox Labs., USA). Os resultados foram expressos como média ($\pm$ DP). A administração aguda do EM de *R. officinalis* (100 mg/kg) induziu um aumento na atividade da SOD no hipotálamo (salina $0,57 \pm 0,04$; etanol $0,52 \pm 0,10$; EM 30 mg/kg $0,52 \pm 0,08$; EM 100 mg/kg $0,64 \pm 0,04$ U/ mg proteína; ANOVA seguida de Tukey, $p=0,0197$) e na atividade da GPx (salina $4,74 \pm 2,34$; etanol $5,77 \pm 2,28$; EM 30 mg/kg $4,60 \pm 2,82$; EM 100 mg/kg $8,73 \pm 1,42$ U/ mg proteína; ANOVA seguida de Tukey, $p=0,05$) no córtex frontal. A administração aguda de EM de *R. officinalis* aumentou a atividade das enzimas antioxidantes em diferentes estruturas cerebrais, indicando que compostos desta espécie parecem interagir com a rede de defesas antioxidantes. A atividade antioxidante pode ser relevante para o tratamento da Doença de Alzheimer. **Palavras-Chave:** *Rosmarinus officinalis* ; estresse oxidativo; córtex frontal; hipotálamo; estriado; ratos.

Apoio Financeiro: PIBIC CNPq/UFRGS; BIC/FAPERGS, BIC/UFRGS.

A administração aguda do extrato metanólico de *Rosmarinus officinalis* não altera a atividade da acetilcolinesterase em estruturas cerebrais de ratos

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Espécies da família Lamiaceae, como *Salvia* spp, são usadas no tratamento da Doença de Alzheimer. *Rosmarinus officinalis* L. (Lamiaceae), conhecida popularmente como “alecrim”, é originária da Costa do Mar Mediterrâneo. *Rosmarinus officinalis* é utilizada como condimento, há relatos de uso popular para melhorar a memória. A atividade anticolinesterásica *in vitro* tem sido amplamente descrita. Nosso objetivo foi avaliar o efeito da administração aguda do extrato metanólico de *Rosmarinus officinalis* sobre a atividade da enzima acetilcolinesterase em estruturas cerebrais de ratos. Métodos: O material vegetal, obtido no Mercado Público Central de Porto Alegre (RS, Brasil), foi submetido à maceração com metanol, sendo o extrato conduzido à secura em evaporador rotatório. Foram utilizados 25 ratos Wistar adultos machos. Os animais receberam (v.o.) extrato metanólico de *Rosmarinus officinalis* (30 e 100 mg/kg), salina ou etanol. Após 60 minutos, os animais foram decapitados e as estruturas cerebrais, córtex frontal, cerebelo, hipocampo e estriado, congeladas em N₂ líquido. As estruturas foram homogeneizadas em tampão fosfato e centrifugadas, o sobrenadante foi utilizado. A atividade da enzima acetilcolinesterase foi determinada pelo método de Ellman. Neste ensaio, os homogeneizados foram incubados com o ácido 5,5'-ditio-bis-(2-nitrobenzóico) e, o substrato da enzima, acetiltiocolina (412 nm). A administração aguda do extrato metanólico de *Rosmarinus officinalis* (30 e 100 mg/kg) não alterou significativamente a atividade da enzima acetilcolinesterase nas estruturas cerebrais estudadas, córtex frontal, cerebelo, hipocampo e estriado. Outros estudos deverão ser realizados para determinar o perfil farmacológico desta espécie, especialmente utilizando tratamento crônico. **Palavras-Chave:** *Rosmarinus officinalis*; acetilcolinesterase; córtex frontal; cerebelo; hipocampo; estriado; ratos.

Apoio Financeiro: PIBIC CNPq/UFRGS; BIC/FAPERGS, BIC/UFRGS

Comparação do paradigma evocação livre auditivo-verbal e o paradigma visual-semântico como instrumentos auxiliares no diagnóstico de demência

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Objetivos: Comparar o uso de dois paradigmas de avaliação de memória episódica como instrumentos para diferenciar idosos com e sem demência e caracterizar o funcionamento da memória nestas amostras.

Método: Foram avaliados 306 sujeitos, subdivididos em duas faixas etárias (idade: < 75 e ≥ 75 anos). O grupo com idade ≥ 75 anos incluiu 63 idosos sem demência e 65 em estágio leve de uma síndrome demencial (*Clinical Dementia Rating-CDR:1*). O grupo com idade < 75 anos, teve 117 idosos sem e 61 com demência em grau leve (*CDR:1*). Para o diagnóstico de demência usou-se os critérios do *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) baseados em entrevista clínica, avaliação médica e neuropsicológica abrangente. Os instrumentos utilizados neste estudo foram: o Teste de Aprendizagem Auditivo-Verbal de Rey-RAVLT e o Teste de Memória de Figuras da Bateria Cognitiva Breve-BCSB.

Resultados: No grupo com idade ≥ 75 anos, os pacientes com demência evocaram um número significativamente menor de estímulos que os idosos normais, tanto no RAVLT, quanto no BCSB ($t=4,71$ e $t=7,79$, $p<0,05$). No grupo com idade < 75 anos, o mesmo padrão de desempenho foi observado ($t=7,49$ e $t=13,76$, $p<0,05$). A análise da curva ROC mostrou que o grupo com idade ≥ 75 anos, a evocação livre do BCSB (0,838) apresentou uma área sob a curva superior ao RAVLT (0,725). No com idade < 75 anos, o mesmo padrão foi observado, o BCSB (0,924) apresentou uma área sob a curva maior que o RAVLT (0,812). Para idade ≥ 75 anos, a evocação do RAVLT apresentou 73,7% de sensibilidade e 73,1% de especificidade para um ponto de corte de 0,5. No BCSB foi de 89,5% e 92,3%, ponto de corte de 4,5. Para idade < 75 anos, no RAVLT foi de 77% e 71%, ponto de corte de 2,5. E no BCSB foi de 93,8% e 85,5%, ponto de corte de 4,5. **Conclusão:** O teste de memória de figuras, envolvendo codificação visual e semântica, é uma tarefa com maior capacidade diagnóstica que a do RAVLT, que envolve apenas codificação auditivo-verbal. Os pacientes com demência apresentam um comprometimento na capacidade de evocação de novas informações auditivo-verbais, visuais e semânticas. **Palavras-Chave:** Envelhecimento. Idade. Escolaridade. Memória. Demência.

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Neuronas Von Economo están presentes en el área 9 de la corteza prefrontal humana

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Las células Von Economo son un tipo especial de neuronas, que previamente solo han sido observadas en la lámina Vb de la región anterior del cíngulo y en la región frontal de la ínsula de grandes primates. El estudio de este tipo de células es de especial interés ya que su volumen, número y grado de agrupamiento se correlaciona con el grado de encefalización lo cual podría indicar que son un adelanto evolutivo paralelo al desarrollo de muchas habilidades cognitivas y emocionales en primates. En neurodegeneración el estudio de estas células también es de especial interés por cuanto en etapas iniciales de demencia frontotemporal se ha observado pérdida selectiva de este tipo de neuronas.

Objetivo: Evaluar la presencia de neuronas Von Economo en regiones corticales relacionadas con el área 24 en términos de proyecciones.

Método: se tomaron muestras de tejido cerebral humano post mortem proveniente de 8 sujetos con una edad promedio de 40 años (desviación estándar 9) de las áreas 9, 24, 4, 46, 45, 10 y 17 de Brodman.

Se realizó inmunohistoquímica de acuerdo con protocolos estándar para los anticuerpos NeuN, Map2, Calbindina, Calretina y parvalbúmina.

Resultados: se identificaron neuronas Von Economo en el área 9 de la corteza cerebral humana, allí representan el 0.5% (desviación estándar 0.3) de neuronas de la lámina V mientras que en el área 24 representan el 6% y (Proc. Natl. Acad. Sci. USA 96 5268–5273 1999)

También se observó que las neuronas Von Economo del área 9 presentan un tamaño significativamente mayor ($627.5 \pm 96.92 \mu\text{m}^2$) que el de las neuronas Von Economo del área 24 ($536.3 \pm 161.5 \mu\text{m}^2$) ($p < 0.05$).

No se observaron neuronas Von Economo en las otras áreas corticales evaluadas.

Conclusiones: El presente trabajo se constituye en la primera evidencia de la presencia de este tipo de células en regiones corticales distintas a las reportadas inicialmente por Von Economo y Koskinas en 1925. En caso tal de que en pacientes con demencia frontotemporal estas neuronas se afectaran en el área 9, de la misma manera que se afectan en el área 24, se podrían explicar de alguna manera las alteraciones en atención e inhibición que presentan estos pacientes.

Palavras-Chave: Neuronas Von Economo. Demencia frontotemporal.

Apoio Financeiro: Universidad del Valle.

Amyloid- β Binds to the Extracellular Cysteine-rich Domain of Frizzled and Inhibits Wnt/ β -Catenin Signaling

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The amyloid- β peptide (A β) plays a major role in neuronal dysfunction and neurotoxicity in Alzheimer disease. However, the signal transduction mechanisms involved in A β -induced neuronal dysfunction remain to be fully elucidated. A major current unknown is the identity of the protein receptor(s) involved in neuronal A β binding.

OBJECTIVES: Identify ligands that mediate the neuronal impact of A β

METHODS: Using phage display of peptide libraries, we have identified a number of peptides that bind A β and are homologous to neuronal receptors putatively involved in A β interactions. We report here on a cysteine-linked cyclic heptapeptide (denominated cSP5) that binds A β with high affinity and is homologous to the extracellular cysteine-rich domain of several members of the Frizzled (Fz) family of Wnt receptors. Based on this homology, we investigated the interaction between A β and Fz.

RESULTS: The results show that A β binds to the Fz cysteine-rich domain at or in close proximity to the Wnt-binding site and inhibits the canonical Wnt signaling pathway. Interestingly, the cSP5 peptide completely blocks A β binding to Fz and prevents inhibition of Wnt signaling.

CONCLUSIONS: These results indicate that the A β -binding site in Fz is homologous to cSP5 and that this is a relevant target for A β -instigated neurotoxicity. Furthermore, they suggest that blocking the interaction of A β with Fz might lead to novel therapeutic approaches to prevent neuronal dysfunction in Alzheimer disease.

Palavras-Chave: Alzheimer disease, Amyloid-beta peptide, Frizzled, Wnt signaling, cSP5 peptide

Apoio Financeiro: HHMI, CNPq, FAPERJ

Sirtuin Family: Molecular Identification, Phylogenetics Analysis and Brain Expression Pattern in Zebrafish (*Danio rerio*)Pereira, T.C.B.¹; Rico, E.P.²; Rosember, DB²; Dias, R. D.¹; Bonan, C. D.¹; SOUTO, A. A.³; Bogo, M. R.¹- ¹PUCRS - Departamento de Biologia Celular e Molecular; ²UFRGS - Departamento deBioquímica; ³PUCRS - Departamento de Química Pura

Sirtuins are a family of NAD⁺-dependent protein deacetylases distributed in all phyla of life, but only eukaryotes typically have multiple sirtuins. Accumulating evidence indicates that they play important physiological roles especially in aging and regulation of life span. Zebrafish (*Danio rerio*) is a teleost widely used as a vertebrate model in many research areas, but few is known about *D. rerio* as model in aging-related investigations. **Objectives:** For the first time, this study intended to identify all sirtuins-related genes in zebrafish, to determine their phylogenetic relationship, and expression pattern. **Methods:** The sirtuins members identification was performed in NCBI-BLAST searches, using *Homo sapiens*, *Mus musculus*, and *Gallus gallus* proteins as queries. The obtained sequences were compared with zebrafish protein database (ZFIN) and an alignment was performed using ClustalX. A phylogenetic tree was constructed according to Neighbor-Joining method using proportional (p) distance with MEGA 4.0.2. Total RNA was isolated from adults zebrafish brain (n=5) and cDNA species were synthesized through RT-PCR. Sirtuins and β -actin primers were designed, and PCR optimal conditions were determined. Performed reactions were analyzed on 1% agarose gel with GelRed[®]. The relative mRNA abundance of each *sirtuin* versus *β -actin* was determined by optical densitometry using ImageJ1.37. Results (mean $\pm$ S.E) were statistically compared by one-way ANOVA, followed by Tukey test, considering $P \leq 0.05$ as significant. **Results:** Seven sirtuins-related genes were identified, and their phylogenetics analysis resulted on seven well-resolved terminal clades, corresponding to each one of the sirtuins (SIRT). Two *SIRT3* paralogues were identified. Gene expression patterns were determined for *SIRT2* (0,654 $\pm$ 0,077); *SIRT3* (0,520 $\pm$ 0,042); *SIRT3.2* (0,373 $\pm$ 0,033); *SIRT4* (0,464 $\pm$ 0,027); *SIRT5* (0,553 $\pm$ 0,028); *SIRT6* (0,564 $\pm$ 0,037); and *SIRT7* (0,793 $\pm$ 0,101). **Conclusions:** The significant differences identified for *SIRT2-SIRT3.2*; *SIRT3.2-SIRT7* and *SIRT4-SIRT7* expression patterns suggest that they could exert distinct physiological roles zebrafish brain and contribute differently in aging and brain functions.

Palavras-Chave: Sirtuin, Zebrafish, *Danio rerio*, aging.

Apoio Financeiro: CNPQ; FAPERGS; FINEP Research Grant "Rede Instituto Brasileiro de Neurociência" (IBN-Nef) # 01.06.08.42-00 and PUCRS.

AVALIAÇÃO DO IMPACTO DA EXPOSIÇÃO A AGROTÓXICOS NA SAÚDE DOS IDOSOS NO SUL DO BRASIL.

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Objetivos: A população de idosos no mundo vem aumentando consideravelmente nas últimas décadas. Até 2025, o Brasil será o sexto país do mundo com o maior número de pessoas idosas, segundo dados da Organização Mundial de Saúde (OMS), atualmente, se estimam que existam cerca de 17,6 milhões de idosos. A população com mais de 60 anos é a que mais cresce no país entre as diferentes faixas etárias, apresentou um aumento de 104% de 1980 a 2000. Entre os fatores ambientais responsáveis por alterações na saúde humana podem-se citar os agrotóxicos atuando diretamente, por meio do contato direto do organismo com essas substâncias ou indiretamente, por intermédio do desenvolvimento de algum fator impactante como resultado do uso desses agentes químicos. Com isto este trabalho tem como objetivo estabelecer a prevalência de doenças neurodegenerativas em população idosa do Sul do Brasil sujeita a exposição direta ou indireta a agrotóxicos.

Metodologia: realizou-se um estudo transversal. Os dados foram coletados na zona urbana e rural, com indivíduos de ambos os sexos e acima de 60 anos utilizando questionário semi-estruturado. A amostra foi composta de 226 idosos.

Resultados: A idade média da população entrevistada foi de 73 ± 8 anos para as mulheres e 71 ± 7 anos para homens. 35,8% dos idosos relataram utilizar agrotóxicos, sendo em sua maioria organofosforados (38,3%). Foi observada associação significativa entre gênero masculino e uso de agrotóxicos ($p < 0,05$). Observou-se correlação positiva entre exposição a agrotóxicos com esquecimento e Mal de Parkinson.

Conclusão: os resultados aqui obtidos corroboram com estudos anteriores e demonstram que é fundamental incentivar o conhecimento pormenorizado dos efeitos agudos e crônicos de uso de pesticidas. Além disso, é importante salientar a necessidade de desenvolvimento de programas de saúde pública que auxiliem na promoção de saúde do idoso, salientando a necessidade de considerar a possível exposição dessa população a agrotóxicos.

Palavras-Chave: Agrotóxicos, Saúde, Idosos

Apoio Financeiro: Centro Universitário Univates

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The neuromodulator D-serine decreases with aging in the human brain

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Background: The normal aging is associated with impairment in cognitive function such as learning and memory. The long-term potentiation (LTP) mediated by the NMDA type of glutamate receptors in the hippocampus is considered essential for the formation of memory. D-serine is the dominant endogenous co-agonist of the NMDA receptors in the hippocampus. Decreased levels of D-serine are responsible for decreased LTP in the hippocampus of old rats. The present study assess whether the availability of D-Serine in the brain is affected during human aging.

Methods: Amino acids levels were measured by high-performance liquid chromatography (HPLC) in postmortem brain samples and cerebrospinal fluid (CSF). 35 subjects older than 50 years old with no clinical history of dementia were studied. The expression of the enzymes responsible for D-serine formation, serine racemase, and degradation, D-amino acid oxidase were evaluated by western blotting.

Results: Significant negative correlation between the concentration of D-serine and the increase of age was observed in the hippocampus ($r^2 = 0.17$, $p = 0.015$) and in the parietal cortex ($r^2 = 0.12$, $p = 0.039$). The study of CSF in a subset of subjects ($n=13$) showed a trend to negative correlation as well ($r^2 = 0.26$, $p = 0.071$). There were no correlations between the levels of glutamate, L-serine and glutamine and aging in the samples studied.

Conclusions: The availability of D-Serine in hippocampus and parietal cortex decrease during human aging. The decrease in the co-agonism of NMDA receptors by D-serine in the hippocampus can be associated to the impairment in memory observed in normal aging.

Palavras-Chave: aging, D-serine, NMDA receptor

Hippocampal Extracellular Matrix of Sedentary and Exercised Presenescent Rats: histochemical study by /Wisteria floribunda/ lectin.

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Objectives: The physiological expression of hippocampal contribution for memory consolidation depends on the integrity of perineuronal nets (PN), a specialized region on the extracellular matrix (ECM). In the present report we described the histochemical extracellular matrix architecture of CA1, CA2 and CA3 hippocampal areas, and estimated the number of perineuronal nets in CA1 of exercised or sedentary 16-22 months old, Wistar rats, after Wisteria floribunda histochemistry. The total number of PN of CA1 was estimated by the optical fractionator method, and the neuropil histochemical activity of CA2 and CA3 by densitometry.

Methods: Pre-senescent rats were submitted to 3 months of 20 min daily exercise in the treadmill (n=4), or left sedentary (n=4), and then perfused with aldehyde fixatives and processed for Wisteria floribunda histochemistry. Conspicuous limits between the subicular region, CA1, CA2 and CA3 were detected and used to place the counting boxes in the area of interest (CA1), and to measure transmitted light through the neuropil of CA2 and CA3 in both groups. Stereological investigation was done by optical fractionator (Stereoinvestigator, Microbright Field Inc). PN counts were restricted to oriens, pyramidal and stratum radiatum of CA1.

Results: Two types of perineuronal nets were found and designated: Type I and Type II. Type I present soma, proximal and distal labeled dendrites and type II only somatic perineuronal nets with very short and faint primary dendrites. The total PN numbers estimated by optical fractionator in CA1 were similar in the exercised rats (86677 ± 23537) and in the sedentary ones (76515 ± 32498 ; two-tailed T-test $p > 0.05$). Densitometric measurements in CA2 and CA3 also revealed no differences between the groups ($p > 0.05$).

Conclusions: Since PN is essential for synaptic plasticity and we have found no differences between experimental groups, our data would predict no differences in cognitive performances at this age after 90 days of treadmill exercise at pre-senescence age.

Palavras-Chave: Hippocampus, ageing, perineuronal nets, stereology

Apoio Financeiro: FINEP, CNPq, CAPES, UFPE, PROPESP-UFPA, FINEP/IBN net

Hindpaw cortical representation in the somatosensory S1 area in exercised pre-senescent Wistar Rats.

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Cortical neuron regeneration is an activity-dependent process whereby physical exercise may induce significant plastic changes. In the present report we studied the possible impact of exercise on the somatosensory cortical representation of the hindpaw in pre-senescent (16-22 month old) Wistar rats. Treadmill-exercised (for ninety days at 0.2m/s) (n=4) or sedentary (n=4) subjects, were anesthetized and the extracellular activity of small clusters of neurons was recorded with tungsten microelectrodes in the hind paw area of the primary somatosensory cortex (S1). The multiunit signal was amplified and fed to a monitor and audio systems while delimiting receptive field borders with light stimulation of the skin. At the end of the experiment all animals were perfused through the aorta with saline (0.9%) followed by aldehyde fixatives. Brains were removed, cut and stained with cresyl-violet, Wisteria floribunda histochemistry and immunolabeled for GFAP and Neu-N. Bi-dimensional reconstructions of the penetration series were done for each animal using anatomical landmarks in superimposed sections. There was no significant difference between the exercised and sedentary groups in terms of both the size of receptive fields (5513.3 ± 1556.6 vs 7242.3 ± 2578.5) and the total area of the hind paw representation (45087.0 ± 13370.2 vs 530803.8 ± 100299.1), both expressed in arbitrary units. However the areal measurements of the hind paw footprints in exercised (1.97 ± 0.17) rats were significantly smaller than in sedentary ones (2.23 ± 0.05 ; 2tail, T-test $p < 0.01$). These results could indicate that multiunit recordings may not have the adequate resolution to detect small differences in map changes or that the number of subjects were not sufficient to confirm the small tendency of the receptive field areas of the exercised subjects to be smaller than the sedentary group. In agreement with the electrophysiological recordings, however, we have found similar results in the quantitative analysis of the anatomical markers between the groups.

Palavras-Chave: Aging; Physical exercise; Neural plasticity.

Apoio Financeiro: CNPq, FINEP, CAPES, UFPE, UFPA, IBN-net

A BRAZILIAN HERBAL INHIBITS ACETYLCHOLINESTERASE IN MICE CORTEX, STRIATUM AND HIPPOCAMPUS: *IN VIVO* AND *IN VITRO* ANALYSES.

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Introduction: The decline in cholinergic neurotransmission is partially responsible for the cognitive deficits associated with normal aging and dementias like Alzheimer. The current pharmacotherapy for cognitive deficits, especially Alzheimer, still relies on the use of acetylcholinesterase inhibitors (AChEIs). We showed that a standardized ethanol extract of *Ptychopetalum olacoides* Benth (POEE), medicinal specie used in the Amazon, presents promnesic and anti-amnesic properties. Considering the dose- and time-dependent competitive/noncompetitive AChE inhibitory effect identified for POEE (*in vitro* and *ex vivo*), the purpose of this study was to further characterize this AChEI effect. **Material and Methods:** AChE G1 and G4 isoforms were isolated by centrifugation and activities determined by the Ellman's colorimetric method (1961), with [POEE] from 0- 1000µg/mL, in hippocampus (H), striatum (S) and frontal cortex (FC). Histochemistry was carried out according to Karnovsky and Roots (1964). Mice (n=5) were treated *per oz* with POEE (300 and 800mg/kg), saline or vehicle, and 90 min latter transcardially perfused (4% paraformaldehyde). Coronal brain slices (50µm) were collected in buffer. The optical density (OD) in H (CA1 and CA3), S, and lateral entorhinal cortex (LEC) was measured semi-quantitatively using a Nikon Eclipse microscope coupled to a high definition digital camera and the Image Pro Plus Software 6.0. ANOVA/Duncan. **Results:** POEE, significantly ($P<0.05$) inhibited G4 and G1 in H, S and FC, although effective concentrations varies. The histological analysis revealed that POEE 800 mg/kg significantly ($P<0.05$) reduced OD in H (CA1: 0.08 ± 0.01 ; CA3: 0.14 ± 0.01) and S (0.13 ± 0.01) as compared to controls CA1 (0.10 ± 0.01), CA3 (0.16 ± 0.01) and S (OD: 0.17 ± 0.01). **Conclusion:** These results corroborate the suggestion that POEE is an effective and orally bioavailable acetylcholinesterase inhibitor. An improvement in cholinergic function at cognition relevant areas so obtained is likely to underlie the promnesic results seen behaviorally.

Apoio Financeiro: CNPq Patent: PI0307647-4 (INPI/Br)

C.01.074

Effects of AL0608 ethanol extract on MPTP-induced tremors in mice.

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Introduction: AL0608 is an extract obtained from a medicinal plant used in the Brazilian Amazon, especially by the elders. One of the symptoms associated with the use of this species is tremors.

Objective: The purpose of this study was to examine the effects of AL0608 on a mice model of Parkinson disease.

Methods: Adult C57BL/6 mice were treated twice with MPTP (ip, 25mg/kg, 1h interval). Saline, DMSO 20%, AL0608 100mg/kg and apomorphine 1.5mg/kg were given (ip) 30min before each MPTP administration. Immediately after the second injection of MPTP, tremor was monitored for 45min, every 3min; tremor scores were attributed according to a pre-determined schedule (0-5). The maximum score was considered for each given period (**1**). Kruskal-Wallis/Mann-Whitney.

Results: MPTP induced tremors on saline 4.0 ± 0.0 (maximum at 12-18min) and DMSO 3.8 ± 0.0 (maximum at 18min) treated mice. AL0608 and APO significantly reduced ($p < 0.05$) tremors to 2.4 ± 0.3 (maximum at 9min) and 3.0 ± 0.3 (maximum at 12min), respectively. The duration of tremors was also significantly reduced ($p < 0.05$) to 39min for AL0608 and 42min for APO from over 45min observed for saline and DMSO.

Conclusions: The study suggests that AL0608 counteracts MPTP-induced tremors. Further studies are necessary to determine the relevance of using this extract in the management of this specific neurodegenerative disease.

Reference: 1-Behav. Brain Res. 163:159, 2005.

Palavras-Chave: Tremor, MPTP, Parkinson Disease.

Apoio Financeiro: CNPq

INTRANASAL ADMINISTRATION OF MPTP IN RODENTS CONFIRMS THE OLFACTORY VECTOR HYPOTHESIS OF PARKINSON'S DISEASE

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Objective: Environmental agents, including viruses, prions, and toxins, have been implicated in the cause of a number of neurodegenerative diseases. The presence of smell loss and the pathological involvement of the olfactory pathways in the early stages of Parkinson's diseases (PD) have led to the "olfactory vector hypothesis" that postulates that PD may be caused by agents that enter the brain via the olfactory mucosa. In the present study, we tested this hypothesis through the intranasal (i.n.) administration of the dopaminergic toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in rats. **Methods:** Adult male Wistar rats received a single i.n. administration of MPTP (1 mg/nostril) or saline (i.n.). The animals were submitted to a battery of behavioral paradigms including olfactory discrimination, social recognition, water maze, open field and activity chamber tasks. We also performed HPLC quantification of dopamine levels in several brain areas and immunohistochemical determination of enzyme tyrosine hydroxylase (TH) and the proteins associated to apoptotic cell death c-Jun N-terminal kinase (JNK) and caspase-3 in the olfactory bulb (OB) and substantia nigra (SN) of MPTP-treated rats. **Results:** The MPTP-treated rats suffered progressive impairments in olfactory (%cont:55), cognitive (%cont:65) and motor (%cont:85) functions. I.n. administration of MPTP reduced the levels of TH in the OB (%cont:45) and SN (%cont:55) of rats, resulting in a significant reduction of dopamine concentration in the OB (%cont:53), prefrontal cortex (%cont:65) and striatum (%cont:70), but not in the hippocampus (%cont:85). Moreover, MPTP-treated rats presented higher activation of JNK and caspase-3 in the OB (%cont JNK:750; caspase-3:145) and SN (%cont JNK:400; caspase-3:175). **Conclusion:** These results confirm the "olfactory vector hypothesis" indicating that the olfactory system represents a particularly sensitive route for the transport of neurotoxins into the brain that may be related to the etiology of PD. In addition, the time course of the olfactory, cognitive and motor impairments verified in rats treated intranasally with MPTP, which appears to be correlated with different stages of the human PD, and the activation of proteins associated to apoptotic cell death suggest that the MPTP intranasal model in rats may provide new insights into the underlying mechanisms of PD pathogenesis.

Palavras-Chave: Parkinson's disease, olfactory system, intranasal MPTP, rats

Apoio Financeiro: CNPq, FAPESC, CAPES

IDENTIFICATION OF A β -OLIGOMERS LIGANDS: IMPACTS ON ALZHEIMER'S DISEASE DIAGNOSTIC AND THERAPEUTICS

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder, neuropathologically characterized by the presence of amyloid plaques and by the build-up of soluble oligomers of the amyloid- β peptide (Ab) in the brains of affected individuals. Current evidence supports the notion that Ab oligomers play a major role in neuronal dysfunction and neurotoxicity in AD. However, the mechanisms involved in the deleterious actions of Ab remain to be fully elucidated and there is no method of early diagnostic nor an effective treatment against AD. In the present work, we propose to identify ligands for Ab oligomers using the phage-display library technique. We characterized four peptides that bind to Ab oligomers. Using Nuclear Magnetic Resonance and Fluorimetric assays we show that two of the selected peptides strongly bind to Ab oligomers and were able to block Ab fibrilization. In addition, all four biotinylated peptides were retained on a nitrocellulose membrane when incubated with Ab oligomers, but not with albumin. Finally, the selected peptides could detect low amounts of Ab oligomers in a ligand binding assay. Our results validate the binding between the four selected peptides and Ab oligomers and indicate that these peptides may be used in the development of diagnostic and/or therapeutic tools against AD.

Palavras-Chave: Alzheimers disease; Phage-Display; Abeta oligomers

Apoio Financeiro: FAPERJ

Efeitos do tratamento crônico com guanosina no modelo animal da Doença de Parkinson induzido por 6-OHDA.

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A Doença de Parkinson (DP) é uma doença multifatorial. Seu tratamento é apenas sintomático, o que salienta a importância do estudo de novas terapias. Há evidências que a excitotoxicidade causada pelo aumento da atividade glutamatérgica no corpo estriado seja uma das causas da DP. O nucleosídeo guanosina pode ser encontrado no meio extracelular e evidências apontam para um papel modulador de derivados da guanina (nucleotídeos de guanina e guanosina) sobre parâmetros glutamatérgicos, com possível efeito sobre aprendizado, memória e ansiedade, além de efeitos neuroprotetores. Derivados da guanina têm ainda funções tróficas importantes para desenvolvimento, estrutura e manutenção de células neurais. Quando administrada oralmente, a guanosina protege contra convulsões induzidas por ácido quinolínico, bem como protege fatias de hipocampo submetidas à combinação de hipóxia e hipoglicemia. **Objetivos:** investigar os efeitos do consumo crônico oral de guanosina no modelo animal da DP induzido por 6-hidroxidopamina (6-OHDA). **Métodos:** ratos Wistar machos adultos receberam solução de guanosina 0,5mg/l (n=20) ou água destilada (n=16) para beber durante 4 semanas. Após as duas primeiras semanas de tratamento, receberam infusão de 6-OHDA (5,5 µl, 3 µg/µl) no feixe prosencefálico medial direito. Após 2 semanas do término do tratamento, os animais foram submetidos ao Teste de Motricidade sobre Grade [TMG (*Footfault Test*)] que teve duração de 3 minutos. Imunohistoquímica para tirosina hidroxilase (TH) em células nigrais também foi realizada. **Resultados:** Treze de 16 animais do grupo controle apresentaram comportamento rotacional no TMG, o que diferiu do grupo guanosina, onde apenas 9 de 20 animais mostraram o mesmo comportamento (Teste Exato de Fisher, $P=0,041$). Não houve diferença no nível de TH nigral entre os grupos (teste t para amostras independentes, $p=0,615$; controle, $n=3$; 78 ± 8 , média $\pm$ E.P.; tratado, $n=5$; 70 ± 11). **Conclusões:** a guanosina reverte a expressão comportamental no TMG induzida pelo modelo, mas não altera os níveis de TH nigral. **Referências:** Pharmacol Ther. 3:401, 2007; Br J Pharmacol. 8:963, 2007; Neurochem Int. 51:333, 2007; Mol Neurobiol. 12:73, 1996.

Palavras-Chave: Doença de Parkinson, 6-OHDA, guanosina, footfault

Apoio Financeiro: PRONEX, CNPq, Rede Instituto Brasileiro de Neurociências (IBN - Net) - FINEP

Comparação entre os Peptídeos Beta-Amilóide $A\beta_{1-40}$ e $A\beta_{25-35}$ em Camundongos: Avaliação de Alterações Cognitivas, Captação de Glutamato e Viabilidade Neuronal

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Objetivos: Investigar possíveis alterações na modulação da transmissão glutamatérgica e viabilidade celular em fatias de hipocampo de camundongos induzidos a déficit de memória espacial pela infusão intracerebroventricular (i.c.v.) dos peptídeos beta-amilóide $A\beta_{1-40}$ e $A\beta_{25-35}$, principais componentes das placas senis nos pacientes com a doença de Alzheimer. **Métodos:** Camundongos Suíços machos adultos receberam infusão i.c.v. de salina (controle) e dos peptídeos $A\beta_{1-40}$ agregado (400pmol-3ul/animal) e $A\beta_{25-35}$ solúvel (10nmol-3ul/animal). Quatorze dias pós-tratamento os animais foram submetidos a uma versão espacial do teste do labirinto aquático de Morris. No dia posterior, os animais foram sacrificados e seus hipocampus removidos para obtenção das fatias hipocampus. A captação de glutamato foi realizada incubando-se as fatias com 3μCi/ml de [³H]glutamato e a radioatividade quantificada por cintilação líquida. A viabilidade celular foi avaliada pela redução do MTT (0,5mg/ml) e pela captação de IP (iodeto de propídeo, 7μg/ml). A captação do IP foi observada em microscópio de epifluorescência invertido e quantificada com o software Scion Image beta-3. **Resultados:** A infusão i.c.v. de $A\beta_{1-40}$ ou $A\beta_{25-35}$ nos camundongos resultou em déficit de aprendizado e memória [%Tempo quadrante correto: C:36,17±4,22; $A\beta_{1-40}$:17,41±2,94; $A\beta_{25-35}$:23,33±2,7; P<0,05]. Além do prejuízo cognitivo, observamos uma diminuição da captação de glutamato tanto em animais que receberam $A\beta_{1-40}$ (nmol/mg prot/min)(C:0,228±0,020; $A\beta_{25-35}$:0,152±0,011; P<0,05) quanto $A\beta_{25-35}$ (C:0,244±0,021; $A\beta_{25-35}$:0,185±0,012; P<0,05). A infusão de $A\beta_{1-40}$ mostrou redução do metabolismo do MTT (em D.O.)(C:1,962±0,076; $A\beta_{1-40}$:1,657±0,084; P<0,05), e aumento da captação de IP (C:1,900±0,215; $A\beta_{1-40}$:2,608±0,159; P<0,05). Entretanto os animais infundidos com $A\beta_{25-35}$ não apresentaram alterações relativas à viabilidade celular. **Conclusão:** Nossos resultados demonstram que os déficits cognitivos induzidos pela administração i.c.v. dos peptídeos beta-amilóide $A\beta_{1-40}$ e $A\beta_{25-35}$ em camundongos são acompanhados pela alteração da neurotransmissão glutamatérgica, apesar dos distintos padrões de alteração na viabilidade celular hipocampal. **Palavras-Chave:** Peptídeo beta-amilóide, glutamato, viabilidade celular

Apoio Financeiro: CAPES, CNPq, FAPESC, IBN(Net) # 01.06.0842-00.

ANÁLISE POLIMÓRFICA DOS GENES APOLIPOPROTEÍNA-E E GLUTATIONA S-TRANSFERASE EM PARKINSONIANOS NA CIDADE DE BELÉM-PA: DADOS PRELIMINARES.

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Objetivos: Neste estudo apresentamos dados preliminares sobre o polimorfismo genético dos genes apolipoproteína E (APOE) e glutatona S-transferase (GST) entre indivíduos portadores da doença de Parkinson (DP) e um grupo controle (CTRL), residentes na cidade de Belém-PA. Nosso objetivo consiste na investigação da ocorrência de associações entre estas formas alélicas e a prevalência da doença de Parkinson.

Métodos: Coleta de sangue por punção venosa, seguida de extração fenólica de DNA. Utilizou-se o procedimento de genotipagem por RFLP/PCR (1) (DP, n=52; CTRL, n=41), devidamente pareados. Após a tabulação dos resultados, foram feitas as comparações entre os diferentes valores observados através do software BioEstat 5.0.

Resultados: A genotipagem para a APOE mostrou o alelo $\epsilon 3$ como o mais freqüente nos dois grupos (65% DP; 48% CTRL). O genótipo $\epsilon 3\epsilon 3$ foi mais comum entre o grupo DP (40,4%) e o $\epsilon 3\epsilon 4$ no grupo CTRL (53,7%). As análises de significância demonstraram haver diferenças consideráveis entre as amostras investigadas ($\chi^2=20,902$; $p=0,0003$; CI=95%). Nas análises da família gênica da GST foram investigados os alelos M1, P1 e T1. Para os sistemas GSTM1 e T1 o polimorfismo está na presença (+) ou ausência (-) da forma alélica, condicionando um fenótipo de metabolizador lento. As freqüências para GSTM1 foram de 47,1% (+) e 52,9% (-) para os portadores de DP e, 56,1% (+) e 43,9% (-) entre o grupo controle. Os padrões encontrados para o alelo GSTT1 foram 74,1% (+) e 25,9% (-), entre os portadores, e 85,4% (+) e 14,6% (-) entre os controles. Para o gene GSTP1 a forma A foi a mais comum (58% DP; 66% CTRL), e entre os genótipos as maiores freqüências foram para as formas AB (44,4% DP) e AA (48,8% CTRL).

Conclusão: Os resultados obtidos para o gene APOE sugerem a associação diferenciada do alelo $\epsilon 3$ com uma prevalência maior entre os indivíduos portadores da DP em comparação aos indivíduos do grupo controle. Apesar dos diferentes valores de freqüências encontradas entre os alelos GST, não foram observadas, considerando-se nossa amostra limitada, associações significativas entre as formas genotípicas investigadas e a prevalência da DP.

Referência: 1- Mutat Res. 288:113, 1993

Palavras-Chave: doença de Parkinson - polimorfismo - APOE - GST

Apoio Financeiro: SEDECT-PA / FINEP

Co-ocorrência das doenças de Alzheimer e Parkinson em um indivíduo brasileiro portador da mutação p.G2019S no gene *LRRK2*

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OBJETIVOS: Em diferentes populações, mutações no gene *Leucine-rich repeat kinase 2 (LRRK2)* têm sido consideradas o maior fator de risco genético associado tanto à forma familiar como esporádica da doença de Parkinson (DP). A multifuncionalidade deste gene, assim como, as características pleiomórficas associadas às suas mutações sugerem que o *LRRK2* pode estar potencialmente envolvido em outras doenças neurodegenerativas além da DP, principalmente a doença de Alzheimer (DA). Neste trabalho, descrevemos os achados genéticos e clínicos em um indivíduo do sexo masculino (86 anos) que apresenta DA esporádica de aparecimento tardio concomitantemente a sinais de parkinsonismo.

METODOLOGIA: O propósito exibiu uma história de perda de memória progressiva de curto e longo prazo, além de exibir um tremor de repouso brando unilateral. Os critérios DSM-IV e NINCDS-ADRDA foram utilizados para avaliação da DA, enquanto que a escala de Hoehn & Yahr foi utilizada para classificação dos sinais de parkinsonismo. Para a análise do gene *LRRK2*, o DNA do paciente foi extraído a partir do sangue periférico e a análise da mutação c.6055G>A (p.G2019S), a mais frequentemente encontrada no gene *LRRK2*, foi realizada através da técnica de PCR-RFLP, seguida da visualização dos produtos amplificados por eletroforese em géis de poliacrilamida.

RESULTADOS: O paciente foi diagnosticado como provável portador da DA através dos critérios descritos e os testes neurológicos demonstraram deterioração lenta das funções motoras, com sinais de parkinsonismo moderados, evidenciados principalmente por bradicinesia e rigidez assimétricas, sendo classificados como estágio 3 na escala de Hoehn & Yahr. A mutação p.G2019S foi encontrada em heterozigose no probando.

CONCLUSÃO: Embora a ocorrência simultânea da DP e DA seja ocasionalmente esperada, os mecanismos de susceptibilidade comum entre estas duas doenças permanecem desconhecidos. Recentes avanços a despeito do amplo papel do gene *LRRK2* tanto em condições normais como patológicas nos permitem especular que mutações neste gene podem atuar não apenas na etiologia da DP, mas em diferentes desordens envolvendo a alfa-sinucleína e a proteína tau, incluindo a DA. **Palavras-**

Chave: LRRK2, doença de Alzheimer, doença de Parkinson

Apoio Financeiro: CNPq/FAPERJ/PPSUS-MS/CEPUERJ.

C.01.081

Restrição calórica não tem efeito neuroprotetor ao longo do envelhecimento em ratas Donryu.

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Objectives

O nosso objetivo foi avaliar a densidade neuronal no córtex cerebral e no hipocampo, ao longo do envelhecimento e os efeitos da restrição calórica (RC) sobre este parâmetro. Ademais foram avaliados peso corpóreo e encefálico.

Methods

Foram utilizadas 50 ratas Donryu fêmeas (162-639g) divididas em 2 grupos: 1- animais com dieta *Ad libitum* (AL); grupo 2: animais submetidos a RC, estes foram subdivididos em 5 subgrupos (3, 6, 12, 18 e 24 meses).

Os animais foram anestesiados, eutanasiados, seu encéfalo foi retirado, pesado, fixado, seccionado em micrótomo e corado (Nissl). A densidade neuronal foi estimada pela técnica do disector óptico.

Os dados foram expressos em percentagem do controle (devido ao encolhimento tecidual). Quando as diferentes idades eram comparadas entre si utilizava-se o grupo 3M como 100%; quando eram comparados os diferentes tratamentos em animais de mesma idade, os grupos AL eram considerados 100%. A análise estatística foi realizada por ANOVA-Tukey ($p < 0.05$) (GraphPad Prism 4).

O presente trabalho foi aprovado pelo Comitê de Ética em Pesquisa da PUCRS.

Results

Observamos um aumento significativo de peso corpóreo nos animais com dieta AL a partir dos 6 meses de idade ($p < 0.01$). Nos animais submetidos ao protocolo de RC não há nenhuma alteração significativa de peso corpóreo nas diferentes idades analisadas. O peso encefálico se manteve inalterado nos diferentes grupos experimentais. Nos animais 24M foi registrada uma diminuição de densidade neuronal: 1- No córtex cerebral nos animais com dieta AL (72.5%) e nos animais com RC (75.9%); 2- Na região CA1 do hipocampo nos animais AL (41.6%) e nos animais com RC (36%); 3- Na região CA3 do hipocampo nos animais AL (34%) e no RC (24.1%) ($p < 0.01$). No giro denteado não houve perda neuronal.

Conclusions

Observamos uma redução de densidade neuronal ao longo do envelhecimento, esta não foi atenuada pelo protocolo de RC indicando que a RC não tem efeito neuroprotetor ao longo do envelhecimento. A constância do peso encefálico indica que a perda neuronal é seletiva e não afeta de forma significativa o volume encefálico ou que a proliferação glial supre a perda neuronal.

Palavras-Chave: restrição calórica, envelhecimento, perda neuronal

Apoio Financeiro: CNPq; FAPERGS, PUCRS

Sistema diagnóstico de la demencia tipo alzheimer basado en el análisis de comportamientos, funcionalidad de reconocimientos sensoriales e identificación y contexto del disparador del proceso
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Objetivos: evaluar un sistema de diagnóstico para demencia tipo alzheimer y su poder de discriminación de diferentes situaciones. **Métodos:** a partir de la teoría que explica el proceso como combinación de factores estructurales psicológicos, comportamientos psicosociales previos que se continúan hasta el momento del diagnóstico, disfuncionalidad de reconocimientos sensoriales, alteración eventual o sostenida de la atención de rastreo y focalizada que emerge como alteración de la memoria, identificación del disparador que desencadena la enajenación de la realidad actual, la traslocación hacia un pasado infantil idílico que elude las pérdidas penosas con incapacidad de afrontamiento, y eventualmente la fijación de la atención en el deseo de muerte. **Resultados:** el protocolo permite diferenciar el envejecimiento normal, el envejecimiento con rechazo de un pasado de sumisión, el proceso demencial no combinado con envejecimiento, el proceso demencial combinado con envejecimiento, y el de adultos muy mayores. El protocolo fue aplicado a 158 casos diagnosticados y medicados como alzheimer por especialistas médicos, que fueron seguidos durante 18 meses o hasta la rehabilitación en los casos ratificados como alzheimer que pudieron ser abordados. 98 (62%) casos del total de casos abordados inicialmente correspondieron a envejecimientos normales, y de ellos 17 (17%) fueron envejecimientos con rebelión de la condición sumisa (especiales), 3 de ellos hombres. De los 60 casos ratificados como con demencia tipo alzheimer, 14 (9 % del total, 23,3 % de los casos efectivos) no presentaron combinación con comportamientos de envejecimiento normal o especial, todos ellos menores de 58 años, 42 (70 % de los casos efectivos) (edades de 58 a 76 años) y 4 (6,7%) a mayores de 79 años. En éstos últimos no se presentó un disparador del proceso que remita a una pérdida penosa sin capacidad de afrontamiento, a diferencia de los anteriores. **Conclusiones:** la aplicación de este sistema de diagnóstico por historia de vida, comportamientos y reconocimientos sensoriales permite vislumbrar situaciones diferentes consideradas en general equivalentes. La aparente pérdida de la memoria reciente por alteración de la atención es el factor de confusión más frecuente.

Palavras-Chave: Palabras clave: alzheimer, diagnostico, envejecimiento normal, envejecimiento y rebelión, demencia y envejecimiento, demencia en adultos muy mayores.

Apoio Financeiro: Universidad Autónoma de Entre Ríos, BBVA Banco Francés, Fundación Alzheimer

C.01.083

Oxidative damage in skeletal muscle mitochondria from ALS animal models.

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Mitochondrial dysfunction and oxidative stress contribute to motor neuron degeneration in amyotrophic lateral sclerosis (ALS). Motor neurons innervate skeletal muscle and while alterations in mitochondrial activity in the central nervous system have been described, data on mitochondrial function in skeletal muscle is scarce and controversial.

OBJECTIVES: To investigate oxidative damage and mitochondrial dysfunction in skeletal muscle in SOD^{G93A} rats.

METHODS: We investigated the respiratory activity of isolated mitochondria as well as biopsies obtained from early symptomatic SOD^{G93A} rat skeletal muscle, injected with the spin trap DMPO or vehicle. DMPO-protein adducts detection was used as footprint of oxidative damage.

RESULTS: Mitochondrial respiratory activity was reduced in SOD^{G93A} skeletal muscle compared to non-transgenic littermates. Pretreatment with DMPO (48 and 24 hour i/p 200mg/kg) reestablished mitochondrial respiration and lead us to detect DMPO-protein adducts in protein samples.

CONCLUSIONS: Our data show that mitochondrial dysfunction is an early event in skeletal muscles of SOD^{G93A} rat and is associated with oxidative stress damage as detected by the formation of DMPO protein adducts.

Palavras-Chave: oxidative stress, muscle, Amyotrophic Lateral Sclerosis

Genetic deletion of cellular prion protein (PrP^C) confers increased susceptibility to age-related behavioral and neurochemical deficits.

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Objective: The cellular prion protein (PrP^C) is a neuronal anchored glycoprotein that has been associated with distinct functions in the CNS, such as neuroplasticity and formation of senile plaques. The objective of the present study was to determine the role of PrP^C in the behavioral and neurochemical deficits observed during aging in mice.

Methods: Wild-type (*Prnp*^{+/+}) and PrP^C knockout mice (*Prnp*^{0/0}) with 3 or 11 months-old were submitted to a battery of behavioral tasks including the open field, olfactory discrimination, social recognition and inhibitory avoidance tasks. We also performed quantification of seric acetylcholinesterase levels and immunohistochemical determination of the hippocampal proteins associated to apoptotic cell death and synaptic changes, respectively, caspase-3 and synaptophysin.

Results: No significant inter-strain differences were observed in olfactory discrimination ability. However, age-related deficits in the locomotor activity were observed in *Prnp*^{0/0} mice [crossings; *Prnp*^{+/+} 3mo: 47.06±9.37; *Prnp*^{+/+} 11mo: 48.53±7.28; *Prnp*^{0/0} 3mo: 32.00±7.62; *Prnp*^{0/0} 11mo: 18.60±8.61]. The absence of PrP^C also disrupted the short-term social recognition memory [investigation time (s); *Prnp*^{+/+} 3mo: 90.9±12.29; *Prnp*^{+/+} 11mo: 42.15±6.89; *Prnp*^{0/0} 3mo: 94.9±12.78; *Prnp*^{0/0} 11mo: 104.9±14.69] and the long-term memory [latency to step-down (s): *Prnp*^{+/+} 3mo: 180; 115.5/180; *Prnp*^{+/+} 11mo: 144.5; 58/180; *Prnp*^{0/0} 3mo: 41.5; 28/65; *Prnp*^{0/0} 11mo: 33.0; 22/56]. Moreover, aging and PrP^C deletion increased seric acetylcholinesterase levels [AChE (mU/mL); *Prnp*^{+/+} 3mo: 2.51±0.21; *Prnp*^{+/+} 11mo: 3.25±0.39; *Prnp*^{0/0} 3mo: 3.21±0.27; *Prnp*^{0/0} 11mo: 2.31±0.19]. No significant differences were observed in synaptophysin levels whereas 11 mo mice from both strains presented higher activation of caspase-3 [%stained-cells; *Prnp*^{+/+} 3mo: 2.03±0.8; *Prnp*^{+/+} 11mo: 22.84±3.97; *Prnp*^{0/0} 3mo: 2.17±1.31; *Prnp*^{0/0} 11mo: 13.81±0.2].

Conclusion: The present results demonstrate an increased susceptibility of PrP^C null mice to age-related behavioral and neurochemical alterations. These findings suggest PrP^C as potential target to counteract neurological disorders associated to aging.

Palavras-Chave: Cellular Prion Protein, Aging

Apoio Financeiro: CNPq, CAPES, FAPESC.

DOENÇA DE ALZHEIMER EM MÃES JOVENS DE INDIVÍDUOS COM SÍNDROME DE DOWN: ANÁLISE DOS GENES *APOE* E *PSEN1*

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OBJETIVOS: O desenvolvimento de neuropatologias características da doença de Alzheimer (DA) em portadores de síndrome de Down (SD) a partir dos 40 anos de idade e a alta taxa de SD em famílias nas quais a DA está presente sugerem uma susceptibilidade comum entre as duas doenças. Além disso, mulheres jovens que tiveram filhos com a SD têm um risco 5 vezes maior de desenvolverem DA. Esta susceptibilidade compartilhada poderia envolver um processo de envelhecimento acelerado, que levaria à SD em filhos de mães jovens e a um risco aumentado de demência nestas mães. Desta forma, este trabalho teve como objetivo analisar a frequência do alelo e4 no gene *APOE* e do polimorfismo -48C>T no gene *PSEN1*, envolvidos direta ou indiretamente na deposição cerebral de beta amiloide, em mães jovens (<35 anos na concepção) de portadores da SD, em comparação com mães de indivíduos cromossomicamente normais.

METODOLOGIA: Amostras de DNA foram extraídas de 106 mães de portadores da trissomia livre do cromossomo 21 e de 111 mães controle de mesma faixa etária. A genotipagem foi realizada pela técnica de PCR-RFLP, seguida da visualização dos produtos amplificados a partir de eletroforese em géis de poliacrilamida corados com prata.

RESULTADOS: A análise estatística através do teste exato de Fisher revelou que não houve diferenças significativas entre as distribuições genotípicas observadas nas amostras caso e controle tanto para os alelos do gene *APOE*, quanto para o polimorfismo *PSEN1*-48C>T ($\chi^2 = 3,07$; g.l. = 4; P = 0,54; $\chi^2 = 0,9$, g.l. = 2, P = 0,6, respectivamente). O mesmo resultado foi encontrado na interação gênica multiplicativa entre os alelos dos genes *APOE* e *PSEN1*.

CONCLUSÕES: No Brasil, este é o primeiro relato da investigação destes genes entre as mães de indivíduos com SD. Nossos resultados sugerem que os alelos *APOE* e4 e *PSEN1*-48 T não são fatores de risco independente para a DA nestas mães. Entretanto, em virtude da complexidade tanto da SD como da DA, a análise de variantes em outros genes é de fundamental importância para melhor elucidar a o aparecimento insidioso de DA em mães jovens que tiveram filhos com SD.

Palavras-Chave: APOE, PSEN1, síndrome de Down, doença de Alzheimer

Apoio Financeiro: FAPERJ/ CEPUERJ/CAPES.

Lack of association between PSA-NCAM expression and migration in the rostral migratory stream of a Huntington's disease transgenic mouse model.

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Huntington's disease is a neurodegenerative autosomal disorder characterized by selective loss of striatal and cortical neurons. The mammalian brain subventricular zone contains a population of neural precursors involved in postnatal neurogenesis. These newly generated cells migrate from the subventricular zone along the rostral migratory stream and differentiate into mature olfactory bulb neurons throughout adulthood. The establishment of this pathway depends upon a variety of molecules, including polysialylated neural cell adhesion molecule (PSA-NCAM). We used a murine model of Huntington's disease, the R6/2 transgenic mouse, and *in vivo* bromodeoxyuridine administration to label cells undergoing proliferation and to follow their migration along the rostral migratory stream. Bromodeoxyuridine labeling did not show any significant increase in proliferation of progenitor cells in symptomatic R6/2 mice, but migration of neuroblasts along the rostral migratory stream was significantly diminished ($P < 0.05$). The decrease in neuroblast migration was not due to an alteration in the expression of PSA-NCAM along the rostral migratory stream since immunohistochemical analysis showed no significant differences between R6/2 ($1.29 \pm 3.1 \times 10^6$ cells/mm³) and wild type mice ($1.34 \pm 2.5 \times 10^6$ cells/mm³). In addition, we used Fluoro-Jade C to evaluate apoptosis and demonstrated that the number of apoptotic cells in the rostral migratory stream is similar in affected ($1.36 \pm 0.04 \times 10^5$ cells/mm³) and wild type ($1.34 \pm 0.03 \times 10^5$ cells/mm³) animals suggesting that cell death is not responsible for the differences observed in neuroblast migration. We conclude that in the R6/2 mice progenitor cells have an impaired migration in their route to the olfactory bulb, with accumulation of cells in the caudal rostral migratory stream that does not result from changes in PSA-NCAM expression and/or cell death.

Palavras-Chave: Migration, PSA-NCAM, rostral migratory stream, Huntington's disease, R6/2

Apoio Financeiro: CNPq

Gene expression changes in human brain slices treated with beta-amyloid peptide oligomers: possible implications for the pathogenesis of Alzheimer's disease

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Objectives: Alzheimer's disease (AD) is a worldwide, highly prevalent neuropathology in elderly people characterized by progressive memory loss. Cognitive decline in AD appears to be caused by the neurotoxic impact of oligomeric forms of the b-amyloid peptide (Ab), a product of the proteolytic cleavage of the amyloid precursor protein (APP). However, the molecular events and cellular mechanisms that trigger the gain of toxic function of this peptide are incompletely understood. In this work, we have investigated alterations of global gene expression induced by soluble oligomers of Ab in organotypic cultures of adult human brain cortex.

Methods: Human brain slice were treated with abeta oligomers 500 nM for 12 h, and global gene expression profiles were accessed by DNA microarray using a 4.8K universal human chip. Validation experiments using quantitative RT-PCR are currently being carried out to confirm the gene expression alterations identified in the microarray analysis.

Results: Analysis of the results obtained with three different human samples revealed a set of 27 genes that are differentially expressed after Ab oligomers treatment. Most of these genes (70%) were down-regulated, suggesting a major inhibitory effect of Ab oligomers on neuronal gene expression. Functional classification of the genes with differential expression indicates that important neuronal signalling pathways, such as the axon guidance and intracellular transport of vesicles are affected by Ab oligomers.

Conclusion: Once validated, we expect that our results may reveal novel neuronal pathways affected by Ab oligomers and may also suggest pharmacological targets for the development of novel therapeutic approaches in AD.

Palavras-Chave: Abeta oligomers, Alzheimer's disease, DNA microarray, gene expression, neurotoxicity

Apoio Financeiro: HHMI, CNPq, Faperj

Aged murine model of prion disease: impact of enriched environment on early behavioral changes

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Behavioral and neuropathological changes in prion diseases (PD) have been studied in details. However, possible impacts of aging and environmental enrichment (EE) on prion disease progression have not been investigated. We investigated the temporal course of behavioral changes in murine prion disease induced by ME7-infected brain homogenate, in comparison with normal brain homogenate (NBH). Albino Swiss female mice, 22 months old were submitted to enrichment either for short (2 weeks-EES) or long (8 weeks-EEL) periods and injected with ME7 or NBH homogenates. The behavioral impairments induced by prion disease in aged mice were compared with our previous results on young mice. Subjects were grouped as follows: ME7, n=6; NBH, n=9; ME7EES, n=10; NBHEES, n=8; ME7EEL, n=8; NBHEEL, n=8. At the week post injection (p.i.) all subjects were submitted to behavioral tests as follows: water maze (WM), burrowing (B) and open field (OF). WM and OF were analyzed with ANY-maze tracking system (Stölting Science). All subjects learned and remembered the position of the hidden platform in the WM after 5 training days. Probe trials to investigate spatial memory retention at 4th week p.i. revealed that only ME7-injected animals increased swam total distance to find the platform. Burrowing activity decreased from 3rd (198 ± 14.39) to 7th (153.77 ± 15.38) week p.i. only in the ME7 group and remained as such until the day of sacrifice in the 9th week (ANOVA, $p < 0.05$). ME7 and ME7EES decreased OF activity in the 5th week p.i. until the 9th week p.i. (ANOVA, $p < 0.05$). All others EE and NBH groups did not present any change in B and OF activities. Our present and previous results indicate that aged albino Swiss mice exhibit a shorter incubation period as compared with young mice in which the first symptoms (reduction in burrowing activity) start at 16th week p.i. The environmental enrichment seems to protect mice against PD symptoms at least until the 9th week p.i. How long neuroprotection will remain and which mechanisms are involved are open questions that are currently being investigated both at cellular and molecular levels.

Palavras-Chave: aging, prion, enriched environment, behavior, mice

Apoio Financeiro: CNPq, CAPES, UFPA, FINEP/IBN-Net.

Object recognition, CA3 perineuronal nets and neuronal number of hilar region of dentate gyrus changes in C57BL/6 aged mice

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The integrity of perineuronal (PN) nets and neurons seem to be essential for synaptic plasticity in the hippocampal network. The aim of the present report is to estimate the number CA3 PN nets and neurons of hilar region to investigate possible correlations with cognitive performances of aged and young mice. Six months old (young Y, n=4) and twenty months old (aged A n= 4) were housed in standard cages with free access to food and water. All subjects were assessed with non-match-to-sample, -place and -temporal recognition tasks and sacrificed. Video recorded tests were analyzed with ANY-maze tracking system (Stoelting). After perfusion with aldehyde fixatives, brain was removed, cut and processed for *Wisteria Floribunda* histochemistry and immunolabeled for NeuN (*neuronal nuclei*). PN nets and neuronal numbers were estimated by optical fractionator (Stereoinvestigator® MicroBrightField, Willston, VT, USA). Hippocampal fields were conspicuously delimited by lectin labeling. Oriens, pyramidal and stratum lucidum layers of CA3 present two dense PN net populations of lectin labeled cells: Type I and Type II. Type I present soma, proximal and distal labeled dendrites and Type II only somatic PN nets with very short and faint primary dendrites. The number of both types of cells were estimated and total numbers were similar in both groups (A=1133.33±71.65; Y=943.69±148.12 - ANOVA p>0.05) but the proportion and absolute numbers between Type I and Type II PN nets changed significantly: Type II estimation in aged mice was much higher (716.97±94.75) than Type I (396.37±44.47 - ANOVA p<0.025) whereas no difference was found in young mice (Type I=529.87±94.07; Type II=413.82±80.56; ANOVA p>0.05). A significant reduction of the total neuronal number of hilar region was detected in aged (8206.62±712.99) as compared with young mice (13569.09±943.68, 2-tail T-Student test p=0.0039). Aged mice presented lower performances than young ones in all tests: they were not able to recognize spatial, temporal and object identities. Since the integrity of PN nets and neuronal number are essential for synaptic plasticity we suggest that aged changes may be associated with cognitive decline. **Palavras-Chave:** aging, Object recognition, perineuronal nets, neuronal number, hippocampo, mice

Apoio Financeiro: CNPq, CAPES, UFPA, FINEP/IBN-Net

C.01.090

Avaliação funcional da redução da transmissão colinérgica sobre o envelhecimento

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Introdução: além de estar relacionada a processos fisiológicos, a transmissão colinérgica torna-se deficiente na doença de Alzheimer ¹. Para avaliar as consequências da redução da transmissão colinérgica, foi criado um modelo geneticamente modificado, cuja expressão do transportador vesicular de acetilcolina (VACHT) foi reduzida, resultando na diminuição do armazenamento de acetilcolina nas vesículas sinápticas ². A redução do transportador afetou o desempenho dos animais em testes motores e cognitivos no início da vida adulta.

Objetivo: avaliar os efeitos dessa redução da transmissão colinérgica sobre o envelhecimento.

Métodos: camundongos VACHT Knock-down heterozigotos, nas idades médias de 486, 581 e 682 dias, tiveram suas performances comparadas a animais selvagens da mesma linhagem e idades, em testes de RotaRod, WireHang e GripForce, habituação em campo aberto, reconhecimento de objeto, reconhecimento social e esQUIVA inibitória.

Resultados: a avaliação motora revelou mesmo efeito da progressão da idade sobre o desempenho dos dois genótipos, de modo que ambos apresentaram baixo rendimento com grande variabilidade.

Da mesma forma, ambos os grupos mostraram desempenhos semelhantes nos testes cognitivos. Em, praticamente, todos os testes realizados, tanto em versões referentes à memória de curto como de longo prazo, não houve efeito da idade, nem do genótipo e tampouco uma interação entre esses fatores. Os animais VACHT Knock-down apresentaram limitada capacidade mnemônica, assim como demonstrado anteriormente em heterozigotos mais jovens ². No entanto, os animais selvagens também mostraram desempenho ínfimo nesses testes. Apenas na versão de curto prazo do teste de esQUIVA inibitória, os animais transgênicos de 486 dias apresentaram melhor desempenho que os selvagens ($p=0,044$), determinando um efeito da idade sobre o genótipo ($p=0,011$).

Conclusão: ao envelhecer, os animais selvagens atingiram a mesma capacidade mnemônica que os transgênicos apresentavam no início da vida adulta. Desta forma, a redução da expressão de VACHT pode ser, potencialmente, um modelo de envelhecimento precoce.

Bibliografia: 1 - Learn. Mem. 11, 2004. 2 - Neuron, 51:5, 2006

Palavras-Chave: Acetilcolina, envelhecimento, memória

Apoio Financeiro: CNPq e FAPESP

EFFECT OF HOMOCYSTEINE ON THE IN VITRO PHOSPHORYLATION OF INTERMEDIATE FILAMENTS FROM HIPPOCAMPUS OF RATS DURING DEVELOPMENT

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Objectives: The amino acid homocysteine is an important factor of risk for several neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Increased homocysteine levels induce neuronal cell death in a variety of neural cell types. However, the effects of homocysteine in neural cytoskeleton remain to be demonstrated. In this study we investigated the effects of Hcy on the *in vitro* phosphorylation of the medium and low molecular weight neurofilament subunits (NF-M and NF-L) and glial fibrillary acidic protein (GFAP), neuronal and glial intermediate filaments, respectively, in slices of hippocampus of young rats of.

Methods: Tissue slices of 9, 12, 17, 21 and 29-day-old rats were incubated with ³²P orthophosphate with or without addition of 500 mM Hcy. The intermediate filament enriched cytoskeletal fraction was isolated and the radioactivity incorporated into the NF-M, NF-L and GFAP was measured. In some experiments we used phosphatase inhibitors as follows: okadaic acid (OA) was added at the concentrations described to inhibit protein phosphatase 2A (PP2A), protein phosphatase 1 (PP1) or PP2B respectively (0.05, 0.5, or 5 mM) and 100 mM FK 506 an immunosuppressant described to inhibit PP2B.

Results: When hippocampus slices were incubated in the presence of 500 mM Hcy we observed a significantly decreased *in vitro* ³²P incorporation into NF-M, NF-L and GFAP from 17-day-old rats. This effect was prevented by 0.05, 0.5 and 5 mM OA and FK 506.

Conclusions: Hcy induced *in vitro* hypophosphorylation of neuronal and glial cytoskeletal proteins, NF-M, NF-L and GFAP, respectively, in hippocampus slices of 17-day-old rats and this effect was mediated by the protein phosphatases 1, 2A and B (PP1, PP2A and PP2B). We propose that the alteration of cytoskeletal-associated phosphorylating system induced by Hcy is a developmentally regulated process and it may be related to the neurological dysfunction characteristic of homocysteinemia.

Palavras-Chave: homocysteine, phosphorylation, cytoskeleton

Apoio Financeiro: : CNPq, CAPES, FAPERGS, PROPESq-UFRGS.

Decorin and IGF-R1 expression in astrocytes expressing the ALS-linked SOD1 mutation

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Astrocytes plays a role in the progressive motor neuron degeneration observed in Amyotrophic lateral sclerosis (ALS). Mutations in the Cu/Zn superoxide dismutase (SOD1) are found in about 20% of patients with familial ALS. We have previously found that SOD1G93A-expressing astrocytes induced apoptosis of co-cultured motor neurons instead of supporting survival. This prompted us to investigate whether the effect on motor neuron survival was related to a change in the gene expression profile.

Through high-density oligonucleotide microarrays, we found changes in the expresión of genes involved in transcription, signaling, cell proliferation, extracellular matrix synthesis, response to stress and steroid and lipid metabolism. The most up-regulated gene was decorin (*Dcn*), a small multifunctional extracellular proteoglycan. Downregulated genes included the insulin-like growth factor-1 receptor (*Igf-1r*) and the RNA binding protein ROD1. We confirmed the up-regulation of decorin in SOD1G93A astrocytes at messenger (RT-PCR) and protein (Western Blotting) level. Changes in the mRNA expression of *Dcn* and *Igf-1r* were also found in the spinal cord of asymptomatic animals, suggesting these changes occur before overt neuronal degeneration and potentially influence astrocyte-motor neuron interaction in the course of the disease. Western blotting analysis showed that whereas the increase in decorin messenger correlated with an increase in protein content in the spinal cord, no apparent change in IGF-1R protein was observed. Changes in the expression of extracellular matrix-related genes in CNS affect several biological processes including proliferation, neuronal migration and axon guidance. Thus, up-regulation of decorin may influence matrix remodeling, glial reactivity and inflammation associated to neurodegeneration occurring in ALS.

Palavras-Chave: Astrocytes, ALS, Decorin, IGF-1R.

DOENÇA DE ALZHEIMER: POLIMORFISMO 844INS68 NO GENE DA CISTATIONINA BETA-SINTASE E SUA RELAÇÃO COM OS NÍVEIS DE HOMOCISTEÍNA

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Objetivos: Altos níveis de homocisteína (Hcy) podem estar associados a polimorfismos em genes que codificam enzimas participantes do metabolismo do folato. A homocisteína é um metabólito da metionina, que é neurotóxica em altas concentrações e, recentemente, tem sido considerada como um dos fatores de risco em potencial para a Doença de Alzheimer (DA). A enzima cistationina beta-sintase é responsável pela conversão da homocisteína à cistationina por uma via de transulfuração. Polimorfismos no gene que a codifica (*CBS*) podem levar à diminuição da atividade da enzima e ao conseqüente aumento nos níveis de homocisteína. O objetivo do presente estudo foi analisar a influência do polimorfismo *CBS* 844ins68 e os níveis plasmáticos de Hcy no risco do desenvolvimento da DA.

Metodologia: Amostras de DNA foram extraídas de 108 pacientes com DA provável diagnosticada segundo os critérios do NINCDS-ADRDA e 62 idosos saudáveis. A amplificação das amostras foi realizada por reação em cadeia da polimerase (PCR) e a visualização dos produtos em gel de poliacrilamida a 6% corado por prata. Os níveis plasmáticos de Hcy foram avaliados por imunoenensaio e comparados entre as duas amostras através do teste de Mann-Whitney.

Resultados: Com relação ao polimorfismo *CBS* 844ins68, a análise estatística pelo teste de χ^2 revelou que não houve diferença significativa entre as distribuições genotípicas observadas nas amostras caso e controle ($\chi^2_{(2)}=0,05$; IC 95%; $P=0,98$). Entretanto, os níveis de Hcy encontraram-se significativamente aumentados nos pacientes com DA em relação aos controles ($P=0,0001$). Além disso, os níveis aumentados de Hcy não foram associados à presença do alelo *CBS* 844ins68 ($P=0,71$).

Conclusões: De acordo com nossos resultados os níveis de homocisteína, ao contrário do polimorfismo *CBS* 844ins68, podem ser considerados como um fator de risco independente substancial para o desenvolvimento da DA em nossa população. Estes níveis de Hcy aumentados na amostra caso apontam que polimorfismos em outros genes participantes do metabolismo do folato, associados ou não a distúrbios nutricionais, podem estar envolvidos.

Palavras-Chave: doença de Alzheimer, homocisteína, CBS, folato, neurodegeneração

Apoio Financeiro: PPSUS-MS/CNPq/FAPERJ, CNPq, CEPUERJ

Study of association of brain-derived neurotrophic factor val66met polymorphism with late onset depression in elderly people.

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OBJECTIVES:

Major depression disorder is a common psychiatric. In elderly people, depression causes suffering, family disruption, and disability, compromises the outcome of many medical illnesses, and increases mortality. Late onset depression refers to depressive syndromes that begin after 60 years old (1). One of the hypotheses that might underlie the pathogenesis of depression involves the brain-derived neurotrophic factor (BDNF). BDNF is a neurotrophin that has a role in the neurogenesis and also regulates the apoptosis of neurons. Some evidences suggest that reduction of BDNF levels might increase the susceptibility to develop depression. An association between a variant at nucleotide 196 that results in a Val66Met (rs6265) substitution in BDNF gene and mood disorders (MD) is the most frequent study involving BDNF. The methionine expression seems to promote a reduction in BDNF activity (2). Few are the studies that investigate genetics of the late-onset depression, with this study we aim to clarify the role of the Val66Met polymorphism on the BDNF gene in late life depression (3).

Methods:

Our study involves 98 patients with late-onset depression and 63 controls from Geriatric Clinics of Hospital das Clínicas of Universidade Federal de Minas Gerais. Patients were evaluated by a multidisciplinary group trained in assistance of the elderly. All these patients were genotyped for BDNF Val66Met polymorphism. The rs6265 is a probe provided by Applied Biosystems (Foster, CA) in a mode made-to-order. The statistical analyses were performed by UNPHASED v3.0.

Results:

We genotyped 151 individuals with more than 60 years and, when compared controls with late onset depression subjects, we detect a statistically significant difference for genotype (p-value = 0.03579) and allele analysis (p-value = 0.002868).

Conclusions:

Our study suggests an association between the BDNF Val66Met and late onset depression. This finding is consistent and seems in accordance with findings of major depression.

References:

- 1- Lancet 365: 1961, 2005;
- 2- Cell 112 (2): 257, 2003;
- 3- Biol. Psychiatry 60: 84, 2006.

Palavras-Chave: BDNF, Late life depression, polymorphism, elderly

Apoio Financeiro: CNPq, Guggenheim Foundation

CONVERGENCE OF GENETIC AND ENVIRONMENTAL FACTORS IN AN ANIMAL MODEL OF PARKINSON'S DISEASE.

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Objective: Although the primary cause of Parkinson's diseases (PD) is still unknown, evidences suggest a multifactorial etiology involving genetic and environmental factors. In the light of the "olfactory vector hypothesis" we investigated the interaction of genetic and environmental factors in PD through the intranasal (i.n.) administration of MPTP in Wistar (WIS) and Lewis (LEW) rats.

Methods: Male adult WIS and LEW rats (n=20 per group) received a single i.n. administration of MPTP (1 mg/nostril) or saline (i.n.). The animals were submitted to a battery of behavioral paradigms including social recognition, water maze (working memory version), step-down inhibitory avoidance, open field and rota-rod tasks.

Results: Neither MPTP administration nor strain influenced the locomotor performance of rats in the open field. LEW rats presented a reduced performance in the rota-rod, with no significant effects of MPTP treatment [latency to fall (s): WIS/CONT: 118.7±31.24; LEW/CONT: 36.87±7.7; WIS/MPTP: 116.1±28.1; LEW/MPTP: 39.2±14.6]. WIS and LEW rats presented short-term social memory impairments induced by MPTP [investigation time (s): WIS/CONT: 1st exp: 64.33±11.09; 2nd exp: 44.33±9.06; LEW/CONT: 1st exp: 77.33±9.46; 2nd exp: 44.55±6.66; WIS/MPTP: 1st exp: 99.72±14.33; 2nd exp: 71.36±16.04; LEW/MPTP: 1st exp: 49.1±12.16; 2nd exp: 48.4±17.31]. Moreover, LEW rats presented a better performance than WIS in the water maze and MPTP selectively disrupted WIS behavior in this task [escape latency (s): WIS/CONT=21.35±3.41; LEW/CONT=15.9±2.96 and WIS/MPTP=32.81±3.68; LEW/MPTP=15.55±2.66]. Conversely, in the step-down LEW performed poorly when compared to WIS rats, and the i.n. administration of MPTP did not caused any disturbance in this task [latency to step-down (s): WIS/CONT: 1.5h=51.0; 24h=170.0; LEW/CONT: 1.5h=11.08; 24h=5.12; WIS/MPTP: 1.5h=20.0 ; 24h=86.0; LEW/MPTP: 1.5h=17.85; 24h=19.25].

Conclusions: These findings indicate strain differences in learning, memory and motor coordination in rats. Our results demonstrate a reduced susceptibility of LEW rats to MPTP-induced working memory deficits suggesting a convergence of genetic and environmental factors in an animal model of PD.

Apoio Financeiro: CAPES, FAPESC and CNPq.

STUDY OF ASSOCIATION BETWEEN TRYPTOPHAN HYDROXYLASE 2 AND DEPRESSION OF LATE-ONSET

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Introduction and aims: Depression is one of the most common psychiatric diseases, being considered late-onset depression when only happens after 60 years. The population above this age has increased significantly in Brazil and it is estimated that will reach in the year 2025, about 32 million of elderly people (Tamai, 1999). The depression in this population has been worsening in the course of other diseases and also has a great negative social impact. Several lines of research have shown that depressive symptoms are associated with functional changes of the serotonin system. Thus, the genes involved in the synthesis, transport and degradation of serotonin have received special attention in the effort to discover the genetic basis of depression (Christiansen, 2007). Tryptophan hydroxylase-2 is an enzyme involved in the biosynthesis of serotonin in the brain (Walther, 2003) and polymorphisms in this enzyme can interfere in the synthesis of the neurotransmitter. Some variants of TPH2 have been associated with various psychiatric disorders, especially major depression and suicide (Zill, 2004), but few studies have investigated the role TPH2 gene polymorphisms and susceptibility of the late-onset depression. **Methods:** In this study, we investigated polymorphisms of the TPH2 gene (eight tag SNPs) in 118 elderly patients with late-onset depression and 63 controls attending the Reference Center for the Elderly of Minas Gerais State Professor Benjamin Caio Dias. **Results:** Data showed an association between the genotype c/c of the polymorphism located in the intronic region of the TPH2 gene (rs1487275 - global p-value = 0.008015) and the presence of late-onset depression. **Conclusions:** The result strengthens the hypothesis of neurobiological involvement of serotonin system in the etio-pathogenesis of depression, not only in the major depression as well as in the late-onset depression. **References** Biol Psychiatry 61: 223,2007. Metabolism 54 (Suppl 1): 10,2005. Lemos Editorial:p11,1999. Biol Psychiatry 56: 581, 2003.

Palavras-Chave: Tryptophan hydroxylase-2, serotonin, elderly, SNP, late-onset depression

Apoio Financeiro: CNPq Universal grant, Programa Institutos do Milênio/CNPq/FINEP and John Simon Guggenheim Foundation

a-synuclein oligomerization in HeLa cells: role of mitochondria on aggregation?

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a-Synuclein belongs to a small group of natively unfolded proteins that can transiently bind to lipid membranes and acquire a partial α -helical conformation. Under certain pathogenic conditions, a-synuclein associates to form oligomers and insoluble fibrils with increased β -sheet configuration. Nitration of a-synuclein mediated by ONOO⁻ may play a mechanistic role in PD pathogenesis, i.e. i) nitration induces the formation of a-synuclein oligomers, ii) low concentrations of nitrated protein facilitate the nucleation step of a-synuclein, but iii) at high concentrations, these species inhibit filament assembly, iv) Tyr39 nitration displaces a-synuclein from lipid vesicles, and v) nitrated a-synuclein exhibits a reduced proteosomal degradation. a-synuclein was found in the mitochondria of different cells, and there it regulated mitochondrial NO production, and thus free radical formation. Some authors found that oligomeric a-synuclein bound to mitochondria, but not the monomer. This raises the question of whether mitochondria are capable of accelerating a-synuclein oligomerization, or if a-synuclein binds mitochondria as a pre-formed oligomer. Here we showed that overexpressed a-synuclein entered the mitochondria of HeLa cells, though the endogenous protein was not present in the organelle, but was rather distributed in membrane and nuclei. In addition, by confocal microscopy we observed colocalization of the oligomeric form with mitochondria, but not of the monomer upon over-expression. We have previously engineered a recombinant a-synuclein with a 12 amino acids tag that encodes a binding site for a fluorescent dye, FLASH. With this construct we were able to map the protein to the mitochondria by flow cytometry on the isolated organelle. In future studies we attempt to elucidate whether the mitochondria induces a-synuclein oligomerization or if it is the oligomerized protein that enters the organelle and induces oxidative stress. We also want to elucidate the role of mitochondrial radicals in a-synuclein aggregation. These findings altogether may support a pivotal role for mitochondria and a-synuclein in oxidative stress and apoptosis.

Palavras-Chave: a-synuclein, mitochondria, oxidative stress, Parkinson Disease

Sistema diagnóstico de la demencia tipo alzheimer basado en el análisis de comportamientos, funcionalidad de reconocimientos sensoriales e identificación y contexto del disparador del proceso

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Objetivos: evaluar un sistema de diagnóstico para demencia tipo alzheimer y su poder de discriminación de diferentes situaciones. **Métodos:** a partir de la teoría que explica el proceso como combinación de factores estructurales psicológicos, comportamientos psicosociales previos que se continúan hasta el momento del diagnóstico, disfuncionalidad de reconocimientos sensoriales, alteración eventual o sostenida de la atención de rastreo y focalizada que emerge como alteración de la memoria, identificación del disparador que desencadena la enajenación de la realidad actual, la traslocación hacia un pasado infantil idílico que elude las pérdidas penosas con incapacidad de afrontamiento, y eventualmente la fijación de la atención en el deseo de muerte. **Resultados:** el protocolo permite diferenciar el envejecimiento normal, el envejecimiento con rechazo de un pasado de sumisión, el proceso demencial no combinado con envejecimiento, el proceso demencial combinado con envejecimiento, y el de adultos muy mayores. El protocolo fue aplicado a 158 casos diagnosticados y medicados como alzheimer por especialistas médicos, que fueron seguidos durante 18 meses o hasta la rehabilitación en los casos ratificados como alzheimer que pudieron ser abordados. 98 (62%) casos del total de casos abordados inicialmente correspondieron a envejecimientos normales, y de ellos 17 (17%) fueron envejecimientos con rebelión de la condición sumisa (especiales), 3 de ellos hombres. De los 60 casos ratificados como con demencia tipo alzheimer, 14 (9 % del total, 23,3 % de los casos efectivos) no presentaron combinación con comportamientos de envejecimiento normal o especial, todos ellos menores de 58 años, 42 (70 % de los casos efectivos) (edades de 58 a 76 años) y 4 (6,7%) a mayores de 79 años. En éstos últimos no se presentó un disparador del proceso que remita a una pérdida penosa sin capacidad de afrontamiento, a diferencia de los anteriores. **Conclusiones:** la aplicación de este sistema de diagnóstico por historia de vida, comportamientos y reconocimientos sensoriales permite vislumbrar situaciones diferentes consideradas en general equivalentes. La aparente pérdida de la memoria reciente por alteración de la atención es el factor de confusión más frecuente.

Palavras-Chave: alzheimer, diagnostico, envejecimiento normal, envejecimiento y rebelión, demencia y envejecimiento, demencia en adultos muy mayores.

Apoio Financeiro: Universidad Autónoma de Entre Ríos, BBVA Banco Francés, Fundación Alzheimer

***In vivo* NGF response in Niemann-Pick type C (NPC) nule mice with mild cholinergic pathology**

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Niemann-Pick type C (NPC) disease is an autosomal recessive neurovisceral lipid storage disorder. NPC1 and NPC2 control intracellular lipid trafficking and the dominant feature of NPC1 and NPC2 deficiencies is a severe and progressive neurodegeneration. The endocytic pathway and lipid raft stability are important regulators of neurotrophin signaling suggesting that in NPC, neurotrophin signaling could be altered. In different cognitive disorders there is neurodegeneration of cholinergic neurons in the basal forebrain which positively correlates with the dementia symptoms and are responsive to Nerve Growth Factor (NGF). **To date there are no proves on the status of neurotrophin signaling and the cholinergic system in NPC brain in vivo.** To evaluate *in vivo* if neurotrophin receptors are activated normally by neurotrophins in septal basal forebrain cholinergic neurons (SBFN) we first study the histopathology of SBFN in NPC hypomorphic mice. Subsequently, **we performed an in vivo lesion of the fimbria-fornix to axotomize cholinergic neurons of the basal forebrain.** Normally these neurons respond with cell degeneration and atrophy to the axotomy that is reverted by intraventricular infusion of NGF directly to the brain.

Our studies show that in NPC brain there is normal number of cholinergic neurons; however neurons appeared hypertrophic and with reduced neuropil. In addition, cholinergic neurons are filled with cholesterol and the whole septal area showed positive immunostaining for phosphorylated TAU (AT8). Surprisingly, NPC cholinergic neurons responded normally to axotomy and to NGF infusion. This is consistent with the fact that PC12 cells treated with U18666A -a drug that mimics the accumulation of cholesterol in the late endocytic pathway observed in NPC- are able to differentiate in response to NGF. These results suggest that the lipid raft and traffic abnormalities observed in NPC are not impairing NGF-mediated signaling; therefore, neurotrophins could be good candidates for therapeutically interventions. **Palavras-Chave:** Niemann-Pick type C, NPC, neurodegenerative disorders

Apoio Financeiro: FONDECYT 1085273 to FCB, FONDAP 13980001 to NCI and FBC. Ara Parseghian Medical Research Foundation to SZ, AA, FCB.

Characterization of Recombinant PrP Aggregates and Its Effects on Cultured Neuroblastoma Cells

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Prion diseases can be infectious, sporadic or inherited. Regardless of their origin, they are related to modifications of a ubiquitous membrane-anchored protein, the prion protein (PrP). Through a poorly understood process, the cellular PrP isoform (PrP^C), an α -helical-rich protein, undergoes a profound conformational change, acquiring higher β -sheet content; the latter isoform is named PrP^{Sc} (Sc from *scrapie*) and is the only known component of the infectious prion particle. The protein-only hypothesis postulates that PrP^{Sc} 'multiplies' by catalyzing the conversion of PrP^C into a likeness of itself, thus becoming responsible for its own propagation. It is also suggested, however, that an additional unknown factor could influence the PrP^C to PrP^{Sc} conversion. In this field, a great number of biological macromolecules have emerged as candidates for conversion catalysts. Cellular adhesion molecules, nucleic acids (NAs), basal membrane molecules, and glycosaminoglycans (GAGs) have been reported to interact with PrP^C and to induce its conversion into a β -sheet-rich structure similar to the infectious prion protein. In this work we report data on the biochemical and biophysical characteristics of PrP aggregates formed upon recombinant murine PrP (rPrP23-231) interaction with biomolecules, such as DNA, RNA and GAGs, using spectroscopic techniques, such as nuclear magnetic resonance. Morphology and toxicity were observed by transmission electron microscopy and viability assays. Our results show that aggregates of rPrP23-231 formed upon incubation with the above cited biological polyanions share some characteristics with PrP^{Sc}, such as partial resistance to proteolysis. Toxicity to cells in culture is observed when rPrP is incubated with some RNA and DNA molecules, but not with GAGs, suggesting that a still unidentified nucleic acid sequence(s) may be responsible to the generation of a toxic PrP species.

Palavras-Chave: PrP, DNA, RNA, GAG, aggregation

Apoio Financeiro: CNPq, CAPES, FAPERJ, LOREAL, FUJB, PRONEX

ANTAGONISTS OF KININ RECEPTORS FOR THE TREATMENT OF ALZHEIMER'S DISEASE. NEW EVIDENCE FROM ANIMAL MODELS.

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Objective: Increased brain deposition of amyloid beta-protein (A β) and cognitive deficits are classical signs of Alzheimer's disease (AD) that have been associated to neuroinflammatory response. We have recently demonstrated that the genetic deletion or blockage of kinin receptors improves cognitive deficits induced by intracerebroventricular injection of A β protein(1). Here we investigated the effects of the antagonism of hippocampal kinin B1 and B2 receptors in the age-related cognitive deficits in rats.

Methods: Male Wistar rats with 3 or 24 months-old received a single intrahippocampal injection of the selective kinin receptor antagonists des-Arg9-[Leu8]-BK (DALBK, B1 antagonist) or Hoe 140 (B2 antagonist) and were then trained to locate an escape submersed platform based on spatial cues. Retention was tested 24 h later on a single probe trial of 60 s without the platform and the time spent in the correct quadrant was recorded.

Results: As expected, aged rats presented pronounced learning and memory deficits, as indicated by increased latencies to find the platform during training trials [(s) 3 mo:17.4 $\pm$ 4.1; 24 mo:45.0 $\pm$ 4.3] and reduced target quadrant preference during the probe trial [%time: 3 mo:47.3 $\pm$ 1.3; 24 mo:29.4 $\pm$ 2.0]. The treatment with the selective B1 and B2 antagonists did not interfere with the performance of 3 mo animals. However, the intrahippocampal injection of DALBK (B1 antagonist), but not Hoe 140 (B2 antagonist), improved the spatial learning and memory deficits observed in aging rats [training escape latency (s): Cont: 44.0 $\pm$ 7.7; DALBK: 22.0 $\pm$ 2.0. Hoe: 39.3 $\pm$ 7.0]; [%time in the correct quadrant: Cont: 16.6 $\pm$ 0.6; DALBK: 23.8 $\pm$ 1.8. Hoe: 16.5 $\pm$ 1.1].

Conclusion: This is the first study indicating the involvement of the hippocampal kinin receptors in the age-related cognitive deficits. These findings suggest that kinin antagonists, specially the new orally active non-peptide antagonists, might have therapeutic potential for the treatment of AD.

References: 1- Neuroscience 151:631, 2008.

Palavras-Chave: Alzheimer disease; aging; kinin receptor antagonists, neuroinflammation, learning and memory

Apoio Financeiro: CNPq, FAPESC, CAPES

Impacto da estimulação cognitiva da memória no estado emocional e nos desempenhos verbais de pacientes idosos com quadros de demência

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Objetivo: Foi desenvolvida uma oficina ambiental de jardinagem com a finalidade de estimular a memória e avaliar sintomas cognitivos e comportamentais de idosos com quadros de demência. **Método:** Onze idosos com demência leve-moderada participaram da oficina ambiental de jardinagem desenvolvida no Centro de Medicina do Idoso do Hospital Universitário de Brasília, comparecendo semanalmente durante o período de dois meses. Os participantes, antes do ingresso na oficina e após seu término, foram submetidos a uma avaliação neuropsicológica com o objetivo de conhecer o perfil cognitivo dos idosos e analisar o efeito da oficina ao longo das semanas. Entre as atividades específicas da jardinagem se destacam: a escolha de um avental, pintura de um vaso, escolha de uma planta e aprendizagem dos cuidados com a planta ao longo das semanas. No final da oficina foi avaliada a aprendizagem da sequência das atividades realizadas mediante ao ordenamento de cartões que ilustravam essas atividades.

Resultados: Uma prova t para amostras pareadas demonstrou uma diferença significativa na Escala de Depressão Geriátrica antes e depois da oficina ($p=0,018$). Uma análise de Correlação de Pearson não paramétrica revelou uma correlação positiva entre os melhores desempenhos na tarefa de *Organização Correta da Sequência dos Cartões* que ilustravam a sequência das atividades realizadas na oficina com os testes *Fluência verbal FAS* ($r=783$, $p=0.004$) e o sub-teste *WAIS Dígitos Direitos* após a oficina ($r=642$, $p=0.033$). **Discussão:** De forma geral, os resultados comparativos da avaliação neuropsicológica antes e após a participação na oficina ambiental de jardinagem indicaram uma diminuição nos valores da Escala de Depressão Geriátrica sugerindo uma melhora no bem-estar, na auto-estima e na inter-relação dos participantes; demonstrando a utilidade deste tipo de atividade no tratamento de pacientes com demência leve a moderada. Ademais os melhores desempenhos de reconhecimento de cartões ilustrativos associaram-se aos melhores desempenhos nos testes de fluência verbal e repetição verbal de dígitos diretos.

Palavras-Chave: Estimulação Cognitiva - Memória - Idosos

COMPARAÇÃO COMPORTAMENTAL E NEUROQUÍMICA DE TRÊS MODELOS ANIMAIS DE PARKINSONISMO

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Objetivos: Avaliar o comportamento motor e alterações dos níveis de dopamina (DA) em modelos animais de parkinsonismo.

Método e resultados: Ratos Wistar foram infundidos bilateralmente com 1µl na substância negra de MPTP (100mg/ml), ou 6-OHDA (6mg/ml) ou LPS (2mg/ml). Para avaliação motora foi empregado o teste Campo Aberto com 58 ratos divididos em 5 grupos, controle (n=18), SHAM (n=10), MPTP (n=11), 6-OHDA (n= 9) e LPS (n=10). Foram avaliados: frequência de locomoção (LO), latência para início do movimento (LA), frequência de levantar (LE) e imobilidade (TI). Este procedimento foi realizado 24h, 3 e 7 dias após a cirurgia estereotáxica. A investigação neuroquímica avaliou os níveis de DA estriatal e seus metabólitos em 78 ratos Wistar (n=5-7/grupo) feita por HPLC-ED. Os resultados foram expressos pela média ± EPM, avaliados por ANOVA, seguida do teste de Newman-Keuls, considerando p<0,05 como significativo. Os resultados mostraram que o grupo MPTP apresentou diminuição das frequências de LO (52,1±8,5) e de LE (17,9±2,3) 24h após a cirurgia em comparação aos animais dos grupos controle e SHAM. Os ratos do grupo 6-OHDA mostraram redução em todos os parâmetros motores (LO - 24h: 17,2±6,6; 3dias: 35,6±9,6; 7dias: 36,5±11,1; LE - 24h: 9,3± 3,2; 3dias: 10,5±2,3 e 7dias: 11,5±2,6) em comparação aos ratos controles. Os animais do grupo LPS não mostraram nenhuma diferença nos parâmetros motores quando comparado aos grupos controle e SHAM. Os dados da análise neuroquímica mostraram um aumento significativo dos níveis de DA estriatal (ng/g de tecido) 24h (10920,6±788,1) após a infusão de 6-OHDA, seguido de uma redução de DA nos dias 3 (2593,1±604,6) e 7 (1543,0±821,1) após cirurgia. Os níveis de DA no grupo MPTP mostraram redução apenas no tempo de 3 dias após a lesão (3997,5±555,3). Não ocorreu alteração dos níveis de DA nos animais do grupo LPS em nenhum dos tempos analisados.

Conclusões: os dados do presente trabalho mostram que os modelos induzidos por MPTP e 6-OHDA em ratos, mas não o LPS, foram capazes de causar sinais motores e alterações neuroquímicas mimetizando uma síndrome parkinsoniana.

Palavras-Chave: Dopamina, Parkinsonismo, Modelos animais, 6-OHDA, MPTP, LPS

Apoio Financeiro: CNPq E CAPES

EVIDÊNCIAS DE GANHOS APÓS ESTIMULAÇÃO COGNITIVA MEDIANTE UMA PROPOSTA DE ANÁLISE DE DESEMPENHOS NEUROPSICOLÓGICOS EM PACIENTES COM DEMÊNCIAS

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Objetivo: O estudo propõe avaliar em pacientes demenciados ganhos cognitivos obtidos em testes neuropsicológicos após estimulação cognitiva (EC) que possam se evidenciar mesmo abaixo do ponto de corte. **Método:** Onze idosos diagnosticados com quadro de demência submeteram-se a EC durante dois meses com encontro semanal. Para observar mudanças dentro dos limites de capacidade cognitiva adotamos os pontos de corte de cada teste neuropsicológico como uma capacidade cognitiva possível de ser conseguida após estimulação. Desta maneira o ponto de corte equivaleu a 100% do que passamos a denominar percentual mínimo de capacidade cognitiva (PMCC) em cada teste ou sub-teste neuropsicológico, quando se tratava de uma avaliação individual. Para uma avaliação global do sujeito o PMCC, utilizamos uma média de desempenho geral (a soma dos PMCC em cada teste dividido pelo número de testes realizados). Foram utilizados: Teste Mini-Mental (MM) e para o PMCC: Teste do Desenho Livre de um Relógio, Escala de Memória de Wechsler, Teste de Fluência Semântica e Verbal FAS aplicados antes e após a EC. **Resultados:** Quando comparamos o PMCC de cada paciente obtido na AN anterior a EC com a que ocorreu depois, podemos constatar que dos 11 pacientes demenciados, 2 reduziram seus escores entre 3,9 a 9,7%, outros 2 mantiveram os seus escores, e os outros 7 aumentaram os seus escores entre 2,9 a 13,5%. O Teste T apontou que os PMCC antes [$X = 60,26 (17,18)$] e depois [$X = 62,90 (13,26)$] da EC, demonstraram um estabelecimento do perfil cognitivo desses pacientes durante o todo o período que estavam sobre EC. Ademais as correlações de Pearson apontaram uma significância positiva entre os melhores desempenhos no Teste MM ($0,832 p < 0,01$) e no PMCC ($0,948 p < 0,01$) antes e depois da EC, associando os maiores escores de MM com os PMCC que mais se elevaram. **Conclusão:** O estudo demonstrou a estabilização do perfil cognitivo desses pacientes durante o todo o período que estavam realizando EC, assim como ganhos cognitivos após as oficinas analisados mediante a esta proposta de avaliação por PMCC em acordo aos resultados do MM.

Palavras-Chave: Estimulação Cognitiva; Idosos; Demência; Avaliação Neuropsicológica

Apoio Financeiro: UNB

Stereological analysis of the microglial inflammatory response in dentate gyrus in murine prion disease

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Objective: This work aims to investigate microglial inflammatory response in the granular layer of dentate gyrus (GRDG) in different time windows of murine prion disease.

Methods: 16 mice C57BL6, female, 6 weeks old, were grouped as follow: control (n=3) or prion (n=13) groups. They were submitted to a bilateral intra-hippocampal injection of normal brain homogenate or ME7 prion strain. Prion subjects were sacrificed in different time windows after inoculation: 9, 11, 18 and 20 weeks. Animals were aldehyde and brains were cut and histochemically stained for tomato lectin, *Lycopersicon esculentum*, and counterstained by Nissl. Morphological analyses were done using an optical microscope, Nikon, with low and high power objectives. The number of microglial cells in GRDG of hippocampus was assessed using the optical fractionator technique with StereoInvestigator software (MicroBrightField, USA).

Results: The number of microglial cells in the granular layer of dentate gyrus showed no statistical differences between control group (1998.94 ± 469.46) and ME7 groups at 9 (1947.05 ± 363.55) and 11 weeks (2856.20 ± 580.29) postinjection (ANOVA, one-way, Bonferroni *a priori*, $p > 0.05$). ME7 animals at 18 and 20 weeks postinjection presented an increased number of microglial cells when compared with animals in earlier stages of prion disease (5493.82 ± 1333.28 and 4795.03 ± 298.09 , respectively) (ANOVA, one-way, Bonferroni *a priori*, $p > 0.05$).

Conclusion: The present report described, by the first time, by stereological procedures, the quantitative progression of the microglial response in GRDG in murine prion disease, and explain, at least in part the reduction in the cell number of dentate gyrus previously detected.

Palavras-Chave: Prion, ME7, hippocampus, microglia, murine model

Relação entre distúrbios de marcha e equilíbrio e o escore no mini-exame do estado mental em uma população de idosos.

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OBJETIVO: As quedas são frequentes em todas as épocas da vida. Contudo, representam um problema de saúde mais sério na terceira idade devido ao maior risco de lesões graves. Um dos fatores de risco para quedas são as alterações de marcha e equilíbrio, que por sua vez são influenciados pelo envelhecimento normal e patológico. Com o objetivo de avaliar se distúrbios de marcha e equilíbrio podem estar associados à redução da capacidade cognitiva, no presente trabalho estudamos a associação entre os resultados obtidos no Teste de Tinetti - Performance-oriented assessment of mobility problems in elderly patients (1) e os escores obtidos no mini-exame do estado mental - MEEM (2) em um grupo de idosos.

MÉTODOS: Dados são apresentados na forma de média $\pm$ erro padrão da média. O Teste de Tinetti e o MEEM foram realizados em 90 homens (idade: $79,6 \pm 0,6$ anos; 65 a 92) e 191 mulheres ($78,8 \pm 0,5$ anos; 65 a 94). Os escores de Tinetti foram $25,2 \pm 0,4$ para homens (H) e $24,2 \pm 0,3$ para mulheres (M) enquanto que os escores de MEEM foram $28,3 \pm 0,2$ para H e $28,0 \pm 0,1$ para M. Não foram observados efeitos significativos de gênero para a idade nem para os escores (ANOVA: $P > 0,05$ em todos os casos). Todos os sujeitos apresentaram escore maior que 22 no MEEM. Foi observada uma correlação significativa entre os escores de Tinetti e os do MEEM ($r=0,3$; $P < 0,001$). Foi observado efeito significativo dos escores do MEEM sobre os do Tinetti (ANOVA: $F=3,3$; $gl=8$; $P=0,001$; co-variado com a idade). A análise post hoc indicou que para um escore de 28 a 30 no MEEM, não são observadas diferenças no Tinetti. No entanto, para escores progressivamente menores do que 28 no MEEM ocorrem reduções progressivas e significativas nos escores do Tinetti.

CONCLUSÕES: Nossos resultados indicam que há uma associação significativa entre os escores do Tinetti e os do MEEM. Interessantemente, as alterações de marcha e equilíbrio parecem apresentar piora significativa em escores do MEEM em que um quadro de demência leve ($MEEM < 26$) ainda não pode ser claramente sugerido.

REFERÊNCIAS: 1- J Am Geriatr Soc 34:119, 1986; 2- J Psychiatr Res 12:189, 1975.

Palavras-Chave: Tinetti, MEEM, Envelhecimento, Cognição, Marcha, Equilíbrio

Apoio Financeiro: FAPERJ, SR2-UERJ, Unidade Integrada de Prevenção - Hospital Silvestre RJ

Efeitos da idade no desempenho de ratos Wistar nos labirintos em T- Elevado e no aquático de Morris: aspectos cognitivos, emocionais e bioquímicos

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O envelhecimento fisiológico está envolvido com alterações em aspectos cognitivos e emocionais. Dados obtidos por nosso grupo demonstraram um efeito significativo da idade sobre a velocidade de aquisição e memória de tarefas de navegação espacial e uma correlação desses parâmetros com a atividade serotoninérgica no núcleo dorsal da rafe. A hipótese de que o déficit observado no desempenho desses animais poderia ser devido a uma disfunção em um componente emocional, importante para a execução da tarefa cognitiva, baseia-se em dois fatores: (i) o teste, Labirinto Aquático de Morris (LAM), utilizado envolve uma tarefa aversiva e, ii) existem evidências de que circuitos serotoninérgicos estão envolvidos com aspectos emocionais. No presente trabalho estudamos os efeitos da idade sobre: i) aspectos emocionais avaliados no Labirinto em T- elevado (LTE); a aprendizagem e memória espacial e iii) parâmetros serotoninérgicos. Foram utilizados 22 ratos divididos em 2 grupos, adultos (A) e idosos (I). Todos os ratos foram submetidos ao LTE e, após 48 horas, ao LAM. As concentrações de serotonina (5-HT) e do metabólito ácido 5-hidroxi-indolacético (5-HIAA) foram determinadas por cromatografia (HPLC). Verificamos diminuições significativas nas concentrações (ng/g) de 5-HT no córtex orbito-frontal (A= 280 e I= 140, $p= 0,0045$) e 5-HIAA na amígdala (A= 1300 e I= 800, $p= 0,0055$). A ANOVA com medidas repetidas mostrou um efeito significativo da idade na velocidade de aquisição da tarefa de navegação espacial. No entanto, os animais de ambos os grupos foram capazes de aprender a tarefa. No LTE houve efeito significativo do fator tentativa, indicando aquisição da tarefa de esquiva, bem como do fator idade. Não houve interação significativa entre os dois fatores. Ambos os grupos retiveram a aprendizagem, quando testados 48 horas após. Como os animais (A e I) nadaram com velocidade semelhante no LAM, a diferença observada no LTE parece não ser devida a déficit motor. Aventa-se a hipótese de déficit motivacional que não interferiu no aprendizado de esquiva, mas poderia, além de uma provável disfunção no hipocampo, contribuir para o déficit no desempenho no LAM.

Palavras-Chave: Idade, LTE, Labirinto Aquático, memória, aprendizagem e motivação

Apoio Financeiro: CNPQ

AUTOFLUORESCENT AGGREGATES OF CHALCEDONY IN THE HUMAN BRAIN FROM ELDERLY PATIENTS

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Objectives.- Aluminum and silicon accumulations were detected in neurofibrillary tangle-bearing neurons and in normal elderly brains by a combination of scanning electron microscopy and X-ray spectrometry (EDS-SEM) (1). These inorganic elements are related to pathological changes in the cerebral cortex. The presence of chalcedony (SiO₂) was also documented in the human brain, by using a petrographic microscope (2). Chalcedony is a microcrystalline fibrous form of silica consisting of nanoscale intergrowths of quartz and optically length-slow fibrous silica polymorph moganite (3). Based on this evidence we decided to document the presence of autofluorescent mineral chalcedony in autopsy-derived brain tissue from aged patients (mean, 81 years) by using a Leica TSC – TS2 laser scanning confocal microscope (LSCM). **Methods.-** For these experiments, haematoxylin-eosin sections from routine neurohistologic preparations at the Pathology Department of the Instituto Nacional de Enfermedades Neoplásicas (INEN) Lima-Perú, were analyzed. **Results.-** A slight oversaturation of silicon is necessary for to allow chalcedony precipitation from the solution. Some quartz continues developing, forming large, euhedral overgrowths. Prismatic quartz belong to the rhombohedral crystal system, they are often twined or druse. Aggregates of prismatic quartz overgrowths are shown in this paper. These crystals show the characteristic autofluorescence with different excitation wavelengths, using a LSCM. **Conclusions.-** Fibrous minerals in biological environments generally grow either from an aqueous fluid in open spaces such cavities, or during retrograde metamorphism and alteration of preexisting minerals in the presence of an aqueous fluid. The fibrous habit of chalcedony might be worrisome for it can bind directly to DNA and cause DNA damage and neoplastic transformation. This is the first experimental evidence of biologically-produced crystalline silica mineral (chalcedony) and its degree of maturation, in human CNS from aged patients, using a LSCM. **References:** 1. Science 208: 297, 1980. 2. Micron, doi:10.1016/j.micron.2007.12.005. 3. Science 255: 441, 1982

Palavras-Chave: AChR, Neurodegeneration, Oxidative stress, Permineralization, Quartz, Silicon.

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Schwann cell transdifferentiation into myofibroblast: possible role of *Mycobacterium leprae* and TGF β 1

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Introduction: Neural fibrosis is one of the causes of deformities during leprosy, which remains a major global health problem. Neural degeneration progresses after the patient healing and, as suggested by others, the Schwann cells (SC) are involved in the degeneration and regeneration of the nerve. Our group showed, in a human Schwannoma lineage (ST88-14), increased mRNA levels of the cytokine TGF β 1 and its receptors after *Mycobacterium leprae* (ML) stimulus. Since TGF β 1 neutralization reduces fibrosis, and this cytokine promotes transdifferentiation of the SC into myofibroblast, which in turn, overproduce extracellular matrix (ECM) components, resulting in neural degeneration, the aim of this study was to investigate the possible role of TGF β 1 on the SC leprosy lesion context. **Methods:** ST88-14 cells were stimulated with ML and supernatants were transferred to non-infected cells to evaluate TGF β 1 and its receptor (TGFRII) expression. In order to evaluate myofibroblast transdifferentiation (a-smooth muscle actin expression, a-SMa) and ECM deposition, ST88-14 were stimulated with TGF β 1 and/or ML. The cytokine and its receptor expression at the cultures and ECM deposition components were analyzed by ELISA, RT-PCR, flow cytometry or immunofluorescence. **Results:** Our data suggest that ML modulates TGF β 1 and its receptor expression. TGF β 1 was also able to induce expression of a-SMa and upregulation of fibronectin, tenascin, collagen IV and laminin. **Conclusion:** These partial results suggest the involvement of TGF β 1 in the cellular answer modulation to ML, and its role in SC transdifferentiation into myofibroblast which could contribute to neural degeneration.

Palavras-Chave: Schwann cell, myofibroblast, leprosy, M. leprae

Apoio Financeiro: FioCruz, CNPq

PERCEPÇÃO VISUAL DE CONTRASTE EM CRIANÇAS COM HISTÓRIA DE DESNUTRIÇÃO

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Objetivo: Uma nutrição adequada é essencial para organização, formação e desenvolvimento do Sistema Nervoso Central (SNC). A ocorrência de deficiência nutricional durante o período crítico de formação do SNC resulta em alterações morfológicas, neuroquímicas e comportamentais. O objetivo deste estudo foi verificar possíveis alterações na percepção visual de contraste relacionadas à desnutrição energético-protéica (DEP) em crianças.

Método: Participaram dos experimentos 10 crianças de ambos os sexos, cinco com DEP e cinco sem história de desnutrição e com peso normal conforme os parâmetros antropométricos de Waterlow. A percepção visual foi avaliada utilizando a função de sensibilidade ao contraste (FSC) que é uma das principais técnicas na avaliação clínica e teórica do sistema visual humano. As medições de sensibilidade ao contraste foram realizadas com o paradigma psicofísico da escolha forçada, binocularmente, à distância de 150 cm, utilizando estímulos visuais de grade senoidal com frequências de 0,25; 1,0; 2,0 e 8,0 ciclos por grau de ângulo visual (cpg). Durante cada sessão experimental, pares de estímulos eram apresentados, um com uma das frequências de teste e outro apenas com a luminância média a 42 cd/m². O participante tinha a tarefa de escolher sempre o estímulo com a frequência de teste. A cada três acertos consecutivos decrescia-se uma unidade de contraste e a cada erro acrescia-se a mesma unidade (20 %). A ordem de apresentação dos estímulos era aleatória e controlada pelo programa.

Resultados: Os resultados demonstraram que a FSC das crianças com DEP foi menor do que a das crianças sem histórico de desnutrição para as frequências espaciais mensuradas. As análises com a ANOVA para medidas repetidas mostraram diferenças significantes entre os grupos [$F(1, 118) = 17,86$; $p < 0,0001$]. Entretanto, a análises com o teste post-hoc Tukey HSD mostrou diferença significativa entre os grupos apenas na frequência de 8,0 cpg. ($p < 0,001$).

Conclusões: Esses resultados sugerem que a DEP altera a percepção visual, principalmente nas frequências mais altas, que estão relacionadas à percepção de detalhes.

Palavras-Chave: percepção visual; desnutrição; desenvolvimento visual; sensibilidade ao contraste; crianças.

Apoio Financeiro: CNPQ

AVALIAÇÃO DAS VIAS PARVO E MAGNOCELULAR EM CRIANÇAS COM HISTÓRIA DE DESNUTRIÇÃO

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Objetivo: O objetivo deste estudo foi determinar a função de sensibilidade ao contraste visual (FSC) de crianças com desnutrição energético-proteica (DEP) em condições de luminância fotópica (42 cd/m²) e escotópica (0,03 cd/m²) e verificar se a DEP altera a resposta das vias visuais parvo e magnocelular de maneira discreta ou difusa.

Método: A FSC escotópica e mesópica foi medida por cinco crianças de ambos os sexos, na faixa etária de 7-9 anos, e com história de DEP, conforme os parâmetros antropométricos de Waterlow. Todas as crianças apresentavam acuidade visual normal ou corrigida e passaram pelas duas condições. As medições de sensibilidade ao contraste escotópica e fotópica foram realizadas com o paradigma psicofísico da escolha forçada, binocularmente, à distância de 150 cm, utilizando estímulos visuais elementares de grade senoidal com frequências de 0,25; 1,0; 2,0 e 8,0 ciclos por grau de ângulo visual (cpg). Durante cada sessão experimental, pares de estímulos eram apresentados, um com uma das frequências de teste e outro apenas com a luminância média a 0,03 cd/m² ou 42 cd/m². A criança tinha a tarefa de escolher sempre o estímulo com a frequência de teste que a cada três acertos consecutivos decrescia uma unidade de contraste e a cada erro acrescia a mesma unidade (20 %). A ordem de apresentação dos estímulos era aleatória e controlada pelo programa.

Resultados: Os resultados demonstraram que a FSC das crianças com DEP em condição escotópica foi menor do que em condições fotópicas para as frequências espaciais mensuradas. As análises com a ANOVA para medidas repetidas mostraram diferenças significantes entre as duas condições os grupos [$F(1, 118) = 134,94$; $p < 0,001$]. Entretanto, as análises com o teste post-hoc Tukey HSD mostrou diferença significativa entre os grupos apenas na frequência de 2,0 ($p < 0,05$) e 8,0 cpg ($p < 0,001$).

Conclusões: Esses resultados sugerem que a DEP altera a percepção visual de modo seletivo, principalmente nas vias magnocelular para frequências médias e altas, que estão relacionadas principalmente à percepção de detalhes.

Palavras-Chave: desnutrição; visão escotópica; visão fotópica; vias visuais; sensibilidade ao contraste.

Apoio Financeiro: CNPQ

C.02.003

Chronic early postnatal glutaric acid administration causes cognitive deficits in the water maze task in rats

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Objectives: Glutaric acidemia type I (GA I) is an autosomal recessive metabolic disorder caused by glutaryl-CoA dehydrogenase deficiency leading to predominant accumulation of glutaric acid (GA) in body fluids and tissues. The clinical manifestations of GA I are predominantly neurological. Although the pathophysiological mechanisms responsible for the brain damage of this disease are virtually unknown, they are thought to be due to the neurotoxic actions of GA. Therefore, in the present work we investigated whether chronic exposure of GA during early development could alter the cognitive performance of adult rats in behavioral tasks.

Methods: Saline-buffered GA, pH 7.4 (5 $\mu\text{mol. g of body weight}^{-1}$) was administered subcutaneously, twice a day, from the 5th to the 28th day of life to produce brain concentrations of GA of around 0.6 $\mu\text{mol g}^{-1}$. Control animals received saline in the same volumes and frequency. We evaluated the rat performance in the Morris water maze, open field and elevated plus maze tasks.

Results: GA administration provoked an impairment of spatial performance in the water maze since adult rats pretreated with GA were not able to remember the previous location of the platform spending significantly less time in the training quadrant (GA-treated: $30.4\% \pm 12.2$; controls: $45.5\% \pm 9.5$) and more time in the opposite quadrant (GA-treated: $28.3\% \pm 12.1$; controls: $18\% \pm 8.9$). GA-treated rats also had a lower number of correct annulus crossings than the control group (GA-treated: 1.37 ± 2.19 ; controls: 4.3 ± 1.6) and presented a higher latency to cross over the platform position (GA: 43.8 ± 8.46 s; controls: 12.4 ± 2.40 s). In contrast, GA chronic administration did not affect rat performance in the open field and elevated plus maze tasks, indicating that motor activity and anxiety were not changed by GA.

Conclusion: The results provide evidence that early chronic GA treatment induces long-lasting spatial behavioral deficit in rats.

Palavras-Chave: Glutaric acid, Morris water maze, Glutaric acidemia

Apoio Financeiro: CNPq, FAPERGS, PRONEX and the FINEP research grant Rede Instituto Brasileiro de Neurociência (IBN-Net) # 01.06.0842-00.

C.02.004

Consequences of maternal alcoholism on the radial glia.

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Prenatal ethanol exposure (PEE) induces structural disorders in the developing central nervous system (CNS). The relationship between radial glia cells (RGC) and migrating neuroblasts is crucial for the establishment of normally laminated structures. This study was conducted to determine RGC temporal vulnerability as a function of exposure to EtOH during brain development. Pregnant Wistar rats, 200-250 g, were fed one month before and during pregnancy and lactation a liquid diet with 5.9% (w/w) EtOH; their fetuses were compared with that of mothers fed the control liquid diet. Fetal brains were morphologically studied at embryonic days E12, E14, E16 and E18. Vimentin (VIM) cortical expression (an RGC cytoskeletal marker) and S-100b protein (a neurotrophic factor, cytosolic marker of RGC during embryonic development) were decreased in all gestational ages (for VIM control vs EtOH; E12: 27.99 ± 0.90 vs 11.85 ± 1.27 [$P < 0.0001$]; E14: 23.9 ± 0.67 vs 13.44 ± 0.55 [$P < 0.0001$]; E16: 19.04 ± 0.83 vs 10.07 ± 1.05 [$P < 0.0001$]; E18: 16.59 ± 0.88 vs 6.71 ± 0.33 [$P < 0.0001$]; for S100b control vs EtOH; E12: 19.55 ± 1.25 vs 10.56 ± 1.41 [$P < 0.0001$]; E14: 21.23 ± 0.52 vs 14.55 ± 0.41 [$P < 0.0001$]; E16: 15.47 ± 0.87 vs 8.28 ± 0.85 [$P = 0.0002$]; E18: 13.69 ± 1.02 vs 6.71 ± 1.23 [$P = 0.0007$]). Treated fetuses showed cerebral cortex thickness reduction at embryonic days E16 and E18 (control vs EtOH; E12: 0.40 ± 0.02 vs 0.37 ± 0.01 [$P = 0.1787$]; E14: 0.59 ± 0.02 vs 0.57 ± 0.02 [$P = 0.548$]; E16: 1.55 ± 0.07 vs 1 ± 0.07 [$P < 0.0001$]; E18: 1.97 ± 0.09 vs 1.59 ± 0.0 [$P = 0.0015$]). Also, at birth, treated offspring showed body weight reduction. Our data suggest that PEE strongly affects RGC, neuronal migration and corticogenesis. These morphological alterations could be involved in the pathophysiology of neurodevelopment disorders observed in the children of alcoholic mothers affected by the fetal alcohol syndrome.

Palavras-Chave: prenatal ethanol exposure, corticogenesis, cortical dysplasia, radial glia

Apoio Financeiro: Grants PICT 31964, UBACYT M-072, PIP 5034

C.02.005

The effect of aseptic prenatal inflammation on schizophrenia-like behaviours depends on the developmental stage and strength of maternal immune activation

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OBJECTIVES: Maternal infection during pregnancy has been hypothesised to increase the risk of developing schizophrenia in the offspring. Immune responses to infection change as pregnancy progresses and this, along with the stage of foetal development, are proposed to determine the nature of the behavioural alterations in the offspring. To understand the contribution of maternal immune activation during pregnancy on the offspring's behavioural alterations, turpentine (TURP), an aseptic inflammatory agent was used. The use of TURP allows testing the hypothesis that the inflammatory response *per se*, rather than the exogenous immunogen, contributes to the aetiology of psychiatric disorders. **METHODS:** TURP or saline (SAL) were injected at gestational day (GD)15 or 18 (i.m., 100ml/rat, n=4-9/group). Maternal fever was measured. The adult male offspring's behaviour was evaluated using pre-pulse inhibition (PPI) of acoustic startle and amphetamine (AMPH)-induced locomotion (AIL, 2mg/kg). Tyrosine hydroxylase (TH) levels in the mesocorticolimbic dopaminergic regions were determined by Western blot in the adult offspring. **RESULTS:** Fever response to TURP was greater at GD15 than at GD18 (38.05 ± 0.16 vs. 37.39 ± 0.13 °C, $p < 0.01$). The offspring from G18 TURP treated mothers showed a significant decrease in PPI at the highest pre-pulse intensity (0.79 ± 0.07 vs. 1 ± 0.04 a.u. in controls $p < 0.01$); in contrast, a global decrease in PPI was found in adult offspring of mothers treated at GD15 (21 ± 3.9 vs. $34 \pm 3.7\%$ PPI in control offspring, $p < 0.05$), which correlated with the maternal febrile response ($r^2 = 0.39$, $p < 0.05$). AIL was not affected in any of the TURP-treated offspring, however it positively correlated with maternal febrile response at GD 15 ($r^2 = 0.7$, $p < 0.01$). The offspring treated at GD 15 presented as well decreased levels of TH in the ventral tegmental area ($9 \pm 2.2\%$ in TURP vs. $100 \pm 17\%$ in SAL treated offspring, $p < 0.01$). **CONCLUSIONS:** The effects of the immune challenge were more marked at GD 15 than those at GD 18, suggesting that the consequences of maternal immune activation in the adult offspring vary with the stage of pregnancy and the magnitude of the maternal febrile response.

Palavras-Chave: inflammation, fever, pregnancy, schizophrenia, behaviour, dopamine, tyrosine hydroxylase

Apoio Financeiro: CIHR Neuroinflammation training program, NARSAD

**CONGENITAL HYPOTHYROIDISM LEADS TO CYTOSKELETON
HYPERPHOSPHORYLATION AND MAPK ACTIVATION IN CEREBRAL CORTEX OF RATS**

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Objectives: Congenital hypothyroidism is associated with mental retardation and several neurological deficits. The aim of this study was to evaluate the effect of congenital hypothyroidism in the intermediate filament (IF) phosphorylation and immunocontent, as well as the involvement of extracellular signal-regulated kinase 1/2 (ERK1/2), Jun N-terminal kinase (JNK) and p38^{MAPK} activation in cortical neural cells.

Methods: Congenital hypothyroidism was induced in rats by adding 6-propyl-2-thiouracil in the drinking water from gestation day 9, and continually up to post-natal day 15. Cerebral cortex slices were incubated with ³²P orthophosphate for 30 min. The IF-enriched cytoskeletal fraction was obtained and the ³²P incorporated into IF proteins was measured. The immunocontent of IF proteins, ERK1/2, JNK and p38^{MAPK} was analyzed by Western blot. Results are expressed as % of controls.

Results and discussion: Congenital hypothyroidism induced hyperphosphorylation of neuronal intermediate filament (IF) proteins, neurofilaments of high (135.07±3.8%), medium (155.8±4.9%) and low (132.9±5.3%) molecular weight (NF-H, NF-M and NF-L, respectively) without altering the phosphorylation level of astrocyte IF proteins, glial fibrillary acidic protein (GFAP= 112.1±5.1%) and vimentin (109.9±6.7%) in cerebral cortex of rats. Furthermore, the immunocontent of GFAP, NF-L, NF-M and NF-H was down-regulated (76.6±2.7, 65.8±2.5, 48.8±1.5 and 55.2±1.5%, respectively), while vimentin was unaltered in tissue homogenate of hypothyroid animals. Moreover, we verified the activation of mitogen-activated protein kinases (MAPKs) in hypothyroid rats. Results demonstrated increased ERK1/2 phosphorylation (138.9±3.1%) without altering JNK and p38^{MAPK} phosphorylation. Taken together, these evidences suggest that the thyroid status could modulate the integrity of neuronal cytoskeleton acting on the endogenous NF-associated phosphorylating system and that such effect could be related to ERK1/2 modulation. These events could be somehow related to the neurological dysfunction described in hypothyroidism.

Palavras-Chave: hypothyroidism; intermediate filaments; phosphorylation; MAPK pathway.

Apoio Financeiro: CNPq, CAPES, FAPERGS, PROPESq-UFRGS

TRATAMENTO *in vivo* COM ÁCIDO QUINOLÍNICO ALTERA A FOSFORILAÇÃO DOS FILAMENTOS INTERMEDIÁRIOS EM ESTRIADO DE CÉREBRO DE RATOS

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O ácido quinolínico (AQ) é produzido no metabolismo do triptofano e está envolvido na etiologia de algumas doenças neurodegenerativas. Sua neurotoxicidade é atribuída à ação agonista de receptores N-metil-D-aspartato, peroxidação lipídica, entre outras. Os filamentos intermediários (FI) são importantes constituintes do citoesqueleto e a fosforilação das subunidades dos FI é o principal mecanismo regulatório de suas funções celulares. Metabólitos que se acumulam em várias doenças neurodegenerativas são capazes de alterar a fosforilação de proteínas do citoesqueleto. Portanto, o objetivo do presente estudo é verificar o efeito *in vivo* do AQ sobre a fosforilação das subunidades de FI (neurofilamentos, vimentina e proteína glial fibrilar ácida -GFAP) em fatias de estriado de cérebro de ratos de 30 dias de idade. O estriado de ratos de 30 dias de idade sofreu uma injeção de AQ (150 μ moles), após 30 minutos os animais foram decapitados, o estriado dissecado e fatiado. As fatias de tecido foram incubadas com ortofosfato radioativo (³²P-ortofosfato) e a fração citoesquelética foi obtida por extração em tampão de alta força iônica na presença de inibidores de proteases. As proteínas fosforiladas foram analisadas em SDS-PAGE e a radioatividade incorporada na vimentina, na GFAP e nas subunidades de médio e baixo peso molecular dos neurofilamentos (NF-M e NF-L, respectivamente) foi medida por densidade óptica das bandas correspondentes nos autoradiogramas. Os resultados mostraram que a injeção de AQ aumentou significativamente a fosforilação da vimentina (132,5 $\pm$ 12%), GFAP (140 $\pm$ 11%) e NF-L (135 $\pm$ 14%), quando comparados com o grupo controle (100%). Também se verificou que não houve diferença significativa na fosforilação do NF-M (111 $\pm$ 12%) quando comparado com o grupo controle. Com base nos resultados preliminares podemos concluir que a injeção intraestriatal de AQ causa hiperfosforilação dos FI de astrócitos (vimentina e GFAP), bem como da subunidade NF-L dos neurofilamentos. Considerando a importância da fosforilação na função das células neurais, podemos propor que o AQ pode contribuir para a disfunção neuronal em diversas doenças neurodegenerativas.

Palavras-Chave: Fosforilação, ácido quinolínico, citoesqueleto, filamentos intermediários.

Apoio Financeiro: CNPq, CAPES, FAPERGS, PROPESq-UFRGS, Rede Instituto Brasileiro de Neurociência

Mild cognitive deficits associated to neocortical microgyria in mice with genetic deletion of cellular prion protein

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Objective: Cellular prion protein (PrP^C) has been implicated with the modulation of neuronal apoptosis, neurite outgrowth and maintenance – all processes involved in the neocortical development. Malformations of cortical development are frequently associated with neurological conditions including mental retardation and epilepsy. The aim of the present study was to investigate the behavioral performance of mice lacking the PrP^C submitted to the neonatal freezing lesion model of microgyria.

Methods: On postnatal day 1, unilateral neonatal freezing-lesion was induced in the right hemisphere of wide type (*Prnp*^{+/+}) and knockout (*Prnp*^{0/0}) mice. Behavioral performance on olfactory, locomotor, anxiety, learning, and memory tasks were evaluated in the adulthood stage of mouse life (3 months old).

Results: No significant differences regarding the locomotor activity and olfactory discrimination ability were observed in animals with microgyria independently of their genetic background. Lesioned mice presented a tendency to increased anxiety-related responses in the elevated plus-maze [%open-arm time - *Prnp*^{+/+} sham: 12.1±4.3; CL: 6.3±2.5; *Prnp*^{0/0} sham: 10.0±3.9; CL: 7.3±2.8] and [%open-arm entries - *Prnp*^{+/+} sham: 19.7±4.2; CL: 11.1±3.3; *Prnp*^{0/0} sham: 17.5±5.6; CL: 10.4±3.6]. The freezing-lesioned mice performed poorly when compared to sham-operated animals in the social recognition task [investigation time - *Prnp*^{+/+} sham: 90.90±12.29; CL: 101.71±8.04; and *Prnp*^{0/0} sham: 104.90±14.69; CL: 118.14±13.22]. In the step-down inhibitory avoidance task, the genetic deletion of PrP^C disrupted the long-term retention of inhibitory avoidance. However, no significant effects of freezing lesion procedure were observed [latency (median and interquartile range) to step down (s) - *Prnp*^{+/+} sham: 144.5±58/180; CL: 180±50/180; and *Prnp*^{0/0} sham: 33.0±22/56; CL: 22.5±4.5/81.5].

Conclusion: Genetic deletion of PrP^C disrupted short- and long-term memory processes, these findings reinforce the involvement of PrP^C in learning and memory processes and provide convincing evidence that malformations of cortical development induced by freezing lesion in mice are associated to specific cognitive deficit in adulthood.

Palavras-Chave: Cellular prion protein; Freezing lesion; Malformations of cortical development; Polimicrogyria; Behavior; Cognition; Knockout mice

Apoio Financeiro: CNPq, CAPES, FAPESC

ARBORIZAÇÃO DENDRÍTICA DE NEURÔNIOS ESPINHOSOS MÉDIOS DO ESTRIADO DORSAL DE RATOS SUBMETIDOS À HIPÓXIA-ISQUEMIA NEONATAL E A NOVE SEMANAS DE ENRIQUECIMENTO AMBIENTAL

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Objetivo: A hipóxia-isquemia neonatal (HIN) provoca injúria encefálica e déficits cognitivos e o enriquecimento ambiental (AE) resulta em efeitos benéficos sobre a performance comportamental e atenua danos estruturais cerebrais. O estriado é uma das regiões primárias das lesões encefálicas provocadas pela HIN tanto em ratos como em seres humanos. O objetivo desse trabalho foi estudar, utilizando a técnica de Golgi, a arborização dendrítica dos neurônios espinhosos médios do estriado dorsal contralateral à oclusão arterial, de ratos HIN submetidos à estimulação por AE.

Métodos: No 7º dia de vida, ratos Wistar machos tiveram a artéria carótida comum direita ocluída e foram expostos por 90 min a uma atmosfera hipóxica (8% oxigênio e 92% de nitrogênio). Grupos: controle (CTAP, n=11 neurônios) e hipóxico-isquêmicos (HIAP, n=12) mantidos em ambiente padrão; e controle (CTAE, n=11) e hipóxico-isquêmicos (HIAE, n=5) submetidos ao AE (desde o desmame, 1 h/dia, 6 dias/semana/9 semanas). Três dias após o período de estimulação, os animais foram anestesiados e perfundidos com paraformaldeído 4% e ácido pícrico 1,5% em tampão fosfato 0,1M e pH 7,4. Os encéfalos foram removidos e fixados na mesma solução por 24 h, sendo então coronalmente seccionados com vibratomo (200 µm) e submetidos à técnica de Golgi. Os neurônios espinhosos médios do estriado dorsal contralateral foram desenhados com auxílio de uma câmara clara acoplada a um microscópio e os ramos dendríticos foram contados.

Resultados: A ANOVA de uma via não mostrou diferenças significativas ($p > 0,05$) no número de ramos dendríticos primários (média $\pm$ EP; 6,6 $\pm$ 0,7; 3,8 $\pm$ 0,3; 4,4 $\pm$ 0,2; 4,2 $\pm$ 0,4; CTAP, CTAE, HIAP, HIAE, respectivamente), secundários (7,9 $\pm$ 0,7; 7,4 $\pm$ 0,4; 7,7 $\pm$ 0,5; 8 $\pm$ 0,9), terciários (10,7 $\pm$ 1; 8,8 $\pm$ 0,9; 9,1 $\pm$ 0,9; 10,4 $\pm$ 1,5), quaternários (7,7 $\pm$ 1,1; 7,2 $\pm$ 1,1; 8 $\pm$ 1,3; 5,6 $\pm$ 1,3), quinta ramificação (3,6 $\pm$ 0,8; 2,2 $\pm$ 0,2; 3,6 $\pm$ 0,7; 3,2 $\pm$ 0,5) e sexta ramificação (1,1 $\pm$ 0,6; 0,2 $\pm$ 0,2; 0,5 $\pm$ 0,4; 0,8 $\pm$ 0,5) nos grupos analisados.

Conclusão: A hipóxia-isquemia e a estimulação por enriquecimento ambiental não afetaram o número de ramos dendríticos dos neurônios espinhosos médios do estriado dorsal contralateral à oclusão arterial.

Palavras-Chave: Hipóxia-isquemia neonatal; enriquecimento ambiental; estriado dorsal; neurônios espinhosos médios; arborização dendrítica; técnica de Golgi

Apoio Financeiro: CNPq

PREVENÇÃO PRIMÁRIA DA SÍNDROME DE DOWN: ANÁLISE DE POLIMORFISMOS EM GENES PARTICIPANTES DO METABOLISMO DO FOLATO.

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OBJETIVOS: Apesar da alta incidência da síndrome de Down (SD), os mecanismos moleculares que levam à não-disjunção do cromossomo 21 e à trissomia deste cromossomo não foram totalmente elucidados. Estudos recentes demonstraram uma possível ligação entre a SD e polimorfismos em genes maternos envolvidos no metabolismo do folato, micronutriente essencial para a biossíntese de nucleotídeos, o reparo do DNA e as reações de metilação. Desta forma, polimorfismos em genes maternos participantes da via do folato poderiam predispor à instabilidade genômica, à hipometilação do DNA e à segregação anormal do cromossomo 21, levando à formação de um embrião trissômico. Neste estudo, comparamos a frequência dos polimorfismos *CBS* 844ins68, *MTR* 2756A>G e *RFC*-1 80G>A em mães jovens de indivíduos com SD em comparação com mães de indivíduos cromossomicamente normais, de maneira a observar se estas variantes funcionam como fatores de predisposição para a SD.

METODOLOGIA: Amostras de DNA foram extraídas a partir do sangue periférico de 114 mães jovens (<35 anos) de crianças com SD e de 110 mães controle de mesma faixa etária. Para a triagem dos polimorfismos foi realizada a técnica de PCR-RFLP, seguida da visualização dos produtos amplificados a partir de eletroforese em géis de poliacrilamida.

RESULTADOS: Não houve diferença estatisticamente significativa entre as distribuições alélica e genotípica encontradas nas mães caso em relação às mães controle ($\chi^2_{(2)} = 2,15$; IC 95%; $P = 0,34$, $\chi^2_{(2)} = 4,38$; IC 95%; $P = 0,11$ e $\chi^2_{(2)} = 0,93$; IC 95%; $P = 0,63$, respectivamente). Além disso, ao realizarmos a interação multiplicativa entre os três polimorfismos entre as amostras caso e controle, também não foram observadas diferenças significativas.

CONCLUSÃO: Nossos resultados sugerem que o risco de nascimento de uma criança com SD parece não estar relacionado à presença dos três polimorfismos analisados em nossa população. No entanto, a complexidade do metabolismo do folato, o qual envolve a participação de vários genes, não exclui a participação de outros polimorfismos a ele relacionados. Esta contradição, também observada por outros autores, deve ser esclarecida mediante a realização de demais estudos.

Palavras-Chave: Síndrome de Down, MTR, CBS, RFC-1, folato, metilação,

Apoio Financeiro: CNPq, FAPERJ, CEPUERJ

CALIDAD DE VIDA EN PACIENTES CON TRASTORNO BIPOLAR EUTIMICOS

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Indroduccion: La calidad de vida (CV) se comienza a considerar como una variable a tener en cuenta en los trabajos sobre eficacia terapéutica. **Objetivo:** Este trabajo evaluó la calidad de vida en los individuos con trastorno bipolar en fase de eutimia. **Metodos:** Se trata de un estudio de diseño transversal y comparativo compuesto por dos cohortes, uno de pacientes y otro control, entre los 20 y 79 años, de ambos os sexos. La muestra se obtuvo de 36 pacientes con diagnóstico clínico de trastorno bipolar según el DSM IV, eutímicos, provenientes de la planta de Psiquiatría del Hospital Clínico Universitario Lozano Blesa de Zaragoza, valorados durante 1997 a 2005. Cohorte de sujetos controles: constituido por 36 sujetos sanos provenientes da Provincia de Zaragoza, residentes durante os años 2004 y 2005. Los instrumentos utilizados incluí la Escala de Bienestar General (EBEG), la Escala de Adaptación al Estrés (ACOP) y el Cuestionario Calidad de Vida (QLOC). Los tests estadísticos utilizados fueran: test de Kolmogorov-Smirnov; Test paramétrico de ANOVA; Test de Kruskal-Wallis y Test Chi-Cuadrado. El nivel de significación fijado para todas las pruebas fue 5% ($p < 0,05$). **Resultados:** Los resultados de este trabajo sugieren que las adaptaciones laboral y social son muy deficientes en gran parte de estos pacientes. **Conclusiones:** Los pacientes bipolares presentan una más baja adaptación al estrés y frente a situaciones psicológicamente intensas, presentan disfunción social y peor socialización. En la calidad de vida o factor más deteriorado es el interpersonal. **Palavras-Chave:** calidad vida. trastorno bipolar

IMAGEM CORPORAL EM PACIENTES COM TRANSTORNO BIPOLAR EM FASE DE EUTIMIA

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Introdução: A Imagem Corporal está relacionada aos elementos perceptivos, atitudes, emoções e reações da personalidade dos indivíduos ante seu corpo. Pesquisas apontam como problemas que acompanham a imagem corporal: baixa auto-estima, problemas com identidade de gênero, ansiedade, problemas nas relações sexuais e depressão. **Objetivo:** Avaliar a imagem corporal em indivíduos com transtorno bipolar em fase de eutimia. Foram incluídos no estudo 36 sujeitos com transtorno bipolar (TB) e 36 controles (GC), com idades entre 20 e 79 anos da cidade de Zaragoza (Espanha). Como Instrumento de Medida foi utilizado o Questionário da Figura Corporal (Body Shape Questionnaire – BSQ, adaptado para a população espanhola por Raich et al. 1996). **Resultados:** No grupo TB, 33 sujeitos relataram satisfação com a imagem corporal, enquanto que no GC, 35 sujeitos relataram satisfação. Não foi encontrada diferença estatisticamente significativa entre os grupos (ANOVA; $p=0,5649$ y/o Kruskal-Wallis; $p=0,8522$). A média do BSQ no grupo TB foi 59,25. O valor mínimo encontrado foi 32 e o máximo de 153. No GC a média encontrada foi 55,89. O valor mínimo encontrado foi 34 e o máximo 114. Em nosso estudo os resultados diferem de estudos anteriores, onde nos pacientes bipolares a média foi de 59,2 e no GC a média de 55,9, sugerindo que os dois grupos do presente estudo assinalaram uma menor insatisfação com sua imagem corporal. **Conclusões:** Apesar do grupo TB mostrar uma menor satisfação com a imagem corporal do que o grupo controle, não encontramos diferenças significativas entre os grupos. Outros estudos referentes à imagem corporal nesses pacientes deveriam ser realizados.

Referência:

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Palavras-Chave: Imagem corporal. Transtorno bipolar

EFEITOS TARDIOS DO ENRIQUECIMENTO AMBIENTAL SOBRE OS NÍVEIS DE BDNF E O ESTADO OXIDATIVO EM HIPOCAMPO DE RATOS SUBMETIDOS À HIPÓXIA-ISQUEMIA NEONATAL

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Objetivos: Resultados prévios do nosso grupo demonstraram que o enriquecimento ambiental (EA) recuperou o déficit mnemônico sem alterar a atrofia em hipocampo de ratos submetidos à hipóxia-isquemia (HI) neonatal. No presente estudo investigamos se os níveis do Fator Neurotrófico derivado do Encéfalo (BDNF) e a modulação do estado oxidativo no hipocampo estão relacionados com a neuroproteção cognitiva do EA em animais hipóxico-isquêmico adultos.

Métodos: Ratos Wistar machos foram distribuídos nos grupos: controle mantidos em ambiente padrão (CTAP); hipóxico-isquêmicos mantidos em AP (HIAP); controle enriquecido (CTEA); e HI enriquecido (HIEA), tendo ambos os grupos 5 animais. No 7º dia de vida, os animais tiveram a artéria carótida comum direita ocluída e foram expostos a uma atmosfera hipóxica. A partir do desmame, foram submetidos ao EA (60 min, 6 dias/semana, por 9 semanas). Após 24h do EA, sacrificaram-se os animais e dissecaram-se os hipocampus (cada hemisfério foi analisado separadamente). O estado oxidativo foi avaliado pela determinação dos níveis de radicais livres, pela lipoperoxidação e pela atividade da enzima superóxido dismutase (SOD). O nível de BDNF foi avaliado por meio de ensaio imunoenzimático (ELISA).

Resultados: A ANOVA seguida do teste de Duncan não indicou diferenças entre os grupos nos níveis de radicais livres e na lipoperoxidação. No entanto, os resultados revelaram que a atividade da SOD foi aumentada no hipocampo ipsilateral à isquemia no grupo HIAP ($0,471 \pm 0,03$) em comparação com os grupos controle (CTAP: $0,357 \pm 0,02$ e CTEA: $0,342 \pm 0,01$); o grupo HI enriquecido ($0,411 \pm 0,02$) não foi diferente estatisticamente dos demais grupos. Da mesma forma, foram encontrados maiores níveis de BDNF no hipocampo ipsilateral dos animais do grupo HIAP (105 ± 33) em relação aos grupos CTAP ($36,6 \pm 4$), CTEA ($34,8 \pm 6$) e HIAE ($27,6 \pm 2$). No hipocampo contralateral não foram identificadas quaisquer diferenças nos parâmetros observados.

Conclusões: O efeito neuroprotetor cognitivo do enriquecimento ambiental pode ser dependente da diminuição da expressão de BDNF e da atividade da enzima superóxido dismutase no hipocampo de ratos adultos submetidos à hipóxia-isquemia neonatal.

Palavras-Chave: Hipóxia-isquemia; Enriquecimento ambiental; Hipocampo; BDNF; Estresse oxidativo

Apoio Financeiro: CNPq

PERCEPÇÃO VISUAL DE CRIANÇAS COM DESNUTRIÇÃO PREGRESSA

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Objetivo: Pesquisas vêm enfocando alterações no sistema nervoso central (SNC) decorrentes da desnutrição energético-proteica (DEP). Este estudo objetivou medir a função de sensibilidade ao contraste e avaliar possíveis alterações no sistema visual (SV) de crianças com DEP pregressa, uma das modalidades de desnutrição (conforme Classificação de Waterlow) caracterizada pela baixa estatura de acordo com a idade e sexo. A FSC é uma ferramenta útil para avaliar atributos ópticos e neurais da visão.

Método: Participaram da pesquisa 10 crianças de ambos os sexos, sendo cinco com DEP pregressa e cinco sem DEP e peso normal. Todas estavam livres de doenças oculares e tinham acuidade visual normal. Foram utilizados estímulos de frequências senoidais de 0,25; 1; 2 e 8 cpg, circulares, com um diâmetro de 7,2 graus de ângulo visual. Estes foram gerados em tons de cinza num monitor LG de 19 polegadas, com resolução de 1024 x 768 pixels. As medidas foram obtidas, binocularmente, a uma distância de 150 cm da tela do monitor, usando um delineamento com medidas repetidas e o método psicofísico da escolha forçada. Nesse paradigma, apresentam-se pares de estímulos na tela, um com uma das frequências acima e o outro homogêneo com a luminância média (42 cd/m²). A tarefa da criança era escolher, entre os dois estímulos, aquele que continha a frequência de teste.

Resultados: As análises utilizando a ANOVA para medidas repetidas revelaram diferenças significantes entre os grupos, com $[F(1; 118) = 25,85 \text{ e } p < 0,001]$. No entanto, o Teste Post Hoc Tukey HSD mostrou diferença significativa nas frequências de 0,25 e 2 cpg ($p < 0,001$). As crianças com DEP pregressa precisaram da ordem de 1,8; 1,9; e 2,2 vezes mais contraste para perceber as frequências de 0,25; 1; e 2 cpg, respectivamente, e da ordem de 1,1 vezes menos contraste para perceber a frequência de 8 cpg, do que as crianças sem DEP.

Conclusão: Os resultados sugerem que a DEP pregressa altera a percepção visual de contraste de crianças, principalmente nas frequências senoidais baixas e médias.

Palavras-Chave: Palavras Chave: desnutrição pregressa; sensibilidade ao contraste; crianças; sistema visual.

Apoio Financeiro: CNPQ

SENSIBILIDADE AO CONTRASTE DE CRIANÇAS COM DESNUTRIÇÃO ATUAL E PREGRESSA

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Objetivo: A desnutrição energético-protéica (DEP) é um dos principais problemas de saúde infantil nos países em desenvolvimento. Poucos estudos investigam os efeitos da DEP no desenvolvimento dos processos sensoriais em humanos. O objetivo do presente estudo foi medir a função de sensibilidade ao contraste (FSC) de crianças com DEP atual e pregressa e avaliar os efeitos da DEP na percepção visual de contraste.

Método: Participaram do estudo dez crianças de 7 a 10 anos de ambos os sexos, cinco com desnutrição pregressa e cinco com desnutrição atual. As mesmas foram classificadas de acordo com os parâmetros antropométricos de Waterlow. Todas as crianças apresentavam acuidade visual normal ou corrigida. Foram utilizados estímulos de frequências espaciais de 0,25; 1; 2 e 8 cpg. Estes eram circulares com um diâmetro de aproximadamente 7,2 graus de ângulo visual e foram gerados em tons de cinza em um Monitor LG de 19 polegadas, com resolução de 1024 x 768 pixels. As medidas foram obtidas, binocularmente, a uma distância de 150 cm da tela do monitor, usando um delineamento experimental com medidas repetidas e o método psicofísico da escolha forçada. Nesse paradigma, apresentam-se pares de estímulos na tela, um com uma das frequências acima e o outro com a luminância média (42 cd/m²). A tarefa do participante foi escolher, entre os dois estímulos, aquele que continha a frequência espacial.

Resultados: As análises (ANOVA) mostraram diferenças significantes entre os grupos [$F(1; 118) = 5,9247$ e $p = 0,016429$; $p < 0,05$], já o teste post-hoc Tukey HSD mostrou diferença significativa na frequência de 2 e 8 cpg ($p < 0,001$). As crianças com DEP pregressa foram da ordem de 1,5 menos sensíveis do que as com DEP atual na frequência de 2,0 cpg, enquanto as crianças com DEP atual foram da ordem de 1,5 menos do que as com DEP pregressa na frequência de 8,0 cpg.

Conclusão: Esses resultados indicam que a desnutrição atual e pregressa interagem discretamente com os mecanismos sensoriais que processam frequências espaciais médias e altas.

Palavras-Chave: desnutrição pregressa; desnutrição atual; função de sensibilidade ao contraste.

Apoio Financeiro: CNPQ

Nicotinamide prevents the long lasting effects of perinatal asphyxia on spatial and working memory, anxiety and delayed cell death.

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The present study investigated whether perinatal asphyxia induces behavioural and morphological alterations in adult male rats. The possible neuroprotection of nicotinamide on the consequences of perinatal asphyxia was also investigated. Asphyxia was induced *in vivo* by immersing foetuses-containing uterine horns, removed from ready-to-deliver rats into a water bath at 37 °C for 20 min. The caesarean-delivered siblings, not exposed to perinatal asphyxia, were used as controls. Saline or nicotinamide (0.8 mmol/kg, i.p.) was administered to control and asphyxia-exposed animals 24, 48, and 72 h after birth. Approximately, 3 months after birth, control and asphyxia-exposed animals were examined by a behavioural test battery. A parallel group of rats was used for morphological studies, focusing on mossy fibres sprouting and cell death.

At adulthood, asphyxia- exposed rats displayed: (i) deficits in non-spatial working memory, assessed by a novel object recognition task; (ii) deficits in spatial memory, assessed by a Y maze; (iii) increased anxiety, assessed by an elevated plus-maze test; (iv) apoptosis assessed in whole hippocampus by nuclear Hoechst staining; (v) mossy fibres sprouting restricted to the *stratum oriens* of dorsal hippocampus, assessed by the Timm's staining technique. The effect of perinatal asphyxia on spatial and working memory, anxiety and apoptosis in the hippocampus was prevented by nicotinamide treatment. However, no effect of nicotinamide was observed on mossy fibres sprouting.

It is proposed that inhibition of over activated Poly(ADP-ribose) polymerase with nicotinamide constitutes a promising neuroprotective strategy against the long lasting effects induced by perinatal asphyxia.

Palavras-Chave: Perinata asphyxia, behaviour, nicotinamide, plasticity, apoptosis, sprouting

Apoio Financeiro: Fondecyt 11070192, 1080447.

Repetitive cortical electrical stimulation facilitates spreading depression in adult rats submitted during brain development to different nutritional conditions

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It has been previously demonstrated that the repetitive cortical electric stimulation (rCES) increases cortical spreading depression (CSD) propagation in the stimulated hemisphere of early severe malnourished rats (IINN-ELS Annals 51:35, 2007). We here tested the possibility that another kind of nutritional restriction, which results in a moderate degree of malnutrition, could influence the rCES-effect on SD. The nutritional status was manipulated during lactation by increasing the litter size from 6 (control size) to 12 pups (large-size litters; group LS12). This moderate malnutrition group was compared with the severe malnutrition model (M-BRD), induced in sucklings by feeding their dams a *low-protein* diet ("Basic Regional Diet", BRD, with 8% of protein). After weaning, nutritional recovery was performed by feeding the pups the standard lab-chow diet (with 23% protein). At 60 days of age, CSD was recorded on 2 cortical points of each hemisphere. After a 2-h "baseline recording", rCES (600 μ A, 5ms, 1 Hz) was applied for 20 min on the left cortex with a bipolar electrode placed between the CSD recording electrodes. After rCES, CSD recording continued for 2 h. Compared to the control group, (mean $\pm$ sd: 284.03 $\pm$ 15.18g), the malnourished ones presented lower body weights (210.81 $\pm$ 16.69g and 272.41 $\pm$ 13.76g for the M-BRD and LS12, respectively), on the CSD recording day. Malnutrition also increased CSD velocities of propagation (M-BRD > LS12 > control). In all groups, rCES increased the CSD velocity of propagation, in the stimulated hemisphere (mean $\pm$ sem: D-DBR, 13.19% $\pm$ 1.02; LS12, 10.13% $\pm$ 1.70; control, 25.70% $\pm$ 5.05). The data suggested a similar effect of rCES on CSD irrespective of the previous nutritional condition, during brain development. They also indicated that the moderate malnutrition model does not influence the rCES-effects on CSD. This effect seems to be due to the increase in the cortical excitability provoked by the electric stimulation. The data represent scientific support to the understanding of the electrical stimulation effects in some neurological pathologies, associated or not to nutritional alterations during the brain development.

Palavras-Chave: Cortical electrical stimulation; spreading depression; nutritional status

Apoio Financeiro: CNPq, CAPES, UFPE, FINEP/IBN-Net

PERCEPÇÃO VISUAL DE CRIANÇAS COM DESNUTRIÇÃO ATUAL

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Objetivo: Estudos indicam que a desnutrição energético-protéica (DEP) afeta o sistema nervoso central. Este estudo teve como objetivo medir a função de sensibilidade ao contraste (FSC) de crianças com DEP e verificar possíveis alterações na percepção visual relacionada à DEP atual.

Método: Participaram do estudo 16 crianças de 7 a 10 anos de ambos os sexos, oito sem desnutrição e oito com desnutrição atual, classificadas conforme os parâmetros antropométricos de Waterlow. Todas as crianças apresentavam acuidade visual normal ou corrigida e estavam livres de doenças identificáveis, com exceção da desnutrição. Os estímulos com frequências espaciais de 0,25; 1; 2 e 8 ciclos por grau de ângulo visual (cpg) eram circulares, com diâmetro de 7,25 graus de ângulo visual. Os mesmos foram apresentados a 150 cm de distância da tela de um Monitor LG de 19 polegadas, com resolução de 1024 x 768 pixels. As medidas foram realizadas binocularmente, utilizando um delineamento experimental com medidas repetidas e o método psicofísico da escolha forçada. Durante o experimento, eram apresentados pares de estímulos, sendo que um deles continha a frequência espacial, enquanto o outro era um padrão homogêneo com a luminância média (42,6 cd/m²). A tarefa dos participantes foi escolher sempre o estímulo com a frequência espacial. Para cada três acertos consecutivos era decrescida uma unidade de contraste (20%) e para cada erro era acrescida a mesma unidade.

Resultados: As análises com a ANOVA para medidas repetidas apresentaram diferenças significantes entre os grupos [$F(1, 190) = 38,5$ e $p < 0,0001$], porém o teste post-hoc Tukey HSD só mostrou diferença significativa na frequência de 8,0 cpg ($p < 0,0001$). As crianças com desnutrição precisaram da ordem de 1,3; 1,3; 1,5 e 1,4 vezes mais contraste para perceber as frequências de 0,25; 1; 2 e 8 cpg, respectivamente.

Conclusão: Os resultados sugerem que a desnutrição atual altera a percepção visual de contraste na faixa de frequência espacial mais alta.

Palavras-Chave: desnutrição atual; percepção visual; sensibilidade ao contraste.

Apoio Financeiro: CNPQ

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Evidence that the medium-chain fatty acids accumulated in MCAD deficiency cause oxidative damage in rat brain

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Objectives: Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most frequent disorder of fatty acid oxidation. Affected patients present tissue accumulation of the medium-chain fatty acids octanoic (OA), decanoic (DA) and cis-4-decenoic acids. Clinical presentation is characterized by a wide spectrum of neurological alterations accompanied by lethargy that may develop into coma or even death. The aim of the present work was to investigate the effect of OA and DA on oxidative stress parameters.

Methods: The effects of OA and DA (0.5, 1 and 3 mM) on lipoperoxidation, protein oxidative damage and non-enzymatic antioxidant defenses were evaluated in brain from young (30-day-old) male *Wistar* rats.

Results: It was verified that both DA and OA significantly induced lipoperoxidation, evidenced by increased chemiluminescence (DA: up to 38%, $F(3,19)=3.26$, $p<0.05$, $n=5$; OA: up to 31%, $F(3,23)=3.35$, $p<0.05$, $n=6$) and thiobarbituric acid reactive species levels (DA: up to 147%, $F(3,23)=40.7$, $p<0.001$, $n=6$; OA: up to 36%, $F(3,23)=8.18$, $p<0.001$, $n=6$). However, only DA caused protein damage, as observed by an increase in carbonyl content (up to 340%, $F(3,15)=25.9$, $p<0.001$, $n=4$) and oxidation of sulfhydryl groups (up to 39%, $F(3,19)=15.5$, $p<0.001$, $n=5$). Moreover, non-enzymatic antioxidant defenses, measured by the decreased total antioxidant capacity (up to 43%, $F(3,15)=22.9$, $p<0.001$, $n=4$) and glutathione (GSH) levels (up to 36%, $F(3,19)=9.09$, $p<0.001$, $n=5$), were decreased only by DA.

Conclusion: Our present results show that medium-chain fatty acids cause oxidative stress in rat brain, suggesting that oxidative damage may be involved in the pathophysiology of the brain injury found in patients affected by MCADD.

Palavras-Chave: MCAD deficiency, oxidative stress

Apoio Financeiro: CNPq, PRONEX, FINEP research grant Rede Instituto Brasileiro de Neurociência (IBN-Net) # 01.06.0842-00.

***trans*-Glutaconic acid provokes oxidative stress in brain from young rats**

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Objectives: Glutaric acidemia type I (GAI) is an autosomal recessive metabolic disorder caused by glutaryl-CoA dehydrogenase deficiency leading to chronic tissue accumulation of glutaric, 3-hydroxyglutaric and also of *trans*-glutaconic (*tGA*) during encephalopathic crisis. The clinical manifestations of GAI are predominantly neurological. Considering that the pathophysiological mechanisms responsible for the brain damage of this disease are not yet fully established and nothing has been described about *tGA* effects on the CNS, in the present work we investigated the effect of *tGA* on various parameters of oxidative stress in brain of young rats.

Methods: The effect of *tGA* (0.01, 0.1 and 1 mM) on lipoperoxidation, protein oxidative damage and non-enzymatic antioxidant defenses were evaluated in brain from young (30-day-old) male *Wistar* rats.

Results: We observed that *tGA* significantly induced lipoperoxidation, evidenced by increased chemiluminescence (up to 34%, $F(3,23)=5.26$, $p<0.01$) and thiobarbituric acid reactive species (TBARS) levels (up to 36%, $F(3,23)=3.76$, $p<0.05$). *tGA*-induced increased TBARS levels were prevented by trolox and reduced glutathione ($F(6,27)=17.3$, $p<0.001$). Moreover, the non-enzymatic antioxidant defenses, measured by total antioxidant capacity were decreased by *tGA* (up to 21%, $F(3,19)=3.56$, $p<0.05$). However, *tGA* did not cause protein oxidative damage, evaluated by carbonyl content ($F(3,15)=0.79$, $p>0.05$).

Conclusion: The results provide evidence that *tGA* cause oxidative stress in rat brain and this may be possibly related to the pathophysiology of the brain injury found in GAI-affected patients

Palavras-Chave: glutaric acidemia type I, *trans*-glutaconic acid, oxidative stress

Apoio Financeiro: CNPq, FAPERGS, PRONEX and the FINEP research grant Rede Instituto Brasileiro de Neurociência (IBN-Net) # 01.06.0842-00

AVALIAÇÃO DA EMPATIA EM PESSOAS COM TRANSTORNO INVASIVO DO DESENVOLVIMENTO E UM GRUPO CONTROLE ATRAVÉS DO RECONHECIMENTO DE EXPRESSÕES FACIAIS

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INTRODUÇÃO: Os Transtornos Invasivos do Desenvolvimento (TID) são um grupo de condições caracterizadas pelo início na primeira infância de atrasos e déficits no desenvolvimento das habilidades sociais, comunicativas e comportamentais. No Manual de Diagnóstico e Estatística das Perturbações Mentais revisado DSM IV-R (1995), os TID comportam os quadros de autismo clássico, Transtorno Invasivo do Desenvolvimento sem outra especificação (TID-SOE), Transtorno Desintegrativo da Infância, Síndrome de Rett, Síndrome de Asperger. Um aspecto bastante marcante do endofenótipo de TID é o prejuízo da capacidade de empatia. Esta habilidade social se desenvolve nos primeiros anos de vida e permite a pessoa interagir adequadamente com o outro, compreendendo sinais faciais, e emoções do grupo social no qual está inserido. Estudos vêm buscando explicações para esta questão através de experimentos que buscam compreender como esta habilidade ocorre em pessoas com TID (Baron-Cohen, 2002). **OBJETIVO:** Avaliar e comparar empatia em pessoas com TID e de um grupo controle através de um baralho de cartas desenvolvido pelos pesquisadores, contendo expressões faciais básicas (Ekman, 1999). **MÉTODOS:** Foram avaliadas 7 participantes com TID, do sexo masculino, com idades variando de 7 a 15 anos, pareados por idade, sexo e inteligência com indivíduos com desenvolvimento considerado normal, sem TID. Os critérios de inclusão foram QI mínimo de 70, avaliado pelo Wisc III e preencher critérios para diagnóstico de TID (DSM IV). **RESULTADOS:** O grupo com TID apresentou 33,33% de respostas erradas no julgamento das expressões em fotos (66,66% de acertos), enquanto que o controle errou apenas 7,14% (92,85% de acertos). Nos desenhos, o grupo com TID errou em 35,71% das expressões (64,28% de acertos) e o controle, 4,76% (92,85% de acertos). Não foram encontradas diferenças de desempenho entre figuras e fotos nos dois grupos. Houve diferença significativa entre controle e TID nas expressões de dúvida ($p=0,031$) e nojo ($p=0,005$) no Baralho de Desenhos, e nas Fotos, na expressão de nojo ($p=0,018$), sempre com maior índice de erros do grupo TID. Quando analisado o desempenho nas Fotos e Desenhos juntos, aparece diferença estatisticamente significativa entre expressões de dúvida ($p=0,012$), nojo ($p<0,001$) e surpresa ($p=0,006$). **CONCLUSÃO:** O grupo com TID demonstrou uma dificuldade maior em reconhecer e identificar empaticamente expressões faciais consideradas básicas, do que o grupo controle. Este resultado parece reforçar pesquisas que apontam para um prejuízo na capacidade de Empatia, associada ao quadro de TID.

Palavras-Chave: Empatia - Faces - Autismo

Apoio Financeiro: MackPesquisa

C.02.022

Reconhecimento dos conceitos de cor, forma, tamanho e posição em 10 crianças com Síndrome de Rett através de rastreamento ocular

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Objetivo: Partindo-se do pressuposto de que pessoas com síndrome de Rett (SR) apresentam severos prejuízos psicomotores, verbais e cognitivos, e perda das habilidades manuais proposicionais (Hagberg et al., 2002, Schwartzman, 2003), mas utilizam o olhar com finalidade intencional (Tetzchner et al., 1996), o objetivo deste estudo foi avaliar se crianças com SR, após exposição aos conceitos de cor (vermelho, amarelo e azul), forma (círculo, quadrado e triângulo), tamanho (grande e pequeno) e posição espacial (em cima e embaixo), manifestam o reconhecimento desses conceitos com o olhar.

Método: Foram avaliadas 10 crianças com diagnóstico de SR, com idades entre 4 anos e 8 meses e 12 anos e 10 meses, através de equipamento computadorizado de rastreamento ocular.

Resultados: Os resultados não indicaram diferenças significativas no tempo de fixação do olhar das crianças quando comparados os conceitos solicitados e os não solicitados. Nas categorias "cores" e "formas" foram comparadas as porcentagens médias de fixação do olhar em cada figura-conceito a cada ordem dada. A Prova de Friedman evidenciou que nenhuma diferença significativa foi observada nas ordens relativas a cores e formas. A Prova de Wilcoxon revelou tempo de fixação significativamente maior nos estímulos "não vermelho" à ordem "vermelho" ($p = 0,067$), e aos estímulos "não quadrado" à ordem quadrado ($p = 0,066$). Na comparação dos conceitos de tamanho e de posição espacial solicitados, a Prova de Wilcoxon evidenciou tempo significativamente maior de fixação no estímulo "grande" à ordem "pequeno" ($p = 0,046$), e no estímulo "em cima" à ordem "embaixo" ($p = 0,034$).

Conclusão: Concluiu-se que, com o método utilizado, as crianças não manifestaram com o olhar o reconhecimento dos conceitos avaliados.

Referências: Hagberg et al. Eur. J. Pediatr. Neurol. 6: 293, 2002; Schwartzman. Rev. Bras. Psiquiatr. 25: 110, 2003; Tetzchner et al. Dev. Med. Child Neurol. 38: 212, 1996.

Palavras-Chave: Síndrome de Rett; Cognição; Movimentos oculares.

C.02.023

Mutation located in the centromeric edge of mouse chromosome 17 causes ataxia and abnormal formation of the cellular cerebellar layers

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Spontaneous or chemically induced mutations are useful to elucidate how a mutated gene gives rise to a given illness phenotype. Although the current mouse mutant database, which is composed largely of knockout animals, has been a valuable source of models, only a small proportion of the likely total number of mammalian genes is represented. This gap is being partially filled by the establishment of new mutagenesis programs using potent mammalian mutagens like N-ethyl-N-nitrosourea (ENU). Through an ENU mutagenesis project, recessive mutant mice bearing varied phenotypes were isolated, one being a mutant mouse presenting balance problems, with characteristics of lack of motor coordination, named equilibrio (eqlb). The phenotype led us to hypothesize that the generated mutation could be affecting the development of the cerebellum, region of the central nervous system that controls the execution of the movements and balance. The evaluation of mutant and wild-type adults performance on a rotating bar (rotarod) showed that the time of permanence of eqlb mice was significantly diminished in relation the wild-type. Histological analysis of eqlb mice cerebella shows abnormal formation of the cellular layers, with disorganization of the Purkinje cell layer, the main type of neuron in the cerebellum. Histological analysis also revealed that the external granular cell layer is thicker in eqlb mutant mice, when compared to same age wild-type mice cerebellum. [³H]-Thymidine incorporation showed that the proliferation rate is higher in eqlb mutant cerebella when compared to wild-type. There are no differences between eqlb and wild-type granule neurons migration rate, accessed by BrdU incorporation. Mapping of the mutation, using polymorphic microsatellite markers, indicates that the mutation is located on mouse chromosome 17, in a chromosomal subregion between the marker D17Mit267 (3.31Mb) and the centromeric edge. The region has synteny with a region of human chromosome 6, where recessive ataxias have been mapped suggesting that equilibrio mice may be used as an animal model for a group of human ataxias.

Palavras-Chave: cerebellar development, ataxia, mutant mice

Apoio Financeiro: Granted by FAPESP, CNPq, FADA-UNIFESP.

Reconhecimento de Faces Familiares em Indivíduos com Síndrome de Asperger

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Introdução: A síndrome de Asperger (AS) está inserida nos Transtornos Invasivos do Desenvolvimento (TID) e apresenta prejuízos qualitativos: na interação social; comunicação; padrões restritos estereotipados de interesses e comportamento **(1)**. Atualmente muita atenção tem sido concentrada nos estudos da percepção de faces, cujo papel central é atribuído à percepção do rosto no processo de socialização **(2)**. Evidências crescentes mostram um estilo de processamento visual atípico no autismo; estudos apontam para um processamento de face global (holístico), enquanto outros encontram um estilo de processamento local **(3)**. **Objetivo:** Verificar e comparar o reconhecimento de faces familiares e não familiares em indivíduos com SA (CA) e do grupo controle (CO) através de figuras de faces familiares baseadas em partes (olho e boca) ou holísticas (face inteira e perfil). **Método:** Participaram da pesquisa 14 crianças do sexo masculino, sendo 7 com SA e 7 crianças com desenvolvimento típico, com idades variando de 6 a 12 anos. Os critérios de inclusão foram QI mínimo de 70 avaliado pelo WISC para toda a amostra e, para o grupo com SA os instrumentos de avaliação foram *Autism Screening Questionnaire* (ASQ) e *Screening Questionnaire for Asperger Syndrome* (ASSQ). **Resultados:** Levando-se em consideração o reconhecimento de figuras holísticas e de partes, o grupo CA apresentou mais erros quando comparado ao CO. No grupo CA encontrou-se diferença estatisticamente significativa ($p=0,018$) nos acertos entre figuras de faces familiares (100%) e não familiares (42%). **Conclusão:** Comparando os grupos conclui-se que o CA obteve melhores resultados nas figuras familiares holísticas de face e piores resultados em figuras não familiares holísticas de perfil, enquanto o CO não apresentou diferenças significantes. **Referências:** 1- APA, 1995; 2- Arch Gen Psychiatry, vol.59, n.9 (2002) 809-16; 3- ScienceDirect, vol. 28 (2007) 197-206.

Palavras-Chave: síndrome de Asperger, reconhecimento de faces

Apoio Financeiro: Mackpesquisa

C.02.025

EFEITO DA ADENOSINA DEAMINASE EM MODELO DE HIPÓXIA ISQUEMIA NEONATAL

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OBJETIVOS

O dano cerebral causado pela hipoxia-isquemia (HI) neonatal é a principal causa de mortalidade aguda e morbidade neurológica crônica em crianças. A adenosina deaminase (E.C 3.5.4.4, ADA) é uma enzima responsável pela clivagem hidrolítica da adenosina em inosina(1). O objetivo foi investigar a atividade da ADA em córtex e hipocampo de cérebros de ratos expostos ao modelo de HI neonatal e os níveis séricos de ácido úrico.

MÉTODOS

Ratos Wistar machos de 7 dias foram anestesiados com halotano. Foi realizada a oclusão da artéria carótida esquerda, e após 2 horas, foram submetidos às condições de hipóxia (8% O₂ e 92% de N₂) por 90 minutos. Os animais foram sacrificados imediatamente ou após 8 dias do insulto HI. O córtex e hipocampo foram separados e homogeneizados (Tampão Fosfato), após foram centrifugados (30 min, 14000 x g) e usados para dosagem da ADA. A atividade da ADA foi determinada como descrito por Giusti & Galanti(2). A dosagem do ácido úrico sérico foi realizada por Kit comercial (Bioclin).

RESULTADOS

A atividade da ADA aumentou significativamente no hipocampo e córtex 8 dias após os animais sofrerem o insulto (22,25±4,6/mg prot.) e (8,10±3,36/mg prot.), em relação aos controles (12,43±4,1/mg prot.) e (3,62± 1,46/mg prot.) respectivamente. Imediatamente após o insulto não apresentaram diferenças significativas. O ácido úrico sérico mostrou-se elevado nos animais que sofreram o insulto HI.

CONCLUSÃO

A ADA é uma das enzimas que regulam os níveis de adenosina no cérebro. Em condições de hipóxia isquemia os níveis de adenosina aumentam no cérebro modulando respostas celulares ao insulto. Neste estudo observou-se que logo após a injúria a atividade da ADA não se alterou, porém, em hipocampo e córtex dos animais após oito dias de HI a atividade da ADA aumentou significativamente (p<0.05) o que pode relacionar-se aos elevados níveis de adenosina liberados após o evento ainda presentes nesse período no SNC.

REFERÊNCIAS: 1-J Exp Biol 3149:54,2004; 2-Meth Enz 345:23,1984.

Palavras-Chave: Adenosina deaminase, hipoxia-ischemia

Apoio Financeiro: CNPq e FAPERGS

**THE INFLUENCES OF CHRONIC IMMUNOBLOCKAGE OF 9-O ACETYL GD3
GANGLIOSIDE IN THE *IN VIVO* FORMATION OF CEREBELLAR CORTICAL LAYERS**

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The 9-O acetyl GD3 (9acGD3) expression correlates particularly with periods of cell migration in the retina, superior colliculus, cerebellum, and telencephalon. The expression of this molecule is also observed in regions undergoing neurite outgrowth, such as the developing optic tract, white matter of the cerebellum, dorsal roots, trigeminal system, and olfactory nerve (Mendez-Otero et al, 1988). Furthermore, we demonstrated that the immunoblockage of 9acGD3 ganglioside arrests the *in vivo* and *in vitro* migration of cerebellar granule neurons (Santiago et al, 2001; Santiago et al, 2004).

In the present work we propose to realize a morphologic analysis of the cerebellar cortical layers after the chronic immunoblockage of 9acGD3 *in vivo* in the developing cerebellum.

Postnatal day 7(P7) rats were briefly anesthetized by ether inhalation and injected, with the assistance of a microsyringe, with 20ml of mAb Jones, mAb A2B5 or saline solution (control). The mAb Jones specifically recognizes the 9acGD3 ganglioside and the mAb A2B5 recognizes a group of the c-series gangliosides. Each animal was injected for 7 consecutive days at a 12hr interval. The rats were anesthetized and perfused 12hr after the last injection. Thick cryosections (20mm) were processed for cresyl-violet staining or for immunohistochemistry.

The mAb Jones injected rats showed a significant decrease of cells with migratory- bipolar profiles and an increase of stationary-rounded profiles cells in the molecular layer in comparison with control groups. Moreover, in the immunoblockage animals, we found a higher density of granule cells in the external granular layer (EGL). Furthermore, radial glia cells are not affected by the chronic infusion as revealed by GFAP staining. Our results show that the chronic immunoblockage of 9acGD3 with mAb Jones generates a modification of migratory/stationary profiles in the molecular layer cells, suggesting that this ganglioside is required for cerebellar granule cell migration and that this effect is specific over neuronal cells. The mAb Jones also demonstrates an influence in the EGL cell density indorsing the 9acGD3 importance on cell migration.

Palavras-Chave: Neuronal Migration; Development; Cerebellum; 9-O acetyl GD3

Apoio Financeiro: Capes, FAPERJ e Cnpq

C.03.001

Homer1a expression in the pentylenetetrazol model of seizures

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Aims: Homer1a, a protein that affects the binding properties of mGlu receptors, has been reported in animal models of seizures and epilepsy. It has been hypothesized that Homer1a expression could be associated to antiepileptogenic or anticonvulsant processes. Here we investigate its expression in the pentylenetetrazol (PTZ) model of acute seizures.

Methods: *Seizures* were induced in adult male Wistar rats by PTZ injection (50mg/kg, i.p.). After 2, 8, 24 hours and 7 days of PTZ induced-seizures, brain sections of different animals were evaluated for Homer 1a immunohistochemistry. In addition, the hippocampus and entorhinal cortex were analyzed by semi-quantitative RT-PCR for Homer1a at different times after seizure induction.

Results: Rats were injected with PTZ (n=20, animals with and without seizures) or saline (control, n=8, animals without seizures). Immunohistochemistry showed that the protein Homer1a is not detectable at 2, 8, 24h and 7 days after PTZ-induced acute seizures. In addition, there were also no differences in the semi-quantitative mRNA expression for Homer1a at 2 and 8 hours after PTZ injection when comparing animals with or without seizures and controls.

Conclusion: A single stimulation to PTZ seizures in animals did not alter **protein levels and mRNA expression of Homer1a when evaluated at 2, 8 and 24 hours and 7 days** in hippocampus and entorhinal cortex. Previous studies demonstrate that chronic models of seizures, such as **pilocarpine-induced status epilepticus**, increases levels of **Homer1a** in the hippocampus. Our current data demonstrating that acute seizures induced by PTZ do **not alter Homer1a levels does not favor its proposed functional significance in terms of the epileptic process.**

Palavras-Chave: Epilepsy, Homer1a, Pentylenetetrazol

Apoio Financeiro: FAPESP, CNPq

C.03.002

DIFERENCIAS EN LA EXPRESIÓN DE NR2B DEPENDIENTE DEL AREA Y ESTRATO HIPOCAMPAL EN CONVULSIONES REPETITIVAS

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Objetivo. El receptor NMDA tiene un papel muy importante en la plasticidad sináptica y participa en la iniciación y/o propagación de las descargas epilépticas; este receptor es un complejo heteromérico compuesto por la subunidad NR1, que está siempre presente y subunidades NR2 que determinan distintas propiedades funcionales. En el laboratorio estudiamos la expresión del receptor hipocampal NR2B en el modelo de epilepsia producido por la administración repetitiva del convulsivante ácido 3-mercaptopropiónico (MP).

Métodos. Los lotes de ratas Wistar de 250-300g de peso fueron inyectadas con MP (45mg/kg) durante 4 días (MP4) y 7 días (MP7) y como controles se utilizaron ratas tratadas con solución fisiológica. Se realizaron estudios de Western blot y de inmunohistoquímica por la técnica de peroxidasa-antiperoxidasa, utilizando el anticuerpo antiNR2B. Los resultados se expresaron en unidades de densidad óptica relativa y fueron analizados por ANOVA de una vía y los tratamientos fueron comparados por el test de Student-Newman-Keuls.

Resultados. Una disminución significativa en NR2B (60%, $P < 0.001$) en homogeneizado total de hipocampo se observó en los estudios de western blot, en MP4 con una tendencia a la recuperación en MP7. Los estudios inmunohistoquímicos mostraron una expresión disminuida de NR2B en los estratos Oriens, Piramidal, Radiatum y Molecular de CA1 y CA2/3 (21-73) en MP4, mientras que en MP7 se observó una reversión en el stratum oriens, en la capa de células piramidales de CA1, CA2/3 y en stratum radiatum de CA1. En giro dentado MP4 disminuyó la inmunotinción en la capa granular (20%) y en hillus (71%), mientras que en subicullum se determinó una disminución del 63% (DO Control: 0.37 ± 0.03 , MP4: 0.14 ± 0.06 , MP7: 0.17 ± 0.05). Estas disminuciones se mantuvieron con el tratamiento de MP7.

Conclusiones. Estos resultados sugieren un rol del receptor NR2B en la modulación de la actividad convulsiva hipocampal en las convulsiones inducidas por MP de un modo dependiente de las conectividades de las distintas áreas y capas.

Palabras-Chave: NR2B, Epilepsia, 3-mercaptopropiónico, Hipocampo, Convulsiones

Apoio Financeiro: M033-Universidad de Buenos Aires- CONICET- Buenos Aires-Argentina

DIMINUIÇÃO AGUDA DE CRISES TIPO AUSÊNCIA PELA ADMINISTRAÇÃO SISTÊMICA DE CETAMINA EM RATOS

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Objetivos: Em ratos, as crises tipo ausência constituem uma manifestação patológica de natureza genética, caracterizada por surtos de descargas eletrocorticográficas espícula-onda cuja frequência intrínseca sofre influência de células talâmicas marcadoras de ritmo que, por sua vez, recebem projeções corticais glutamatérgicas excitatórias. O presente estudo investigou se a diminuição global da atividade glutamatérgica suprime a manifestação das crises tipo ausência em ratos geneticamente portadores de descargas espícula-onda.

Métodos: Foram utilizados ratos Wistar machos com 6 meses de idade (n=4), provenientes de uma colônia selecionada de animais portadores de crises tipo epilepsia de ausência. Os animais receberam implante de 8 eletrodos no córtex cerebral, sendo 4 frontais e 4 parietais, bilateralmente. Após uma semana de recuperação, foi realizado eletrocorticograma por 60 min antes e 60 min após a administração intraperitoneal de uma dose única de cetamina (30 mg/Kg), um antagonista não competitivo de receptores de NMDA. A análise estatística foi realizada por meio da ANOVA e Tukey HSD ($\alpha = 0,05$).

Resultados: Os resultados mostraram que o tempo total de crises em estado de vigília foi reduzido significativamente após a administração da droga – $F_{(1,472)} = 13,57$; $p < 0,0003$. Adicionalmente, nos primeiros minutos de registro pós droga de todos os ratos, observou-se a ocorrência de um padrão ictal espiculado de curta duração ($3,76 \pm 1,69$: média $\pm$ desvio padrão) que, com o passar do tempo, aproximou-se cada vez mais em semelhança das descargas espícula-onda.

Conclusões: Em conjunto, os dados do presente trabalho sugerem que as projeções corticais glutamatérgicas para o tálamo influenciam fortemente a frequência de disparos ictais das células talâmicas marcadoras de ritmo que, por sua vez, propagam sua atividade para todo o córtex. O padrão ictal espiculado observado após a administração da cetamina reforça essa idéia, já que parece expressar uma diminuição da excitabilidade das células talâmicas com conseqüente bloqueio temporário das descargas espícula-onda no córtex.

Palavras-Chave: epilepsia, crises tipo ausência, espícula-onda, receptores de NMDA

Apoio Financeiro: CNPq

Interaction nutrition-drug in the brain: neurophysiologic effects of dipyrone on cortical spreading depression in early-malnourished adult rats

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Objectives: The effectiveness of some drugs in mammals can vary as a function of their nutritional status (Ciênc. Cult. 56:32-35 2004). The drug dipyrone (DP), besides its analgesic and antithermic effects, has antiepileptic action in rats (Neuroreport 9:2415-2421 1998). In this species, malnutrition, acting for short periods early in life, can differentially modify the phenomenon of cortical spreading depression (CSD; Braz. J. Med. Biol. Res. 30:663-670 1997). Considering the epilepsy-CSD relationships, as well as the use of DP in children, we here investigated the impact of DP, applied in the 2nd, 3rd or 4th week of life, on CSD, in developing rats previously submitted to different nutritional statuses.

Methods: Malnutrition (M) was produced in 72 rats during lactation by increasing the litter size to 12 pups, as compared to the 72 well-nourished (W) controls (litters with 6 pups). In both nutritional groups half of the animals received, during 7 days, per gavage, DP (300mg/kg/d) and the other half received saline. Both DP- and saline groups were divided in three groups, according to the week of life (2nd, 3rd, or 4th week) in which DP was applied. At 35-45 days of life, CSD was elicited with 2% KCl at the frontal cortex and recorded at 2 points in the parietal region by recording the electrocorticogram and the slow potential change of CSD.

Results: In both W- and M-groups, DP-treatment increased ($P<0.05$) the CSD-velocities of propagation, as compared to the respective saline-controls. This effect was more intense when DP was applied at the fourth week of life, as compared to the second week. For the W and M saline-treated groups, the mean $\pm$ sd velocities (in mm/min) were 3.70 ± 0.11 , 3.77 ± 0.16 and 3.78 ± 0.13 (W-groups) and 4.14 ± 0.09 , 4.13 ± 0.09 and 4.11 ± 0.07 (M-groups), for the animals treated in the 2nd, 3rd and 4th week of life. For the DP-treated groups, the means were 3.99 ± 0.14 , 4.03 ± 0.16 and 4.30 ± 0.14 (W-groups) and 4.46 ± 0.18 , 4.68 ± 0.29 and 4.96 ± 0.28 (M-groups).

Conclusion: Data indicate that DP-treatment in developing rats facilitates CSD propagation, and this effect is not affected by the nutritional status early in life.

Palavras-Chave: Cortical spreading depression, dipyrone, early nutricional status

Apoio Financeiro: CAPES/CNPq/UFPE, FINEP/IBN-Net

C.03.005

Avaliação temporal e migratória do transplante de células de medula óssea no modelo de eletrochoque convulsivo máximo

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Objetivos: O transplante de células de medula óssea (MO) foi capaz de alterar o curso de processos convulsivos induzidos experimentalmente pelo modelo de eletrochoque convulsivo máximo (ECM). Pretendemos avaliar se a ação anticonvulsivante das células de MO é decorrente de um efeito agudo e/ou se as células de MO eventualmente migram e apresentam efeitos tardiamente.

Métodos: A MO de camundongos C57B6/L GFP+ foi removida, células totais de medula óssea, contendo 10^7 células/animal foram injetadas via endovenosa em camundongos hospedeiros (G-MO) e animais controles receberam solução salina (G-CONT). O estímulo elétrico foi aplicado via transcorneal 15min, 1h, 2h, 8h e 24h após o transplante. A frequência e a duração das crises foram determinadas. Os animais foram perfundidos e seus órgãos (cérebro, coração, baço e fígado) removidos para avaliação histológica. Dados expressos como (Media $\pm$ DS) e $P \geq 0,05$.

Resultados: A frequência de crises tônicas induzidas por ECM no grupo MO apresentou uma proteção 64% significativamente diferente ao do G-CONT ($p < 0,05$). A duração dos eventos convulsivos no período pós-ictal foi 34% maior nos animais do G-MO em relação ao G-CONT. A avaliação dos efeitos temporais das células de MO (15min, 1h, 2h, 8h e 24h) mostrou uma significativa proteção (50%) na frequência de crise 24h após o transplante quando comparados aos demais tempos e com G-CONT ($p < 0,05$). A duração do período pós-ictal às 24h no G-MO (125 ± 92) foi significativamente maior que o grupo controle (70 ± 67). Houve marcação positiva de migração de células de MO GFP+, oriundas do transplante, no cérebro nos grupos 8h e 24h, sendo não detectadas aos 15min, 1 e 2 h. Também houve marcação positiva das células transplantadas em todos os tempos no fígado e com maior expressão no baço. Não foram verificadas células GFP+ no coração em nenhum dos tempos avaliados.

Conclusão: Células de MO possuem propriedades de alterar o curso dos processos convulsivos induzidos agudamente. O período da ação protetora coincidiu com a avaliação da marcação positiva de migração das células GFP+ no encéfalo e nos demais órgãos.

Palavras-Chave: Eletrochoque, epilepsia, medula óssea, transplante.

Apoio Financeiro: CNPq e FAPESP.

EFEITO DA EXPOSIÇÃO PRÉ-NATAL AO ÁLCOOL SOBRE ATIVIDADES EPILEPTIFORMES DO TIPO NÃO SINÁPTICAS EM HIPOCAMPO DE RATOS

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Objetivo: Estudos têm demonstrado a importância das conexões não – sinápticas na geração e sustentação de atividades epileptiformes. O hipocampo é um tecido cortical suscetível a crises epiléticas sustentadas por essas conexões e sua maturação e eletrofisiologia podem ser alteradas pela exposição pré – natal ao etanol. O presente trabalho tem como objetivo analisar atividades epileptiformes sustentadas por conexões não sinápticas no hipocampo da prole de ratas alcoolizadas.

Metodologia: Ratas Wistar, a partir da sexta semana de vida, receberam diariamente uma injeção intraperitoneal de etanol (1,5 g/kg) até o último dia de amamentação de seus filhotes. Ratas de três diferentes faixas etárias (P21, P70 e P112) da prole desses animais foram sacrificadas e tiveram o hipocampo removido e fatiado para registro do potencial elétrico extracelular (PEE) na camada granular (CG) do giro denteado (GD). Os animais controles correspondem à prole de fêmeas que não receberam nenhuma dosagem de etanol. Foram analisadas características do PEE como: duração dos eventos (DE), amplitude da variação lenta (DC), intervalo entre os eventos (IE) e valor médio dos *population spike* (PS's). As fatias foram coradas com cresil violeta para quantificação celular do GD.

Resultados: A análise do PEE mostrou que para ratas expostas ao álcool durante a gestação ocorreram principalmente: (i) aumento na DE (variação de $121 \pm 3,31$ % em P21, $133 \pm 2,09$ % em P70 e $167 \pm 5,40$ % em P112) e (ii) diminuição no IE (variação de $76 \pm 1,87$ % em P21, $69 \pm 2,45$ % em P70 e $124 \pm 4,02$ %). A quantificação celular mostrou variações apenas em P112, havendo um decréscimo na densidade (variação de $74 \pm 7,38$ %).

Conclusões: Ratas expostas ao álcool durante a gestação apresentam maior excitabilidade neuronal quando as conexões sinápticas são inibidas o que pode representar maior susceptibilidade a crises epiléticas sustentadas por conexões não sinápticas. Variações do PEE devem estar relacionadas à densidade celular além de outros mecanismos celulares.

Palavras-Chave: Etanol, gestação, atividades epileptiformes, conexões não - sinápticas, giro denteado.

Apoio Financeiro: FAPEMIG

Avaliação do efeito neuroprotetor do agonista adenosinérgico A1 R-Pia através dos métodos autoradiográficos de medida de glicose e fluxo sanguíneo cerebral, no modelo de epilepsia lesional induzida por pilocarpina.

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Objetivos: Caracterizar o efeito neuroprotetor do RPia (R(-)N6-(2-fenilisopropil)adenosina) em ratos com *status epilepticus* induzido por pilocarpina, avaliando o balanço entre a utilização local da glicose cerebral (ULGC) e o fluxo sanguíneo cerebral local (FSCL).

Métodos e Resultados: Três grupos de ratos foram estudados: 1) Pilo: tratamento com pilocarpina (360mg/kg, ip); 2) RPia+Pilo: pré-tratamento com RPia (25µg/kg, ip); e 3) Grupo controle: RPia e salina. Empregou-se o método de Sokoloff (*J. of Neurochemistry*, **28**: 897-916; 1977) para medir a ULGC, e o método de Sakurada (*Am. J. Physiol.*, **234**: H59-H66; 1978) para medir o FSCL, ambos avaliados 4 horas do início do SE. Aumentos significantes na ULGC foram observados em 42 das 44 regiões avaliadas nos grupos Pilo e RPia+Pilo comparados ao grupo controle. No entanto, redução significativa na ULGC ocorreu na substância negra *pars reticulata* (SNPr, p<0.01) e no giro denteado (GD, p<0.01) de ratos do grupo RPia+Pilo versus Pilo. Houve aumento significativo na medida do FSCL em 44 áreas estudadas comparando-se os grupos Pilo e RPia+Pilo com o grupo controle, porém não houve diferença quando comparados os grupos Pilo com RPia+Pilo.

Conclusões: O SE causado pela Pilo e RPia+Pilo promoveu aumento da ULGC na maioria das regiões estudadas. No entanto, um hipometabolismo foi detectado no GD e na SNPr do grupo RPia+Pilo. O SE induziu um hiperfluxo generalizado nas diversas regiões estudadas, e o RPia não alterou esses valores. Os dados indicam que a reduzida atividade metabólica do GD e da SNPr pode contribuir para o efeito neuroprotetor do agonista adenosinérgico A1 RPia.

Palavras-Chave: Epilepsia; Adenosina; Neuroproteção; Metabolismo e Fluxo sanguíneo cerebral

Apoio Financeiro: CNPq, FAPESP, CAPES, FADA

MIGRAÇÃO E PROLIFERAÇÃO DE CÉLULAS DE MEDULA ÓSSEA GFP⁺ NO HIPOCAMPO DE CAMUNDONGOS EPILÉPTICOS TRANSPLANTADOS

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Introdução: Estudos recentes mostram que células precursoras de medula óssea (MO) migram para a área lesada no encéfalo e se proliferam, e que esses processos estão associados a fenômenos do período agudo após eventos lesivos. Nosso objetivo foi analisar o hipocampo de animais epiléticos previamente transplantados com células de MO de animais transgênicos GFP⁺. Verificamos o percentual de co-localização de células GFP⁺ com marcadores de proliferação (BrdU), e quantificação por Nissl, e comparamos com a sobrevivência de células locais após a indução do *status epilepticus* (SE).

Métodos: A MO de camundongos GFP⁺ foi removida e lavada. Células totais de MO foram injetadas por via endovenosa em camundongos irradiados, 10⁶ células por animal. Trinta dias após o transplante, os animais foram injetados com pilocarpina e desenvolveram SE por 30 minutos, e foram perfundidos em diferentes tempos pós crise, 24h, 7 dias e 15 dias. Antes da perfusão esses animais foram tratados com BrdU (50 mg/kg; 6 doses a cada 12 horas). Após a perfusão, os encéfalos foram seccionados e processados para GFP, BrdU e coloração de Nissl.

Resultados: Nos animais epiléticos, células GFP⁺ oriundas da medula óssea estão presentes nas camadas subgranular, CA3 e hilo hipocampal com maior concentração aos 7 dias após o SE, com diferenças significantes quando comparado aos controles ($p < 0,01$) e aos outros tempos ($p < 0,01$). Aos 15 dias, o número de células GFP⁺ diminuiu. O mesmo padrão em termos de distribuição e perfil temporal foi observado para as células BrdU⁺. No entanto, um número muito baixo de células duplo-marcadas (GFP⁺/BrdU⁺) foi observado nessas áreas.

Discussão: Nossos dados indicam que células provenientes da MO têm capacidade de migrar e proliferar em regiões do hipocampo lesadas por indução de crise epilética. O pico de migração e a localização coincidem com a área envolvida com perda neuronal induzida neste modelo e com o tempo descrito para neurogênese presente nestas áreas. Nossos dados sugerem que os processos de migração e proliferação dessas células estão, provavelmente, associados ao período agudo e resposta inflamatória que se segue após eventos lesivos.

Palavras-Chave: Epilepsia, quimera, pilocarpina, medula óssea

Apoio Financeiro: Apoio financeiro: CNPq e FAPESP.

C.03.009

Organotellurium(IV) compound RF-07 neuroproTECTive effect in the pilocarpine model to temporal lobe Epilepsy.

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Objetivos:

The pilocarpine model can reproduce the main characteristics of the Temporal Lobe Epilepsy in humans. Caspase activation is cited among mechanism involved with cell death induced by seizures. Tellurium(IV) compounds present antitumoral, immunomodulatory and neuroprotective effects due to its ability to inhibit cysteine proteases. Our purpose was to characterize the caspase cascade activation during the *status epilepticus*, and to verify if the treatment with RF-07 prior to pilocarpine modifies this caspases profile. The K₂ value to caspase inhibition by tellurium(IV) compounds were determined. Besides, the behavioral and electroencephalographic seizures were evaluated in rats pre-treated with RF-07.

Métodos e Resultados:

Groups: PILO: pilocarpine (360 mg/kg, N=22), RF-07+PILO: RF-07 (0.43 mg/kg, N=20) 15 min prior to pilocarpine, and Controls: RF-07 and saline instead of pilocarpine (N=10) or SALINE (N=4). The treatment with RF-07 prior to pilocarpine, prevented the SE occurrence in >80% of treated rats (p<0.001). The behavioral changes observed in rats injected with pilocarpine were similar to previous description by Turski et al. (Experientia, 1983). Hippocampal homogenates were incubated with substrates for caspase-1, caspase-3, and caspase-8; or pre-incubated with RF-07, or usual inhibitors for caspase-3 and caspase-8. Recombinant caspase-3 and caspase-8 were incubated with usual caspase inhibitors or with RF-07, RF-03 and AS-101. Increases in activity of caspase-1, -3 and -8 were detected in the PILO group. RF-07 was a more potent caspase-3 and caspase-8 inhibitor than AS-101 or Z-VAD-fmk.

Conclusões

According to our results, the caspases are activated as early as 90 min following SE and the Te(IV) compounds exerted an anticonvulsant effect associated with inhibition of caspases. This compound could be a promising antiepileptogenic agent. **Palavras-Chave:** Epilepsy; apoptosis; caspases; neuroprotection; organotellurium(IV) compounds.

Apoio Financeiro: CNPq, FAPESP, CAPES.

C.03.010

LAS CÉLULAS DE PURKINJE PRESENTAN UNA DISMINUCIÓN EN LA EXPRESIÓN DE NR2B EN CONVULSIÓN. EFECTO DEL ANALOGO DE ADENOSINA CICLOPENTILADENOSINA

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La capacidad inhibitoria de la actividad epiléptica del cerebelo puede estar comprometida dependiendo de la frecuencia e intensidad de las convulsiones. La excesiva activación de NMDAR contribuye a la crisis epiléptica y la activación de NR2B incrementa la excitotoxicidad. En las convulsiones se libera adenosina, un neuromodulador endógeno con propiedades anticonvulsivas. Cuando se administra a ratas los análogos de adenosina, se eleva el umbral para alcanzar las convulsiones. En este trabajo se ha estudiado la distribución del receptor NR2B en cerebelo y su expresión luego de la administración del convulsivante ácido 3-mercaptopropiónico (MP) y el efecto del análogo de adenosina ciclopentiladenosina (CPA) previo a MP. La administración de CPA previo a MP demora la aparición y la intensidad de las convulsiones.

Metodos: Los lotes de ratas Wistar (250-300g) se dividieron en 4 grupos y se le administró durante 4 días una dosis diaria: 1) MP, 45mg/kg i.p. (MP), 2) CPA, 2mg/kg ip. (CPA), 3) MP 30 minutos previo a CPA (CPA +MP); 4) solución fisiológica (Control). Un día después de la última inyección, los cerebelos se procesaron para estudios inmunohistoquímicos usando antiNR2B como anticuerpo primario y se aplicó la técnica de peroxidasa - antiperoxidasa. Los resultados se expresaron en densidad óptica relativa y se analizaron por ANOVA una vía y Student-Newman-Keuls.

Resultados. Los estudios de NR2B en cerebelo mostraron una expresión de este receptor en las células de Purkinje, no observándose marca en la capa molecular ni en la capa granular. La administración de MP disminuyó la expresión de NR2B en un 55% respecto del control. (DO, C: 0.191 ± 0.04 ; MP: 0.085 ± 0.07). Cuando el análogo de la adenosina CPA se administró sólo, se determinó un 12% de disminución, (0.137 ± 0.003) mientras que la administración de CPA + MP redujo la expresión de NR2B en un 28% (0.168 ± 0.003).

Conclusión: La disminuida expresión de la subunidad NR2B del receptor NMDA, en las células de Purkinje, podría estar relacionada con una alteración en estas células, o una tendencia a evitar la excitotoxicidad y la administración de CPA previo a MP tiende a evitar o disminuir el efecto de MP.

Palavras-Chave: Cerebelo, Células de Purkinje, Epilepsia, NR2B, Convulsiones

Apoio Financeiro: M033-Universidad de Buenos Aires- CONICET- Buenos Aires-Argentina

C.03.011

EXPRESSÃO DE Cx43 NO HIPOCAMPO DE CAMUNDONGOS SUBMETIDOS AO MODELO DE EPILEPSIA DO LOBO TEMPORAL INDUZIDO POR PILOCARPINA

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Objetivo: Alterações na expressão de conexinas (Cx) têm sido descritas tanto em modelos experimentais de epilepsia como em pacientes epiléticos. Tendo em vista que os canais de junções comunicantes possuem um importante papel em várias funções dos astrócitos (acoplados pela Cx43), incluindo manutenção da homeostase iônica e sinalização celular, avaliamos neste estudo a expressão protéica da forma fosforilada da Cx43 (forma presente nos canais) no hipocampo de camundongos submetidos ao modelo de epilepsia do lobo temporal induzido por pilocarpina.

Métodos: Foram utilizados neste estudo camundongos Balb/c machos, com aproximadamente 2 meses de idade. Os camundongos foram previamente tratados com metil-escopolamina (1 mg/kg; ip) 30 minutos antes da administração da pilocarpina (340 mg/kg; ip). Os animais que desenvolveram *status epilepticus* (SE) foram divididos em dois grupos de estudo: 3h de SE (n=3, animais sacrificados após 3h do início do SE) e 6h de SE (n=3, animais sacrificados após 6h do início do SE). O grupo controle (n=3) recebeu salina ao invés de pilocarpina. Os animais foram sacrificados por decapitação de acordo com os tempos de estudo propostos e os hipocampus foram coletados e submetidos ao protocolo de *immunoblotting* para a detecção da proteína Cx43.

Resultados: Os dados iniciais mostram um aumento significativo (81%; p<0,05) na expressão da forma fosforilada da Cx43 nos animais submetidos a 6h de SE. Nos animais submetidos a 3h de SE houve um aumento de 42% na expressão dessa proteína, porém esse aumento não apresentou significância.

Conclusão: Estes dados parciais revelaram que no modelo de epilepsia do lobo temporal induzido por pilocarpina em camundongos há alterações no estado de fosforilação da Cx43, sugerindo um aumento do acoplamento glial. Este, por sua vez, pode desempenhar um importante papel na epileptogênese nesse modelo devido à íntima relação entre o acoplamento glial e a sinalização de cálcio, fator crítico na patologia em questão.

Palavras-Chave: epilepsia; conexinas; hipocampo; pilocarpina; camundongo

Apoio Financeiro: FAPESP e CNPq.

AVALIAÇÃO DA EXPRESSÃO DE MICRÓGLIAS EM ENCÉFALO DE ANIMAIS SUBMETIDOS AO ELETROCHOQUE CONVULSIVO MÁXIMO APÓS TRANSPLANTE DE CÉLULAS DE MEDULA ÓSSEA

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Objetivos: O transplante de células de Medula Óssea (MO) trinta minutos antes da indução de crise por eletrochoque convulsivo máximo (ECM), em ratos, ocasionou diminuição de frequência de crises convulsivas tônico-clônicas. Sabe-se que a resposta inflamatória pode ser caracterizada pelo aumento do número de macrófagos nas regiões inflamadas após um evento indutor, assim pretendemos, avaliar a relação entre o padrão de marcação de micróglia e efeito evidenciado nos animais tratados com MO e que posteriormente sofreram crise induzida por ECM.

Métodos: Animais pré-tratados com transplante de células de MO (G-MO) e/ou salina (G-CONT) foram submetidos trinta minutos (ECM-1) e cinco dias (ECM-2) depois ao ECM. Após 2 horas do último estímulo com ECM, os encéfalos foram removidos e os cortes encefálicos submetidos a imuno-histoquímica para OX42. Avaliações da frequência, latência e duração das crises e mensuração qualitativas de imuno-histoquímica foram feitas considerando significativo $p < 0,05$.

Resultados: A frequência de crises, no G-MO submetido ao ECM-1, foi significativamente menor 27% (3/11) quando comparado ao G-CONT 90% (9/10) $p < 0,05$. Quanto a frequência de crises após 5 dias do transplante observamos que 90% dos animais convulsionaram em ambos os grupos.

A avaliação qualitativa da marcação de micróglia do G-MO foi similar à do G-CONT. Animais que apresentaram crises de ambos grupos (G-MO + G-CONT), 87% (7/8) tiveram expressão positiva de marcação de micróglia. Sendo que, dos animais do G-MO que apresentaram crises, 80% (4/5), tiveram marcação positiva de micróglia quando comparados ao único animal que não apresentou crises após ambos estímulos de ECM provocados. A marcação para OX42 foi homogênea no encéfalo de forma que não evidenciou regiões específicas diferenciáveis de expressão de micróglia.

Conclusão: O transplante MO como já evidenciado protegeu contra as crises eliciadas por ECM. Sugerimos uma relação entre o aumento de marcação de micróglia e a ocorrência de crises convulsivas, sendo que essa expressão aparentemente não é decorrente dos efeitos do transplante de MO. **Palavras-Chave:** Medula Óssea, Crise Convulsiva, ECM, Inflamação, Micróglia

Apoio Financeiro: FAPESP e CNPq

AVALIAÇÃO DA PERCEPÇÃO VISUAL DE CRIANÇAS E ADOLESCENTES COM EPILEPSIA

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Objetivo: A epilepsia é um distúrbio neurológico que pode estar associado a alterações nas funções sensoriais e cognitivas. Nestes termos, o objetivo principal deste estudo foi determinar a função de sensibilidade ao contraste (FSC) de crianças e adolescente com epilepsia; e verificar a existência de alterações na percepção visual relacionadas a este transtorno. A FSC é uma ferramenta que permite caracterizar a resposta do sistema visual para padrões espaciais em níveis baixos, médios e altos de contraste e avaliar possíveis alterações sensoriais relacionadas ao processamento visual.

Método: Participaram deste estudo oito voluntários com idades entre 08 e 17 anos, de ambos os sexos e com acuidade visual normal ou corrigida. Os mesmos foram divididos em dois grupos com idades equivalentes, sendo quatro com epilepsia (crises tônico-clônicas) e quatro sem nenhuma patologia neurológica. As medidas foram realizadas com o método psicofísico da escolha forçada, utilizando grade senoidal de frequências de 0,25; 2 e 8 cpg (ciclos por grau de ângulo visual). Cada frequência foi mensurada duas vezes em dias diferentes, a 150 cm da tela do monitor, binocularmente e com pupila natural. Durante cada sessão experimental foram apresentados aleatoriamente pares de estímulos (estímulo de teste e neutro), e a tarefa dos participantes foi escolher sempre o estímulo de teste com uma das frequências acima. O critério adotado foi o de três acertos consecutivos para diminuir uma unidade de contraste, e o de um erro para aumentar o contraste na mesma unidade (20%).

Resultados: A análise de variância para medidas repetidas mostrou diferença significativa entre a FSC dos dois grupos [$F(1, 94) = 13,67$; $p < 0,001$]. Já a análise com o teste post-hoc Tukey HSD revelou diferença significativa apenas na frequência de 8 cpg ($p < 0,0001$). Os resultados mostraram que o grupo sem epilepsia foi da ordem de 1,5 vezes mais sensível do que o grupo com epilepsia.

Conclusão: Os dados preliminares indicam que a epilepsia altera a FSC de crianças e adolescentes de 08 a 17 anos nas frequências espaciais mais altas.

Palavras-Chave: percepção visual; epilepsia; função de sensibilidade ao contraste.

Apoio Financeiro: CNPQ

Sulfato de Magnésio administrado intracerebroventricularmente reduz as crises induzidas por pentilenotetrazol em ratos: um estudo eletroencefalográfico e comportamental.

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Objetivos: O Sulfato de Magnésio (MgSO₄) vem sendo utilizado como o tratamento de escolha na profilaxia de crises epiléticas na eclampsia (BMJ. 325:609, 2002; Obstet Gynecol. 108(4):826, 2006; BJOG.114(3):289, 2007) agindo, provavelmente, como um antagonista não-competitivo dos receptores glutamatérgicos do tipo N-Metil-D-Aspartato (NMDA). Este estudo examinou os efeitos centrais do MgSO₄ em diferentes tipos de crises induzidas por pentilenotetrazol (PTZ) em ratos. **Métodos:** Trinta e dois ratos Wistar machos, implantados com cânula intracerebroventricular (icv) e eletrodos nas regiões parieto-temporais bilateralmente, foram submetidos à administração icv de diferentes doses de MgSO₄ (0 - salina, 32, 100 ou 320 mg) seguida por administração intraperitoneal (ip) de 60 mg/kg de PTZ. As respostas comportamentais e registro eletroencefalográfico simultâneo foram observados por 30 minutos após a administração do PTZ. **Resultados:** O tempo de latência para a primeira crise induzida por PTZ foi significativamente aumentado ($p < 0,05$) pela administração icv de 100 mg de MgSO₄ ($651,9 \pm 200,1$ s) quando comparado ao controle (salina seguida por PTZ) ($156,6 \pm 28,8$ s). A duração total do período de crises foi significativamente reduzido ($p < 0,05$) sob a mesma dose de MgSO₄ ($605,4 \pm 140,4$ s) quando comparada ao controle ($1185 \pm 123,4$ s). Além disso, a latência para a primeira crise parcial complexa foi significativamente aumentada ($p < 0,05$) pela menor dose de MgSO₄ (32 mg) (1639 ± 110 s) quando comparada ao controle ($899,1 \pm 206,6$ s). No entanto, a maior dose de MgSO₄ (320 mg) não modificou ou até mesmo agravou os efeitos do PTZ. **Conclusões:** Apesar do uso do MgSO₄ em modelos experimentais de epilepsia ter apresentado resultados anteriores controversos (Epilepsy Res. 4(3): 201-206, 1989; Epilepsia. 32(4): 543, 1991; Am J Obstet Gynecol. 171 (4): 999, 1994) nossos resultados sugerem que, dependendo da dose, o MgSO₄, pode ser considerado como um importante agente adjuvante na profilaxia de crises epiléticas em geral.

Palavras-Chave: crises epiléticas, magnésio, pentilenotetrazol

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Effects of chronic GMP treatment on behavioral tasks and against seizures evoked by quinolinic acid

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Objectives:

The guanine-based purines (GBPs), named the nucleotides GTP, GDP, GMP and the nucleoside guanosine display important extracellular roles in the central nervous system (CNS). In vitro studies have shown that they exert trophic effects on neural cells, protect brain slices in a model of hypoxia and stimulate the removal of extracellular glutamate by astrocytes. In vivo acute studies have shown that GMP act as anticonvulsants in models of overstimulation of the glutamatergic system induced by quinolinic acid (QA), affect the inhibitory avoidance performance, and the facilitatory effect of glutamate on the same task in rats.

This work studied the effect of the chronic treatment of GMP in models of convulsion, amnesia, analgesia, among other behavior tests.

Methods:

Male adult Swiss albino mice (35–45g) were housed in plastic cages with water or GMP (1.5 mg/ml) and commercial food *ad libitum*. All behavioral procedures were conducted between 1:00 PM and 5:00 PM.

Behavior test started at 15th day with Rota Rod (motor co-ordination), and rectal temperature, 16th day – open field, 17th and 18th days - Avoidance Inhibitory Task (memory), 20th and 21st days – Tail flick (analgesia), 22nd day – operation to introduce a cannula guide in the lateral brain ventricle, 25th day - seizure test with ICV quinolinic acid (AQ) infusion.

Results:

Through these experiments we observed that chronic treatment with GMP did not alter significantly the motor co-ordination, the nociception and the memory of the animals. However, the treatment was able to display anticonvulsant effect in front an AQ infusion (40%, $p < 0.05$).

Conclusion:

The chronic treatment with GMP showed some effects similar acute administration (ex: anticonvulsant), but others effects reported in acute treatment were not observed in this study (amnesic effect). Therefore, our work evidenced an important neuroprotective role of GMP treatment in mice.

Palavras-Chave: GMP; anticonvulsant; quinolinic acid; guanine-based purines

Apoio Financeiro: CNPq

Avaliação da atividade de caspases e do Fluoro Jade-B no cérebro de ratos após pré-tratamento com o agonista adenosinérgico A₁ RPia no modelo da pilocarpina.

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Objetivos: Caracterizar o efeito neuroprotetor do RPia (R(-)N6-(2-fenilisopropil)adenosina) no *status epilepticus* (SE) induzido por pilocarpina. Avaliou-se a atividade das caspases 1, 3 e 8 no hipocampo, e a marcação de células em degeneração pela técnica de Fluoro Jade-B (FJB).

Métodos e Resultados: Três grupos de ratos foram estudados: 1) Pilo: tratamento com pilocarpina (360mg/kg, ip); 2) RPia+Pilo: pré-tratamento com RPia (25µg/kg, ip); e 3) Grupo controle: RPia e salina. Para a atividade enzimática das caspases 1, 3 e 8, os hipocampus de ratos dos 3 grupos foram estudados 01h30min e 7 dias após indução do SE. O método de FJB foi aplicado 01h30min, 24h e 90 dias após o SE, nos mesmos grupos descritos. O pré-tratamento com RPia teve efeito anticonvulsivante e diminuiu a mortalidade após a indução do SE. O SE causou aumento na atividade das caspases no hipocampo do grupo Pilo comparado ao grupo controle, e o pré-tratamento com RPia intensificou esta ativação. Na fase latente, a atividade das caspases 1 e 3 permaneceu aumentada no grupo Pilo em relação ao controle, enquanto a caspase 8 foi elevada em todos os grupos avaliados. No grupo RPia+Pilo houve um decréscimo na atividade das caspases 1 e 3 comparado ao grupo Pilo. Uma maior atividade de caspases 1 e 3 foi detectada no grupo Pilo 7 dias quando comparado ao grupo 01h30min. A marcação pelo FJB foi intensa 24h após SE e o RPia reduziu acentuadamente essa marcação nas regiões do hilo, CA1, giro denteado e córtex piriforme. Esta neuroproteção pelo RPia foi também observada no período crônico, comparando-se os grupos Pilo e RPia+Pilo. Não houve marcação no grupo controle ou no grupo Pilo quando estudados 01h30min após SE.

Conclusões: O efeito neuroprotetor e anticonvulsivante causado pelo RPia está relacionado com a diminuição da atividade de caspases no hipocampo e com a redução da morte celular na formação hipocampal e no córtex piriforme. A maior ativação de caspases 1 e 3 na fase aguda, seguida por uma ativação de menor magnitude na fase latente no hipocampo de ratos pré-tratados com RPia, sugere um efeito pré-condicionante deste agente no modelo da pilocarpina.

Palavras-Chave: Epilepsia, caspases, neuroproteção, agonista adenosinérgico A₁

Apoio Financeiro: CNPq, CAPES, FAPESP, FAP-UNIFESP

ALTERAÇÕES ELETROENCEFALOGRÁFICAS E COMPORTAMENTAIS PROMOVIDAS PELO PRÉ-CONDICIONAMENTO COM NMDA CONTRA CONVULSÕES INDUZIDAS POR ÁCIDO QUINOLÍNICO

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Objetivo: Este estudo teve como objetivos verificar os efeitos eletroencefalográficos (EEG) e comportamentais induzidos em camundongos pré-tratados com uma dose sub-convulsivante de NMDA frente às convulsões induzidas por ácido quinolínico (AQ).

Métodos: Camundongos adultos Swiss machos foram submetidos à cirurgia estereotáxica para implantação de uma cânula guia no ventrículo lateral e de eletrodos no hipocampo e córtex cerebral. Após 48 h, foram tratados com uma dose sub-convulsivante de NMDA (7,5 mg/kg, i.p.) ou salina. 24h depois receberam a infusão de AQ (4µL, 9.2mM, i.c.v.) ou PBS. Os registros EEG foram realizados durante 30 min após o pré-condicionamento com NMDA e 10 min após o tratamento com AQ. As alterações comportamentais foram avaliadas no labirinto em cruz elevada (LCE) e no campo aberto (CA) imediatamente após o tratamento com NMDA ou 10 min após o tratamento com AQ.

Resultados: No LCE o pré-condicionamento com NMDA alterou o número de estiramentos corporais protegidos ($13,71 \pm 3,36$) e rearing ($5,57 \pm 0,9$) em relação ao controle ($30,12 \pm 2,95$ e $12,12 \pm 1,3$, respectivamente), enquanto no CA, o NMDA alterou somente o número de rearing ($12,71 \pm 3,37$; $C=26,5 \pm 4,23$). Nenhuma alteração nos parâmetros comportamentais foi observada no LCE e no CA após o tratamento com AQ nos diferentes grupos. Os resultados dos experimentos EEGgráficos mostraram que a dose subconvulsivante de NMDA promoveu alterações epileptiformes de grande amplitude e duração, associadas a um comportamento de hipolocomoção. Além disso, não houveram diferenças estatísticas significativas na latência para primeira crise EEG, bem como, na duração da primeira crise entre os grupos tratados com Sal+AQ e NMDA+AQ que convulsionaram.

Conclusões: Nossos resultados demonstraram que o tratamento agudo com NMDA não altera os parâmetros espaço-temporais avaliados no LCE, mas prejudica a atividade exploratória com um efeito hipolocomotor. Além disso, o tratamento agudo com NMDA promoveu alterações nos registros EEG. Entretanto, novos estudos, como a análise quantitativa dos traçados EEG entre os grupos estão sendo realizados.

Palavras-Chave: NMDA, pré-condicionamento, ácido quinolínico, EEG

Apoio Financeiro: CNPq

RESPOSTA MOTORA DIFERENCIAL DE RATOS PORTADORES DE DESCARGAS ESPÍCULA-ONDA À CETAMINA

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Objetivos: Antagonistas de receptores de NMDA suprimem descargas espícula-onda no eletrocorticograma de ratos Wistar portadores de crises tipo ausência. O objetivo do presente trabalho foi avaliar o efeito agudo da aplicação sistêmica do cloridrato de cetamina (antagonista não competitivo de receptores de NMDA) sobre a mobilidade e a resposta motora à estimulação plantar, a fim de avaliar se há associação entre essas possíveis diferenças motoras e as crises tipo ausência.

Métodos: Sessenta ratos Wistar machos com 6 meses de idade (30 de uma colônia selecionada de ratos portadores de descargas espícula-onda espontâneas / FMUSP e 30 controle) receberam cetamina (50 mg/Kg, 75 mg/Kg ou 100 mg/Kg) intraperitonealmente. Computou-se a latência e a duração da imobilidade e da arreflexia.

Resultados: A ANOVA evidenciou que a duração da imobilidade dos ratos portadores de crises tipo ausência foi significativamente menor em comparação aos animais controle – $F_{(1,51)} = 6,98$; $p < 0,02$. Aqueles também se mostraram menos sensíveis ao efeito de dose em relação à duração da arreflexia – $F_{(2,51)} = 3,43$; $p < 0,05$ – e da imobilidade – $F_{(2,51)} = 53,78$; $p < 0,001$. A duração desses parâmetros aumenta significativamente já na dose intermediária para os animais controle, ao passo que apenas na dose maior para os portadores da patologia (*post hoc*, Tukey: $p < 0,05$ para duração da arreflexia e $p < 0,001$ para duração da imobilidade). Não foi observada diferença significativa entre os grupos em relação à latência para imobilidade e arreflexia, tampouco em relação à duração da arreflexia.

Conclusões: Os resultados mostram que os efeitos da cetamina sobre as descargas eletrocorticais espícula-onda, discutidos na literatura, são acompanhados por resposta diferencial à droga também em relação aos seus efeitos anestésicos. Em conjunto, esses dados sugerem uma importante influência de conexões glutamatérgicas sobre a manifestação das crises tipo ausência, provavelmente em núcleos talâmicos, onde há células marcadoras de ritmo que participam da gênese das descargas espícula-onda e recebem projeções glutamatérgicas corticais.

Palavras-Chave: epilepsia, crises tipo ausência, receptores de NMDA, resposta motora

Apoio Financeiro: CNPq

Estudo da Memória de Reconhecimento de Objetos de Ratos com Epilepsia tratados com Células-Tronco Mesenquimais

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Objetivo: Avaliar a memória de reconhecimento de objetos (R.O) de ratos submetidos ao modelo de epilepsia experimental, utilizando lítio-pilocarpina e tratados com células-tronco mesenquimais (CTMSCs).

Métodos: Ratos Wistar machos foram divididos em quatro grupos: salina, salina+CTMSCs, pilo+salina, pilo+CTMSCs. Vinte e dois animais receberam cloreto de lítio, e 14 horas após foi administrado metilescopolamina e hidrocloreto de pilocarpina. O status epilepticus (SE) foi mantido por 90 minutos, e abortado com diazepam. Após 21 dias de SE, 5 animais do grupo salina e 8 do pilo foram transplantados com CTMSCs GFP+(10⁶/200µl) provenientes de camundongos C57BL6/EGFP, por meio de injeção intravenosa na veia caudal. Decorridos 60 dias de transplante os animais foram testados. Para o experimento de reconhecimento de objetos, os animais foram expostos a um campo aberto (60x40x50 cm, comprimentoxprofundidadexaltura), durante 20 minutos por 4 dias consecutivos para habituação. No dia 1 (Sessão de treino), os ratos foram individualmente colocados no campo aberto contendo 2 objetos (A e B) diferentes, sendo-lhes permitido explorá-los livremente durante 5 min. Vinte quatro horas após (Sessão de teste), os ratos foram colocados novamente no campo aberto, onde um dos objetos foi trocado por um objeto novo (A e C).

Resultados: Nas sessões de treino nenhum dos grupos demonstrou preferência por um dos objetos (ambos desconhecidos). Já na sessão teste os animais dos grupos salina (Obj.A=29,08±5,84 e Obj.C=70,92±5,84), salina+CTMSCs (Obj.A=28,62±3,93 e Obj.C=71,38±3,93) e pilo+CTMSCs (Obj.A=34,91±3,93 e Obj.C=65,09±3,93) mostraram preferência pelo objeto novo ($p < 0,05$ no teste *t* de student), mostrando que estes grupos exibem retenção de memória de longa duração para esta tarefa. No entanto, o grupo pilo+salina (Obj.A=45,89±5,41 e Obj.C= 54,11±5,41) explorou igualmente o objeto novo em relação ao familiar, mostrando que este grupo teve prejuízo na formação da memória de longa duração para a tarefa de R.O.

Conclusão: As CTMSCs são capazes de reverter parcialmente o prejuízo causado pela epilepsia, no reconhecimento de objetos novos.

Palavras-Chave: Epilepsia, células-tronco mesenquimais, lítio-pilocarpina, reconhecimento de objetos

Apoio Financeiro: PUC/RS, CEFET/SC, FUNPAR, Bauducco/PUCRS e Faculdade Gama e Souza/RJ

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Effects of early-life LiCl-pilocarpine-induced status epilepticus on memory and anxiety in adult rats are associated with mossy fiber sprouting and elevated CSF S100B protein

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Purpose: this study investigated putative correlations among behavioral changes, in adulthood, and: neuronal loss, hippocampal mossy fiber sprouting, and reactive astrogliosis in rats submitted to early-life LiCl-pilocarpine-induced status epilepticus (SE). **Methods:** Rats (P15) received LiCl (3 mEq/Kg, i.p.) 12-18h prior pilocarpine (60 mg/Kg; i.p.). Control animals were handled and housed in the same manner as the treated animals and received an equal volume of saline solution (0,9% NaCl). The body weight was checked daily from P15 to P21. At adulthood, animals were submitted to behavioral tasks and after the completion of tasks biochemical and histological analysis were performed, to investigate astrogliosis and mossy fiber sprouting, respectively. **Results:** In SE group, it was observed an increased number of degenerating neurons in the CA1 subfield and in the hilus of animals 24h after SE. At adulthood, SE group presented an aversive memory deficit in an inhibitory avoidance task and the animals that presented lower latency to the step down showed a higher score for mossy fiber sprouting. In light-dark exploration task, SE rats returned less and spent less time in the light compartment and present an increased number of risk assessment behavior (RA). There was a negative correlation between the time spent in the light compartment and the score for mossy fiber sprouting and number of RA. LiCl-pilocarpine-treated animals showed higher levels of S100B immunocontent in the CSF as well as a positive correlation between the score for sprouting and the GFAP immunocontent in the CA1 subfield, suggesting an astrocytic response to neuronal injury. **Conclusion:** In this study we showed that LiCl-pilocarpine-induced SE during development produced long-lasting behavioral abnormalities, which might be associated with mossy fiber sprouting and elevated CSF S100B levels, demonstrating that these effects appear to be associated with a large synaptic reorganization in the hippocampus.

ANÁLISE DO BLOQUEIO DA ATIVIDADE EPILEPTIFORME NÃO-SINÁPTICA DO BROMETO NO GIRO DENTEADO DE RATOS

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Objetivos: verificar o efeito do NaBr sobre as atividades epileptiformes (AE), induzidas por supressão das conexões não-sinápticas por perfusão com líquido cefalorraquidiano artificial (ACSF) 0-Ca⁺⁺ e alto K⁺. **Métodos:** O estudo envolveu medidas experimentais e simulação. Os experimentos com grupo NaBr (n=23), grupo SITS (n=3), grupo controle (n=3) foram realizados em fatias de hipocampo de ratos *Wistar* (250g). Após indução das AE por perfusão com ACSF 0-Ca⁺⁺ e alto K⁺ (8 mM), o Br⁻ foi aplicado nas seguintes concentrações: 5, 7, 9 e 11 mM de NaBr. O SITS também foi aplicado por perfusão.

Resultados: A aplicação do Br⁻ resultou na supressão reversível das AE do giro denteado. Antes da completa supressão das AE, foram observadas as seguintes alterações no potencial elétrico extracelular (PE): (i) redução da amplitude da componente DC; (ii) aumento transitório das amplitudes dos *population spikes*, seguido de redução até completa supressão; (iii) reduções da duração e intervalo entre eventos. Simultâneo ao PE foi registrado o sinal óptico intrínseco (IOS). Em termos temporais, da mesma forma que o PE, observaram-se reduções da duração e do intervalo entre eventos. Espacialmente, o IOS sugere uma partição da região que compreende os eventos, sugerindo uma redução do recrutamento espacial. A reprodução do comportamento espaço-temporal durante o bloqueio foi possível supondo-se o efeito competitivo entre o Br⁻ e o Cl⁻, tanto em canais como em co-transportes. **Conclusões:** 9 mM de NaBr é a concentração mínima necessária para o completo bloqueio das AE, com tempo mínimo de extinção; Simulações computacionais sugerem duas explicações para o efeito inibitório do NaBr sobre as AE: (i) atuação competitiva do Br⁻ com o Cl⁻, decrementando a permeabilidade deste íon que, por sua vez, reduz seus efluxo e influxo durante os períodos interictal e ictal, respectivamente; (ii) o potencial de Nernst do Br⁻ que tem efeito inibitório, favorecendo a redução da excitabilidade. Além disso, a semelhança das regiões do PE e IOS entre NaBr e SITS sugerem possível envolvimento dos canais de Cl⁻ voltagem dependentes.

Palavras-Chave: NaBr, Epilepsia, SITS, Atividade epileptiforme não-sináptica, giro denteado

Apoio Financeiro: CAPES- PROCAD, CNPq, FAPEMIG.

AVALIAÇÃO DA MEMÓRIA EM SAGÜIS (*CALLITHRIX JACCHUS*) EPILÉPTICOS SUBMETIDOS À INJEÇÃO INTRA-HIPOCAMPAL DE VETOR DE ADENOVIRUS ASSOCIADO AO GENE DE NEUROPEPTÍDEO Y

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Objetivos: O neuropeptídeo Y (NPY) atua na regulação de processos cognitivos que geralmente estão desregulados na epilepsia. Neste trabalho, propomos investigar se a expressão de NPY, por vetor de adenovirus associado ao gene de NPY (AAV-NPY), injetado por via intra-hipocampal, pode melhorar a performance cognitiva de animais epiléticos.

Métodos: Sagüis injetados por via intra-hipocampal com AAV-NPY; G-NPY1 ($2,7 \times 10^{13}$ vg/mL, n=4) e G-NPY2 ($1,06 \times 10^{12}$ vg/mL, n=3) e animais controles (G-CTL) foram submetidos, 2 meses depois, ao modelo de pilocarpina (PILO 250mg/kg ip.) para indução de epilepsia. Os animais, nos períodos: antes (período antes), após 1 mês (período durante) da indução com PILO e após a primeira crise espontânea e recorrente (CER) (período depois), foram submetidos a diferentes tarefas de memória (T1, T2, T3, T4, T5 e T6). T1 e T4 servem para habituação ao experimento. T2, T3, T5 e T6 são dependentes de córtex temporal, córtex pré-estriado, hipocampo e córtex ínfimo-temporal, respectivamente. O índice de significância foi $p > 0,05$. Dados expressos: média±EPM.

Resultados: Após a expressão do NPY (período antes), na T2 houve um desempenho significativamente melhor do G-NPY1 ($1,2 \pm 0,2$) e G-NPY2 ($1,7 \pm 0,3$) quando comparados ao G-CTL ($8,5 \pm 2,4$). Na T3, houve um desempenho significativamente pior de G-NPY2 (177 ± 19) quando comparado a G-NPY1 ($2,7 \pm 1,4$) e G-CTL ($9 \pm 4,6$). Quanto à T5, verificou-se um desempenho significativamente melhor do G-NPY1 ($55,8 \pm 5,9$) quando comparado ao G-NPY2 ($347 \pm 20,1$) e G-CTL ($152,5 \pm 9$) e, ainda, os animais do G-NPY2 apresentaram um desempenho significativamente pior que G-CTL. Observações preliminares do G-NPY1, no período durante, não mostraram diferenças em relação ao período antes. No período depois das CER, houve uma aparente piora no desempenho da T5 em relação aos períodos antes e durante.

Conclusões: O NPY na dose maior proporcionou uma melhor performance cognitiva nas tarefas T2 córtex temporal-dependente, T3 córtex pré-estriado-dependente e T5, hipocampo-dependente no período antes. Sugerimos que o NPY manteve o desempenho cognitivo após a indução com PILO, contudo, após o início de CER verificou-se um agravamento no desempenho da tarefa hipocampo-dependente (T5). **Palavras-Chave:** Epilepsia, Memória, Neuropeptídeo Y, Terapia Gênica, Saguis

Apoio Financeiro: CNPq, FAPESP

EFEITO ANTIEPILEPTOGÊNICO DA INJEÇÃO INTRA-HIPOCAMPAL DE VETOR DE ADENOVIRUS ASSOCIADO AO GENE DE NEUROPEPTÍDEO Y EM SAGÜIS *CALLITHRIX JACCHUS* SUBMETIDOS AO MODELO DE PILOCARPINA.

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Objetivos: Durante as convulsões, vários neurônios contendo neuropeptídeos sofrem modificações plásticas, incluindo mudanças na expressão gênica, distribuição e liberação de proteínas e plasticidade dos receptores. A liberação de agentes terapêuticos com ação antiepiléptica duradoura representaria uma alternativa promissora nas epilepsias. Neste sentido, aqui avaliamos se a superexpressão de neuropeptídeo Y (NPY) no hipocampo pela utilização de um vetor associado a um adenovirus recombinante (rAAV-NPY) pode afetar o desenvolvimento de epilepsia.

Metodos: Sagüis, *Callithrix jacchus*, machos de 2-5 anos (250-350g) injetados por via intrahipocampal com AAV-NPY (G-NPY, n=3) e salina (G-CONT, n=5), e após 2 meses foram submetidos ao modelo de pilocarpina (PILO 250 mg/kg ip.). Foi feita análise comportamental por vídeo, eletrocorticográfica por radiotelemetria dos animais avaliados no período de *status epilepticus* (SE) e no período crônico.

Resultados: Os eventos comportamentais, alterações após a injeção da PILO até o início do SE nos animais do G-NPY, não foram diferentes aos evidenciados nos animais CONT. Observando-se uma progressão dos eventos do tipo I/II a escores do tipo III e aparecimento da crise IV-V até o início do SE. Da mesma forma, quanto à duração e perfil de evolução das fases do SE, o G-NPY apresentou susceptibilidade similar aos animais CONT. Contudo, o G-NPY não apresentou o período pós-ictal durante o período agudo de SE quando comparado ao grupo CONT. Dados preliminares das avaliações de CER mostraram que o G-NPY (n=3) apresentou uma latência maior (16 e 32 semanas) para o aparecimento da primeira CER comparado ao G-CONT (1, 5, 7, 15 semanas pós SE).

Conclusões: Podemos sugerir que a administração rAAV-NPY não se mostrou eficaz como anti-*status epilepticus*. Por outro lado, a ausência de período pós-ictal no período agudo minimizou os efeitos decorrentes do SE, assim como, no período crônico da doença retardou o aparecimento das crises espontâneas e recorrentes (CER).

Palavras-Chave: Sagüis, epilepsia, Neuropeptídeo Y, status epilepticus

Apoio Financeiro: FAPESP, CNPq.

EFEITO HORMONAIS EM SAGÜIS *CALLITHRIX JACCHUS* SUBMETIDOS AO MODELO DE PILOCARPINA.

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Objetivos: Hormônios gonadais podem alterar a excitabilidade de neurônios no sistema nervoso central. Evidências demonstram que os estrogênios têm múltiplos efeitos protetores sobre os neurônios. Estudos experimentais sugerem que os esteróides sexuais apresentam efeitos sobre o SNC que potencialmente, afetam a cognição, a susceptibilidade a epilepsia e ansiedade. Vários estudos indicam ainda que os estrogênios atuam melhorando o humor e têm ação antidepressiva e ansiogênica. De modo geral, podemos afirmar que os estrógenos aumentam a excitação neuronal e o risco de crises, e a progesterona diminui. Entretanto, apesar de extensa investigação, a comprovação do papel dos esteróides sexuais no tratamento clínico e prevenção das desordens neuropsiquiátricas e da epilepsia não está bem estabelecida.

Metodos: Sagüis de ambos sexos, castrados grupo (G-CAST, n=4) e normais grupo (G-NOR, n=4) foram submetidos ao Teste de ansiedade denominado de ‘*Marmoset Human Intruder Response Test*’ e posteriormente ao modelo de indução de *status epilepticus* (SE) por pilocarpina (PILO 250 mg/kg ip.). Foi feita análise comportamental por vídeo, eletrocorticográfica por radiotelemetria dos animais avaliados no período agudo de SE.

Resultados: os eventos comportamentais, evidenciados no teste de ansiedade demonstraram que o G-CAST apresentou um aumento na locomoção [$51 \pm 9,2$; $15,8 \pm 2,8$] e diminuição de eventos de *head shake* [$0,3 \pm 0,1$; $5,6 \pm 0,9$]; quando comparados aos animais do G-NOR, respectivamente. As alterações após a injeção da PILO até o início do SE nos animais do G-NOR foram diferentes daquelas evidenciados nos animais G-CAST. Evidenciou-se uma aparente maior susceptibilidade ao SE nos animais do G-NOR (75%) quando comparados aos animais G-CAST (50%). A duração em minutos da crise nos animais do G-NOR foi maior (340 ± 72) que a nos animais G-CAST (87 ± 37), contudo, houve uma aparente maior susceptibilidade à mortalidade nos animais castrados (50%) que apresentaram crise quando comparados aos animais normais (0%).

Conclusões: Podemos sugerir que a ausência de a castração dos animais minimizou os efeitos ansiogênicos e a susceptibilidade epilepsia decorrente da diminuição da duração e precipitação do SE. Sugerimos que os esteróides sexuais influenciam nas modificações das desordens neuropsiquiátricas e na epilepsia. **Palavras-Chave:** Saguis, epilepsia, Hormônios gonadais ,ansiedade

Apoio Financeiro: CNPq, FAPESP.

C.03.025

Aspectos da Dinâmica Temporal do Recrutamento Neural na Ictogênese

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Materiais e Métodos: Foram usados 43 ratos Wistar (250-300 g), submetidos à cirurgia para implante de eletrodos bipolares profundos na amígdala, divididos em 5 grupos: Grupo controle (sem estímulo elétrico profundo), periódico (4 pulsos por segundo, separados entre si por 250 milissegundos (ms)); burst (4 pulsos, separados por 20 ms, posicionados nos últimos 80 ms de cada segundo); não-periódico A (4 pulsos elétricos posicionados aleatoriamente no intervalo de 1 segundo, com proximidade máxima de 20 ms), não-periódico B (4 pulsos distribuídos aleatoriamente, no limite de 1 segundo). Antes da infusão do PTZ (10mg/ml), os animais recebiam 30 segundo de estímulos elétricos e esses permaneciam até o momento da crise tônico-clônica. Foram avaliados o limiar de crise mínima (instante no qual cada animal exibe os primeiros movimentos mioclônicos de membro anterior) e o limiar de crise máxima (crise tônico-clônica). **Resultados:** O limiar, expresso por miligrama de PTZ por grama de peso corporal, para mioclônia de membro anterior foi menor no grupo periódico quando comparado ao controle ($0,0144 \pm 0,0063$ vs $0,0211 \pm 0,0092$; $p < 0,05$) e ao grupo não-periódico A ($0,0144 \pm 0,0063$ vs $0,0238 \pm 0,0074$; $p < 0,05$). O grupo não-periódico B apresentou maior limiar de PTZ para mioclônias de membros anteriores quando comparado aos grupos: controle ($0,0367 \pm 0,0049$ vs $0,0211 \pm 0,0092$; $p < 0,001$); periódico ($0,0367 \pm 0,0049$ vs $0,0144 \pm 0,0063$; $p < 0,001$); burst ($0,0367 \pm 0,0049$ vs $0,0235 \pm 0,0058$ $p < 0,01$); e não-periódico A ($0,0367 \pm 0,0049$ vs $0,0238 \pm 0,0058$ $p < 0,01$). Nenhuma diferença no limiar da crise tônico-clônica foi observada para todos os grupos. **Conclusão:** Os pulsos elétricos periódicos profundos na amígdala de ratos Wistar reduziram o limiar de PTZ aumentando a susceptibilidade para a crise mioclônica. Já pulsos distribuídos aleatoriamente aumentaram o limiar de PTZ diminuindo a susceptibilidade a crise mioclônica. **Palavras-Chave:** Epilepsia, ictogênese, pentilenotetrazol, estímulo elétrico profundo

Apoio Financeiro: Capes, CNPq, FAPEMIG

Early life LiCl-pilocarpine-induced status epilepticus decreases phosphorylation of PKB and GSK-3 β , glutamate uptake and Na⁺/K⁺ ATPase activity in hippocampus.

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Objectives: In this study, we investigated the effect of early life LiCl-pilocarpine-induced SE on phosphorylation of PKB and GSK-3 β , Na⁺/K⁺ ATPase activity and glutamate uptake in hippocampal subfields CA1 and dentate gyrus.

Methods: Rats (P15) received LiCl (3mEq/kg, i.p.) 12-18h prior to pilocarpine (60 mg/kg; s.c.). The biochemical parameters were determined 1.5, 12 and 24 h after the SE.

Results: LiCl-pilocarpine-induced SE decreased the phosphorylation of PKB and GSK-3 β by 35.84% and 38.79%, respectively, 1.5 after SE induction in CA1. However, 24 h after LiCl-pilocarpine-induced SE the phosphorylation of PKB and GSK-3 β returned to control levels. At dentate gyrus, the phosphorylation of PKB was reduced by 35.18% and 31.19% at 1.5 and 24 h after SE induction, respectively. GSK-3 β phosphorylation was reduced by 38.05% and 33.12% at 1.5 and 24 h, respectively, after LiCl-pilocarpine administration. LiCl-pilocarpine treated animals showed decreased levels of glutamate uptake 1.5 h after SE induction. However, 12 and 24 h after SE, the glutamate uptake returned to control levels. LiCl-pilocarpine-induced SE decreased Na⁺/K⁺ ATPase activity by 42% in hippocampal plasma membranes 1.5 h after SE induction. However, at 12 and 24 h after SE there was no difference Na⁺/K⁺ ATPase activity.

Conclusions: Our study provides the first demonstration that the activity of PKB and GSK-3 β is altered in the hippocampus of young rats submitted to early-life LiCl-pilocarpine-induced SE. Moreover, we showed that SE induced early in life decreased hippocampal Na⁺/K⁺ ATPase activity and glutamate uptake 1.5 h after SE induction. These data indicate that LiCl-pilocarpine-induced SE early in life cause short-term biochemical alterations affecting neuronal structure and function. These alterations during development of nervous system may lead to behavioral, morphological and neurochemical alterations in adulthood.

Palavras-Chave: Status epilepticus, glutamate uptake, PKB, GSK-3 β , Na⁺/K⁺ ATPase

Apoio Financeiro: CNPq, FAPERGS, PROPESQ/UFRGS, IBN-Net

Effects of early-life LiCl-pilocarpine-induced status epilepticus on memory and anxiety in adult rats are associated with mossy fiber sprouting and elevated CSF S100B protein

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Purpose: this study investigated putative correlations among behavioral changes, in adulthood, and: neuronal loss, hippocampal mossy fiber sprouting, and reactive astrogliosis in rats submitted to early-life LiCl-pilocarpine-induced status epilepticus (SE). **Methods:** Rats (P15) received LiCl (3 mEq/Kg, i.p.) 12-18h prior pilocarpine (60 mg/Kg, i.p.). Control animals were handled and housed in the same manner as the treated animals and received an equal volume of saline solution (0,9% NaCl). The body weight was checked daily from P15 to P21. At adulthood, animals were submitted to behavioral tasks and after the completion of tasks biochemical and histological analysis were performed, to investigate astrogliosis and mossy fiber sprouting, respectively. **Results:** In SE group, it was observed an increased number of degenerating neurons in the CA1 subfield and in the hilus of animals 24h after SE. At adulthood, SE group presented an aversive memory deficit in an inhibitory avoidance task and the animals that presented lower latency to the step down showed a higher score for mossy fiber sprouting. In light-dark exploration task, SE rats returned less and spent less time in the light compartment and present an increased number of risk assessment behavior (RA). There was a negative correlation between the time spent in the light compartment and the score for mossy fiber sprouting and number of RA. LiCl-pilocarpine-treated animals showed higher levels of S100B immunocontent in the CSF as well as a positive correlation between the score for sprouting and the GFAP immunocontent in the CA1 subfield, suggesting an astrocytic response to neuronal injury. **Conclusion:** In this study we showed that LiCl-pilocarpine-induced SE during development produced long-lasting behavioral abnormalities, which might be associated with mossy fiber sprouting and elevated CSF S100B levels, demonstrating that these effects appear to be associated with a large synaptic reorganization in the hippocampus.

INDUÇÃO DE ATIVIDADES EPILEPTIFORMES NÃO-SINÁPTICAS EM FATIAS DO HIPOCAMPO DE ANIMAIS KINDELIZADOS COM PENTILENOTETRAZOL

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Objetivos: Os modelos kindling de epilepsia experimental são normalmente investigados em termos da atuação ou efeito sobre as conexões sinápticas. Entretanto, como as conexões não-sinápticas são suficientes para geração e sustentação das atividades epileptiformes, o presente trabalho teve como objetivo identificar os efeitos do kindling químico com PTZ sobre a indução de atividades epileptiformes não-sinápticas (AENS) no giro denteado (GD) do hipocampo de ratos *Wistar*.

Métodos: Fatias dos hipocampus de ratos *Wistar* machos de 9 semanas, controle (n=6) e kindelizados com concentrações de 40 (n=5) e 45mg/kg de PTZ (n=7), foram submetidas à indução de AENS por meio de perfusão com solução contendo zero-Ca²⁺ e alto K⁺. Essas fatias foram utilizadas no registro do potencial elétrico (PE) extracelular e na análise morfológica do tecido, corado pelo método de Nissl.

Resultados: Comparando-se o PE dos grupos kindelizados por meio das concentrações de 40 e 45 mg/kg de PTZ com o PE do grupo controle, foi possível verificar aumento da componente DC ($122,1 \pm 8,2\%$, $207,9 \pm 13,2\%$), do intervalo intereventos ($200,5 \pm 22,5\%$, $140,7 \pm 4,9\%$) e dos *population spikes* ($141,7 \pm 12,5\%$, $266,7 \pm 16,7\%$). O grupo kindelizado com a maior dose também apresentou aumento da duração dos eventos ($123,1 \pm 3,7\%$) e redução do parâmetro latência ($60,6 \pm 4,5\%$). As análises morfológicas, indicaram redução da densidade celular no GD dos animais kindelizados tanto com a menor ($64,1 \pm 6,1\%$) quanto com a maior concentração da droga ($48,7 \pm 4,8\%$), além de um quadro de tumefação caracterizado por zonas de palidez.

Conclusão: Os resultados sugerem que o kindling com PTZ, ao promover lesões com características de processos inflamatórios, deve favorecer a excitabilidade neuronal e as conexões não-sinápticas, contribuindo para sustentação das crises epiléticas, mesmo na ausência de mecanismos excitatórios sinápticos. Esse fato pode explicar situações em que o modelo experimental se demonstra refratário à atuação anti-epileptogênica de drogas de ação sináptica.

Palavras-Chave: Epilepsia, Kindling, Pentilenotetrazol, Atividades não-sinápticas, Giro Denteado

Apoio Financeiro: CAPES/FAPEMIG/CNPq

Ligamiento a la región *FEB3* en una extensa familia colombiana con epilepsia autosómica dominante con convulsiones febriles plus, ADEFS+

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Objetivo: El propósito de nuestro estudio fue evaluar ligamiento genético de los loci asociados con convulsiones febriles (CF) y ADEFS+ en una extensa familia de Colombia.

Métodos: Se genotipificaron al menos dos microsatélites estrechamente ligados a cada region candidata. En la region *FEB3* se evaluaron dos marcadores microsatélites adicionales. Los productos de PCR, amplificados con oligos marcados fluorescentemente, se analizaron en un analizador genético ABI-310/377 (Applied Biosystems). Se analizó la segregación mendeliana mediante el programa Pedcheck. Los valores LOD fueron calculados usando el programa MLINK del paquete LINKAGE, bajo distintos valores de penetrancia y tasa de fenocopias.

Resultados y Conclusiones: Se obtuvo exclusión para todos los loci analizados, con excepción del locus *FEB3*. En este locus se evaluaron dos marcadores adicionales (en total 4) con el fin de verificar la presencia de recombinantes. Se obtuvo un máximo Lod score de 5,48 para el marcador D2S2330 a una fracción de recombinación de cero, considerando una tasa de fenocopias del 3% y una penetrancia del 65%. Valores positivos de lod score también fueron observados para los demás marcadores analizados en esta región. Se logró identificar un haplotipo segregando con la enfermedad, el cual está caracterizado por los alelos 2-4-5-1 en los marcadores D2S2380-D2S354-D2S382- D2S2330, respectivamente. No se identificaron individuos recombinantes portadores de este haplotipo, lo cual impidió el refinamiento de la región candidata. *FEB3* es un locus reportado en Convulsiones Febriles que se sobrelapa con *GEFSP2*, uno de los loci en ADEFS+. En este locus se encuentra un cluster de genes que codifican para sub-unidades alfa del canal de sodio dependiente de voltaje. Entre estos se encuentra el gen *SCN1A*, uno de los genes más consistentemente asociados con distintos tipos de epilepsia.

En la actualidad se está llevando a cabo la tamización de mutaciones en los genes de este cluster en búsqueda de la variante responsable de las crisis en esta familia.

Palavras-Chave: Convulsiones Febriles, ADEFS+, FEB3, GEFSP2

Apoio Financeiro: Financiado por Colciencias, proyecto 111534319158

C.03.030

Exposure of Developing Rats to Environmental Hyperthermia Facilitates the Propagation of Cortical Spreading Depression.

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Aims: Cortical spreading depression (CSD) is a brain response related to neural excitability changes and to diseases like epilepsy and migraine. Exposure to a warm environment can alter the neural excitability, changing brain electrophysiological features, which may be relevant to epilepsy genesis. Here we investigated the effects, on brain CSD susceptibility, of exposing developing rats to a warm environment.

Methods

Wistar suckling rats (n=6), suckled by mothers fed a commercial diet with 23% protein, were submitted from day 10st to 29st day of life to 15 daily sessions (5 sessions per week during 3 weeks) of a warm environment (40±2°C), for 15, 20 and 30 min (in the 1st, 2nd and 3rd week, respectively). At 30-40 d of life, they were anesthetized (urethane+chloralose; 1,000+40mg/kg ip) and the ECoG plus slow potential change accompanying CSD were recorded on 2 parietal points for 4h.

Results: Early environmental hyperthermia resulted in higher CSD propagation velocities (P<0.05), as compared to control rats (n=6) submitted to similar sessions at room temperature (25±2°C). Mean CSD-velocities (in nm/min) ranged from 4.08±0.14 to 4.28±0.18 (experimental group) and from 3.74±0.16 to 3.75±0.16 (control group).

Conclusion: Environmental Hyperthermia during the suckling period facilitates CSD propagation in the brain of young weaned rats, as judged by its high velocities, suggesting that the warming episodes occurring during brain development effected cortical susceptibility to CSD. The possible relationship with brain alterations due to febrile seizures in children justify further investigation.

Palavras-Chave: Environmental hyperthermia, Brain development, sensory systems, Spreading depression, Febrile seizures

Apoio Financeiro: CAPES, CNPq, UFPE, FINEP/IBN-Net.

C.03.031

Doublecortin immunoreactivity in dentate gyrus of epileptic patients who underwent epilepsy surgery

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Objectives

Doublecortin (DCX) is a microtubule-associated phosphoprotein involved in neuronal development. In adult neurogenesis newly generated cells express DCX, which has been associated with neurite elongation and synaptogenesis. DCX has been used as a marker of newly born neurons in the adult dentate gyrus (DG). In epilepsy, the role of neurogenesis is controversial and only a few reports determined DCX in epileptic tissue. The aim of this study was to study DCX immunoreactivity in dentate gyrus of human hippocampal sclerosis from patients who underwent epilepsy surgery for intractable temporal lobe epilepsy (TLE).

Methods

Hippocampal sections of 6 patients with hippocampal sclerosis and intractable (refractory) TLE, who underwent therapeutic surgery, were processed using Immunofluorescence and Immunoperoxidase for DCX. Archival material from normal post-mortem hippocampus were simultaneously processed. Staining intensity of neurons expressing DCX was determined by computerized image analysis.

Results

In both control and epileptic hippocampus, DCX immunoreactive (DCX-IR) neurons were found in DG of hippocampus, showing areas with heavy DCX expression. Lower staining intensity was observed in DG of epileptic hippocampus compared with normal hippocampus (transmittance 72, 8 ± 4 / 66, 7 ± 1 ; $p < 0.05$). Cell dispersion of DCX-IR neurons in granular cell layer was found in epileptic tissue. Also longer apical dendrites and slender irregular somas were observed in epileptic GD.

Conclusions

Lower DCX expression, cell dispersion of DCX-IR neurons in granular layer and longer apical dendrites were observed in DG of hippocampal sclerosis. These findings could indicate plastic changes related to epileptic activity in this area. Similar observations determined in experimental models of epilepsy, were related to immature synapses and synapses reorganization.

Palavras-Chave: doublecortin, epilepsy, hippocampal sclerosis, dentate gyrus, neuroplasticity.

Apoio Financeiro: Peruilh Fellowship, CONICET PIP5785, PICT START UP

AVALIAÇÃO DA PERCEPÇÃO VISUAL DA FORMA EM ADULTOS COM EPILEPSIA.

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Objetivo: A função de sensibilidade ao contraste (FSC) pode ser usada para identificar alterações no processamento visual de contraste relacionadas à idade e a patologias. Pesquisas envolvendo a epilepsia e a percepção visual são raras, desta forma, o limiar sensorial de contraste pode contribuir na avaliação de transtornos neuropsicológicos. O objetivo deste estudo foi determinar a FSC de adultos com epilepsia e avaliar possíveis prejuízos na percepção visual.

Método: Participaram deste estudo 16 adultos com idade de 21 a 50 anos de ambos os sexos, sendo oito adultos sem epilepsia e oito adultos com diagnóstico de epilepsia, usando anticonvulsivantes no mínimo há cinco anos. Os grupos apresentaram idades semelhantes e acuidade visual normal ou corrigida. Utilizou-se um delineamento experimental com medidas repetidas e o método psicofísico da escolha forçada para medir a sensibilidade ao contraste de frequências espaciais de 0,25; 2 e 8 ciclos por grau de ângulo visual (cpg). Os estímulos foram gerados e apresentados em tons de cinza com diâmetro de aproximadamente 7 graus de ângulo visual a 150 cm de distância da tela. A tarefa dos voluntários foi escolher entre dois estímulos, qual deles continha a grade senoidal. O outro estímulo era sempre um círculo homogêneo com luminância média de 42 cd/m².

Resultados: Os resultados mostraram que os participantes com epilepsia precisaram da ordem de 1,6; 1,5 e 1,4 vezes mais de contraste para perceber as frequências espaciais de 0,25; 2 e 8 cpg, respectivamente, do que os participantes sem epilepsia. A análise de variância para medidas repetidas (ANOVA) mostrou diferenças entre os grupos ($F_{1, 190} = 18,02$; $p < 0,001$). Já o teste post-hoc Tukey HSD revelou diferença significativa apenas na frequência de 8 cpg ($p < 0,001$).

Conclusão: Os resultados indicam alterações na FSC de grades senoidais na faixa de frequência mais alta, sugerindo alterações relacionadas à epilepsia.

Palavras-Chave: epilepsia; anticonvulsivantes; percepção visual; função de sensibilidade ao contraste.

Apoio Financeiro: CNPQ

PADRÃO HIPOCAMPAL DE EXPRESSÃO DA PROTEÍNA Homer 1a MODELO DE EPILEPSIA DO LOBO TEMPORAL INDUZIDA POR PILOCARPINA EM RATOS

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Objetivos: As epilepsias são um conjunto de distúrbios do sistema nervoso central que leva os indivíduos a apresentarem crises epiléticas espontâneas e recorrentes. O modelo da pilocarpina é capaz de reproduzir em animais de laboratório, aspectos comportamentais e fisiológicos característicos das epilepsias do lobo temporal. A proteína Homer 1a (H1a) liga-se a receptores de glutamato, regulando diversos aspectos fisiológicos envolvidos tanto na transmissão quanto na plasticidade de sinapses glutamatérgicas. Com base nesses resultados, pesquisadores sugeriram que H1a pode participar de importantes mecanismos envolvidos no controle endógeno da iniciação e prolongamento das crises.

Métodos: Ratos Wistar adultos (CEDEME), pesando entre 200 e 250g receberam injeção intraperitoneal (i.p.) de pilocarpina (320mg/kg) para indução de estado de mal epilético (SE). A análise do padrão de expressão de H1a foi feita por meio da técnica de "western blotting" contendo extrato total dos hipocampus dos grupos experimentais 30 minutos, 1, 2, 8 ou 24 horas após a indução do SE (período agudo), 7 e 14 dias (período silencioso) e 30, 60 ou 90 dias (período crônico) após a indução do SE. As membranas foram incubadas com anticorpo anti-Homer 1a e quantificadas por densitometria.

Resultados: No período agudo, a análise da densitometria obtida dos animais pertencentes ao período agudo de SE mostrou um perfil similar nos níveis de expressão de Homer 1a nos grupos, em que apresentou variação de apenas 7,6% no grupo 30 minutos SE, de 10,2% no grupo 1 hora SE e de 10,6% no grupo 2 horas SE quando comparados ao grupo Salina (controle). Observou-se um aumento significativo na expressão de Homer 1a de (21,5%; $P < 0,05$ (Kruskal Wallis) no grupo 8 horas SE e no grupo 24 horas SE (aumento de 19%; $P < 0,05$ (Kruskal Wallis). As médias dos experimentos realizados referentes ao período pós SE mostraram uma pequena e não significativa diminuição de expressão (*down regulation*) nos 3 grupos experimentais, apresentando diminuição de 2,2% no grupo 7 dias SE, 3,9% no grupo 14 dias com crise, e de 3,8% no grupo 14 dias sem crise. No período crônico: Pôde-se observar um leve, mas não significativo, aumento de 7,8% no grupo 30 dias SE de 7,0% no grupo 60 dias SE e um aumento quase inexistente de 3,5% no grupo 90 dias SE, praticamente retornando aos níveis basais de expressão da proteína Homer 1a.

Conclusões: Existem alterações na expressão de H1a no modelo da pilocarpina nos diferentes períodos após o SE visto que H1a está envolvida com o metabolismo e regulação de receptores glutamatérgicos. Qualquer que seja a função desempenhada por esse expressão alterada, ela se estabelece nas primeiras horas após o evento epileptogênico.

Palavras-Chave: Epilepsia, Homer1a, receptores glutamatérgicos

Apoio Financeiro: FAPESP, CNPq, CAPES

PENTYLENTETRAZOL INDUCED SEIZURE INCREASES GLUCOSE AND LACTATE LEVELS IN CEREBROSPINAL FLUID OF RATS

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OBJECTIVE: Seizures induce several morphologic and biochemical alterations in the brain. In experimental studies, pentylenetetrazol (PTZ) has been used as an animal model to induce seizures. Generalized convulsive seizures cause excessive neuronal firing that place large metabolic demands on the central nervous system resulting in a great impact in glycogen, glucose/lactate utilization and availability as energy substrate. To assess the metabolic profile in the brain underwent to an excitotoxic condition we investigated in this study the influence of a single convulsive crisis induced by PTZ on CSF levels of glucose and lactate.

METHODS: CSF levels of glucose and lactate were measured and hippocampal [¹⁴C]-2-deoxy-D-glucose uptake and glycogen content were also evaluated.

RESULTS: animals that presented tonic-clonic seizure had CSF glucose level increased at 10 minutes (96.25 ± 13.19 mg/dL) peaking at 60 minutes (113.03 ± 16.34 mg/dL) and returned to control levels at 24 hours (50.12 ± 12.81 mg/dL), while CSF lactate level also increased at 10 minutes (3.23 ± 1.57 mmol/L) but returned to control level at 360 minutes (1.58 ± 0.21 mmol/L). The [¹⁴C]-2-deoxy-D-glucose uptake decreases 27% at 10 min after seizure and returned to control levels at 30 minutes. Similarly, there was a decrease of 37% in hippocampal glycogen content at 10 minutes, which returned to control levels at 30 minutes.

DISCUSSION: In summary, we shown that acute seizures induced by PTZ elicit a significant temporal increase in CSF glucose and lactate levels and a brief decrease in [¹⁴C]-2-deoxy-D-glucose uptake and glycogen content.

Palavras-Chave: Glucose; Lactate; CSF, Seizure, PTZ.

Apoio Financeiro: CNPq, CAPES and the FINEP research grant "Rede Instituto Brasileiro de Neurociência (IBN-Net)" # 01.06.0842-00.

ESTUDO DO TEMPO DE REFRATARIEDADE À TERAPIA MEDICAMENTOSA NA EPILEPSIA DO LOBO TEMPORAL MESIAL

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Introdução: A epilepsia mesial temporal é a forma mais prevalente de epilepsia em adultos e está, muitas vezes, relacionada à esclerose hipocampal (1). A primeira crise ocorre por volta do fim da infância e as manifestações clínicas são inicialmente autonômicas. Praticamente todos os pacientes têm crises parciais complexas, algumas das quais se generalizam secundariamente. Esse tipo de epilepsia tem uma grande tendência a se tornar refratária ao tratamento (2), o que gerou uma necessidade de se investigar o tempo de instalação da refratariedade, bem como sua associação ao medicamento e dose utilizados, à esclerose hipocampal, à presença de epilepsia mesial temporal (EMT) diagnosticada em familiares e à ocorrência de crises febris na infância.

Métodos: Foi feito um estudo retrospectivo, por análise de prontuários de 50 pacientes do ambulatório de neurologia do Hospital Universitário Antônio Pedro. Os dados pesquisados incluem: Idade na primeira crise, associação a crise febril na infância, presença de caso de EMT da família, características da primeira crise e das subseqüentes, drogas utilizadas, suas doses e tempo de utilização de cada uma, e as características dos exames de imagem e dos eletroencefalogramas no início e ao longo do tratamento.

Resultados: Encontramos média de idade na primeira crise de 13,3 anos, variando de cinco meses a 37 anos. Em 77% dos pacientes, a primeira crise evoluiu para generalização secundária. A refratariedade foi verificada em 80% dos pacientes, sendo a associação inicial de fenobarbital e carbamazepina a terapêutica escolhida para 43% dos pacientes, e a refratariedade notada após uma média de sete anos. 50% dos pacientes que apresentaram ressonância magnética em seus prontuários mostraram esclerose do hipocampo.

Conclusão: Nesta amostra, a EMT mostrou alto índice de refratariedade. A média de troca de medicamento dos pacientes estudados foi de 8,14 vezes, variando de uma a 26 vezes. A associação inicial de drogas mostrou um período de latência maior para a refratariedade do que a monoterapia.

Referências 1- J Epilepsy Clin Neurophysiol 12(1):31-36 2006; 2- J Epilepsy Clin Neurophysiol 12(2):73-74 2006

Palavras-Chave: epilepsia do lobo temporal mesial; refratariedade; esclerose hipocampal

EXTRATO DE *OCIMUM BASILICUM* INIBE A CAPTAÇÃO DE GABA EM CULTURAS PRIMÁRIAS DE RETINA

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Objetivos: Plantas do gênero *Ocimum* são utilizadas popularmente contra diversas doenças do sistema nervoso central, e particularmente extratos de *Ocimum basilicum* já demonstraram efeitos anticonvulsivantes em modelos animais de epilepsia. Assim, o objetivo de nosso trabalho foi elucidar o mecanismo molecular da ação anticonvulsivante da solução extrativa hidroalcoólica de *O. basilicum* (SEHOB) através da análise de captação do neurotransmissor GABA (ácido gama-aminobutírico) em culturas primárias de origem nervoso. **Método:** Células retinianas de galinha White Leghorn foram obtidas de embriões no dia 7 (E7) de desenvolvimento e incubadas em meio DMEM com 10% de soro fetal bovino, antibióticos e glutamina. Após uma semana de incubação a 37°C e 5% de CO₂, as células foram expostas por 30 minutos ao SEHOB nas concentrações de 700 e 1000 µg/ml (equivalentes às doses com melhores efeitos terapêuticos no modelo animal). Como controle positivo foi usada a exposição por 30 minutos com 20 µM de NNC (inibidor do transportador de GABA). Após as incubações, foi realizada a captação de [3H]-GABA (0,5 µCi/500 µl de solução de Hanks) por 15 minutos. As células foram lavadas 3x com solução Hanks e homogenizadas em SDS 1%. Finalmente, foi determinada a radiatividade das amostras de células homogenizadas por meio de cintilação líquida. **Resultados:** O SEHOB na concentração de 700 µg/ml inibiu o transporte de [3H]-GABA nas células de retina em 50,7±15,8% em comparação ao controle. Já a concentração de 1000 µg/ml também reduziu o transporte com uma inibição de 56,0±12,1%, porém não apresentou diferença significativa em relação à concentração de 700 µg/ml. O SEHOB apresentou um efeito semelhante na inibição da captação de [3H]-GABA que o NNC (que diminuiu a captação em 70,2±5,7%). **Conclusão:** O SEHOB é capaz de inibir a captação do neurotransmissor inibitório GABA nas concentrações equivalentes às doses com melhores efeitos terapêuticos no modelo animal, o que poderia representar o principal mecanismo molecular da ação anticonvulsivante.

Palavras-Chave: *Ocimum basilicum*, anticonvulsivante, GABA

Apoio Financeiro: Universidade Federal do Pará (UFPA) e Universidade da Região de Joinville (UNIVILLE)

PARTICIPAÇÃO DO SISTEMA GABAÉRGICO NA INDUÇÃO DE LTP NA AMIGDALA LATERAL DE RATOS WISTAR RESISTENTES E EPILÉPTICOS (WAR)

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Objetivos

Resultados prévios demonstraram diminuição do tônus gabaérgico e menor facilidade de indução de potenciação de longo prazo (LTP) em Wistar Audiogenic Rats (WAR), animais geneticamente selecionados para o fenótipo de crises epilépticas induzidas por estímulo sonoro.

Este trabalho tem o objetivo de verificar a participação do sistema gabaérgico sobre a indução de LTP na amígdala lateral de ratos wistar resistentes e WARs. A hipótese é que o sistema gabaérgico participa da indução de LTP nesta região.

Métodos

Foram preparadas fatias do complexo amigdalóide de ratos Wistar resistentes (n=4) e de WAR (n=6). Iniciou-se o registro do PEPS perfundindo aCSF normal por 20 minutos e então adicionava-se a picrotoxina (50 μ M) e após 30min aplicava-se o estímulo de alta frequência (HFS) e registrava-se por mais 60 min. Estimulava-se a via tálamo-amígdala e os registros extracelulares dos PEPS foram realizados em amígdala lateral. Para os estímulos pré e pós indução de LTP foram aplicados pares de pulsos a 0,05 Hz (intervalo entre os pulso de 50ms) de 100 - 400 μ A, e de 200ms de duração. Para o estímulo indutor de LTP foram aplicados 4 trens de 100 pulsos de 1s de duração separados por um intervalo de 20s.

Resultados

Em ratos Wistar resistentes houve aumento significativo nas amplitudes do PEPS somente nos 5min após a aplicação do HFS (média de aumento 26,7% $\pm$ 15,4) $p < 0,05$. Os WAR não apresentaram aumento significativo nas amplitudes do PEPS. Os resultados foram normalizados pelas amplitudes do PEPS basais antes da indução de LTP (média $\pm$ DPM).

Conclusão

Em presença de picrotoxina não houve indução de LTP na amígdala lateral tanto em animais wistar quanto em WAR, sugerindo que o sistema gabaérgico participa da geração deste fenômeno nesta região da amígdala.

Palavras-Chave: Epilepsia, GABA, LTP

Apoio Financeiro: CAPES, CNPq, FAPEMIG E PRPq/UFMG.

Multiple Subpial Transection in an experimental model of focal epilepsy

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Objectives: To verify the efficacy of Multiple Subpial Transection (MST) in the control the seizure frequency in rats with focal epilepsy induced by topically applied penicillin. **Methods:** Focal epilepsy was induced by topically applied penicillin in thirty-two Wistar male rats with 80-90 days. The animals were anesthetized with ketamin and xilazin then fixed at stereotaxic apparatus; the head skin was open with a straight incision and the cranium was exposed. A left craniotomy was undertaken with a little drill and the bone as well as dura-mater were taken away; five electrodes were fixed on the cortex using the stereotaxic coordinates, making sure the anterior one was positioned on the forelimb coordinate (LL:3mm, AP: 1mm); control video-EEG was registered for 10 min and after that 0.4 micro liters of penicillin G potassic crystalline, was injected at 1mm of subpial depth at the forelimb cortex and then a video-EEG was registered again for 10min. Sixteen animals were submitted to a MST while the other sixteen, to all the procedure but with no MST (control group). The surgical technique was basically two surgical transections 3mm diameter apart in a sagital plane through the cortex with 6-8 mm of length and 1mm of depth, isolating the anterior electrode and then avoiding the spreading of the epleptogenic focal activity; post-surgically a video-EEG was done during 10 min.

Results: The MST decreases considerably and most of the rats stop with generalized seizures and horizontal propagation of electric discharges.

Conclusions: We concluded that MST can be done without any motor deficit in animals and it stops the horizontal propagation of electric discharges, showing to be a very good tool for epilepsy, focusing the motor or eloquent cerebral cortex areas.

Palavras-Chave: Keywords: Epilepsy, Multiple subpial transection, cortex eloquent areas, video-EEG.

Apoio Financeiro: Supported by: CAPES, PUCRS, Fundação Panduratta Ltda e Faculdade Souza Gama.

Behavioral characterization of audiogenic kindling in Wistar rats primed with aminoglycosides.

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Rats, genetically susceptible to audiogenic seizures, develop limbic seizures when audiogenic ones are elicited in a periodic and chronic protocol, which is known as audiogenic kindling (AuK). Kanamycin, an aminoglycoside used in priming protocols, induces susceptibility to audiogenic seizures. In this work, we studied the generation and evolution of audiogenic seizures during chronic acoustic stimulation of rats primed with kanamycin or ampicillin. **Methods:** Wistar male (M) and female (F) rats received one intraperitoneal daily injection from postnatal day (PND) 9 to 12 with the following doses (mg/kg/day) of ampicillin: 100 (A100; n = 4F / 5M), 200 (A200; n = 2F / 1M), 300 (A300; n = 4F / 5M), 600 (A600; n = 5F / 4M) and 900 (A900; n = 1F / 4M); or kanamycin: 100 (K100; n = 9F / 11M). A control group received saline solution 0.9%. After that, beginning on PND 30, rats were submitted to a 30-stimulus AuK protocol. Finally, 30 days after AuK last stimulus, rats were re-stimulated using three stimuli (one/day) in order to test permanence of behavioral alterations induced by AuK. **Results:** 1) Kanamycin-treated and control rats did not present audiogenic seizures; 2) 33% from A100, 0% from A200, 66% from A300, 44% from A600, and 40% from A900 groups became susceptible to AS; 3) proportions of audiogenic susceptible rats that presented limbic seizures during the AuK were: 0% from A100, 80% from A300, 75% from A600 and 50% from A900; 4) all observed limbic seizures presented low severity and no-generalization. **Conclusion:** In contrast with previously published data¹, kanamycin-primed rats did not develop susceptibility to audiogenic seizures. Susceptibility to audiogenic seizures induced by ampicillin-priming is not necessarily associated to development of limbic seizures during an AuK protocol, and if limbic seizures are present, they evolve with less severity than those displayed by rats which are genetically susceptible to audiogenic seizures.

References: Hearing Research 32:1, 1988

Palavras-Chave: audiogenic seizures, neural plasticity, acoustic priming, brainstem seizures

Apoio Financeiro: BIC and BAP FAMERP grants 2007-2008, FAPESP grant 0750339-3

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Prostaglandina E2 facilita convulsões induzidas por metilmalonato

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Objetivo

A acidemia metilmalônica, faz parte de um grupo de erros inatos do metabolismo, sendo ocasionada pelo acúmulo ácido metilmalônico causando convulsões e que existem fatores precipitantes de crises metabólicas nestes pacientes bem documentados, como infecções. Como as prostaglandinas, em especial a PGE₂, é um importante mediador da resposta inflamatória avaliamos o efeito da PGE₂ nas convulsões comportamentais e eletroencefalográficas induzidas pela administração intracerebroventricular de metilmalonato (MMA).

Materiais e Métodos

Ratos adultos machos (270-300 g) foram anestesiados com equitesina (3 mg/ml). Implantou-se uma cânula no ventrículo lateral direito e eletrodos no hipocampo e córtex cerebral através das coordenadas do bregma por meio do aparelho estereotático. Sete a nove dias após o procedimento cirúrgico os animais foram injetados com PBS pH 7,4 e/ou MMA (2,5 µmol/2,5 µl) e/ou PGE₂ (100 ng/2 µl) e monitorado o EEG por 50 min.

Resultados

A injeção de PGE₂ reduziu a latência para as convulsões induzidas por MMA, avaliadas por EEG e comportamentalmente quando comparadas ao grupo controle (controle 281,5 (215,75 – 481,25)s; (PGE₂ 162 (87,75 – 238,25)s, Teste U de Mann – Whitney (U = 5,0 ; p= 0,037). Contudo, o tratamento com PGE₂ não alterou a intensidade medida pela amplitude (F(0.17)=0,846) do registro eletroencefalográfico e a duração (controle 22,66 ± 8,05) (PGE₂ 22,33 ± 5,87) das convulsões induzidas por MMA nas diferentes estruturas.

Conclusão

A PGE₂ facilita a indução das convulsões por MMA, mas não altera a intensidade dos episódios convulsivos, medidas pela duração e amplitude dos registros eletroencefalográficos.

Palavras-Chave: Convulsão, Prostaglandina E2, Metilmalonato

Apoio Financeiro: CNPq, CAPES

O EXTRATO DE *OCIMUM BASILICUM* (MANJERICÃO) INIBE A CAPTAÇÃO DE GABA EM CULTURAS PRIMÁRIAS DE RETINA

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Objetivos: Plantas do gênero *Ocimum* são utilizadas popularmente contra diversas doenças do sistema nervoso central, e particularmente extratos de *Ocimum basilicum* já demonstraram efeitos anticonvulsivantes em modelos animais de epilepsia. Assim, o objetivo de nosso trabalho foi elucidar o mecanismo molecular da ação anticonvulsivante da solução extrativa hidroalcoólica de *O. basilicum* (SEHOB) através da análise de captação do neurotransmissor GABA (ácido gama-aminobutírico) em culturas primárias de origem nervoso. **Método:** Células retinianas de galinha White Leghorn foram obtidas de embriões no dia 7 (E7) de desenvolvimento e incubadas em meio DMEM com 10% de soro fetal bovino, antibióticos e glutamina. Após uma semana de incubação a 37°C e 5% de CO₂, as células foram expostas por 30 minutos ao SEHOB nas concentrações de 700 e 1000 µg/ml (equivalentes às doses com melhores efeitos terapêuticos no modelo animal). Como controle positivo foi usada a exposição por 30 minutos com 20 µM de NNC (inibidor do transportador de GABA). Após as incubações, foi realizada a captação de [3H]-GABA (0,5 µCi/500 µl de solução de Hanks) por 15 minutos. As células foram lavadas 3x com solução Hanks e homogenizadas em SDS 1%. Finalmente, foi determinada a radiatividade das amostras de células homogenizadas por meio de cintilação líquida. **Resultados:** O SEHOB na concentração de 700 µg/ml inibiu o transporte de [3H]-GABA nas células de retina em 50,7±15,8% em comparação ao controle. Já a concentração de 1000 µg/ml também reduziu o transporte com uma inibição de 56,0±12,1%, porém não apresentou diferença significativa em relação à concentração de 700 µg/ml. O SEHOB apresentou um efeito semelhante na inibição da captação de [3H]-GABA que o NNC (que diminuiu a captação em 70,2±5,7%). **Conclusão:** O SEHOB é capaz de inibir a captação do neurotransmissor inibitório GABA nas concentrações equivalentes às doses com melhores efeitos terapêuticos no modelo animal, o que poderia representar o principal mecanismo molecular da ação anticonvulsivante.

Palavras-Chave: *Ocimum basilicum*, anticonvulsivante, GABA

Apoio Financeiro: Universidade Federal do Pará (UFPA) e Universidade da Região de Joinville

Bilateral anterior thalamic nucleus lesions are not protective against spontaneous recurrent seizures in chronic pilocarpine epileptic rats

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Objective: Investigate whether anterior thalamic nucleus (AN) lesions are protective against spontaneous recurrent seizures (SRS) in the chronic phase of the pilocarpine model of epilepsy.

Methods: Adult male Wistar rats, two weeks after pilocarpine induced *Status epilepticus* (SE), underwent to bilateral AN radiofrequency thalamotomies (n=20) or sham surgery (n=19) following the coordinates anteroposterior -1.5; lateral $\pm$ 1.0; depth 3.5 (Paxinos and Watson; 1998). Thalamotomies were performed with 50 mA for 10 seconds, delivered through a radiofrequency (RF) generator (model MFRG01B, Micromar, Brazil). After the lesions, animals were videotaped from the 2nd to the 8th week post SE, 12 hours/day, 5 days/week. Animals from both groups, with the highest number of seizures (n=3 per group) were selected for electroencephalographic (EEG) analysis. Electrodes were stereotactically implanted in the right hippocampus (anterior -3.8; lateral 3.5; depth 6.5) and recording electrodes were placed over the ipsilateral sensorio-motor cortex. The non-parametric Mann-Whitney test, $p \leq 0.05$ was used to compare the frequency and latency for spontaneous seizures. Values are expressed as mean $\pm$ standard deviation.

Results: The latency for monitored SRS was 36 ± 12.7 and 28.8 ± 7.8 days after SE for AN lesions and sham-treated groups, respectively ($p=0.2$). During the 6 week of observation, no differences were noticed in the frequency of spontaneous seizures when animals that had bilateral AN lesions (2.1 ± 1.7 seizures/animal) were compared to sham-treated controls (2.8 ± 2.8 seizures/animal; $p = 0.7$). EEG recordings during SRS (n = 6 seizures) in animals that had bilateral AN lesions or sham-surgery showed ictal activity initially in the hippocampus with a subsequent propagation to the cerebral cortex. Histological section confirmed that lesion compromised most of the thalamus anteroventral nucleus and parts of the anterodorsal and of the anteromedial nuclei.

Conclusion: We concluded that AN thalamotomies are not effective in reducing SRS during the chronic phase of the pilocarpine model of epilepsy.

Palavras-Chave: : Thalamus; Deep brain stimulation; Thalamotomy; Epilepsy; Surgery; Anterior nucleus.

Apoio Financeiro: FAPESP, CNPq.

Involvement of kinin B₂ receptors in the development of the pilocarpine-induced epilepsy in female rats

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Objectives: Kinin, a class of polypeptides represented by bradykinin, kallidin, and their metabolites, acting via B1 and B2 receptors, have been related to cytokines action, glutamate release and prostaglandin production. Several studies indicate that in the central nervous system and peripheral tissues, estrogen regulates the expression of B2 receptor and reduces inflammatory response. Accordingly, the present work aimed to investigate expression of kinin B2 receptors in female rats submitted to the pilocarpine model of epilepsy. Methods: The animals were divided in four groups: OVX + SE ovariectomized female rats, which presented status epilepticus (SE); SE: intact female rats that presented SE; OVX ovariectomized female rats that received saline instead pilocarpine; SAL intact female rats that received saline instead pilocarpine. The hippocampi from all groups were removed 5h (acute group), 5 days (silent group) and 60 days (chronic group). The hippocampi from all groups were used to perform the distribution and quantification of kinin B2 receptor by Western Blotting (n=4 per group) and immuno-histochemistry (n=3 per group). Results: the results showed a decrease of immunoreactivity of kinin B2 receptor in the hippocampal formation during the acute and silent but not in chronic periods of this model in SE group when compared to SAL group. In contrast, the immunoreactivity in OVX + SE group was increased during the acute and silent periods when compared with OVX group. The Western Blotting showed an increased expression of kinin B2 receptor in OVX +SE group during the acute (2872.42 ± 236.82) ($p=0.0034$) and silent periods (2391.32 ± 188.18) ($p=0.0277$), when compared with its proper controls (OVX) (1739.08 ± 425.16). In addition, a decreased expression in SE group during the acute (1171.35 ± 259.73) ($p=0.0039$) and silent periods (1661.87 ± 345.55 , $p=0.0121$) were found, when compared with controls (SAL, 2470.92 ± 509.58). Conclusions: This study showed that the expression of kinin B2 receptor is modified in female rats during epileptogenesis and modulated by steroid hormones.

Palavras-Chave: Bradykinin, Pilocarpine

Apoio Financeiro: FAPESP

C.04.001

Efeitos da fisioterapia aquática e da cinesioterapia em paciente com doença de Charcot-Marie-Tooth: relato de caso descritivo

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Introdução: A doença de Charcot-Marie-Tooth, descrita em 1886, tem como característica uma atrofia muscular neurítica e peroneal que afeta o sistema nervoso periférico. Dentre as abordagens terapêuticas empregadas no tratamento de indivíduos com doenças neuromusculares encontram-se a fisioterapia aquática e a cinesioterapia. *Motivo e propósito:* Como não foram encontrados artigos relacionando a doença de Charcot-Marie-Tooth e a atuação da cinesioterapia e da fisioterapia aquática nestes pacientes, este estudo de caso descritivo analisará os efeitos destas intervenções em uma paciente com Charcot-Marie-Tooth. *Método:* Foram realizadas duas avaliações entre Março e Junho de 2007, sendo realizados os testes *Timed Up and Go*, Velocidade da Marcha, Subir e Descer Escadas, Caminhada de 6 minutos, aplicação do Questionário de Qualidade de Vida SF-36 e da Escala de Equilíbrio de Berg. O tratamento consistiu de duas sessões semanais de 1 hora, envolvendo atividades de circuito, transferência de ajoelhado para de pé, exercício de mini-agachamento no solo e exercícios aquáticos. *Resultados:* Observou-se uma melhora quantitativa da qualidade de vida, equilíbrio, tempo para subir e descer escadas, velocidade da marcha e distância percorrida; e uma melhora qualitativa do padrão de marcha, mudanças de postura e da queixa funcional da paciente. *Conclusão:* a associação da cinesioterapia com a fisioterapia aquática pareceu melhorar a funcionalidade da paciente.

Palavras-Chave: doença de Charcot-Marie-Tooth, fisioterapia, fisioterapia aquática

C.04.002

Associação do treino da musculatura respiratória e do treinamento de condicionamento aeróbio à fisioterapia motora em um paciente com doença de Charcot-Marie-Tooth: relato de caso descritivo.

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A doença de Charcot-Marie-Tooth, uma neuropatia caracterizada por atrofia muscular distal e hipoestesia pode estar associada à hipotonia, fraqueza muscular, diminuição dos reflexos profundos, fadiga e alterações respiratórias. Nós relatamos o caso de uma paciente com atrofia muscular significativa nos músculos da perna e com dificuldade para deambular. O objetivo do estudo foi avaliar e mensurar os efeitos da fisioterapia motora, do treinamento de condicionamento aeróbio e do treino muscular respiratório em uma paciente com essa patologia. METODOLOGIA: As variáveis analisadas foram: Pressão Inspiratória Máxima (PImáx), Pressão Expiratória Máxima (PEmáx), Teste de Caminhada de 6 Minutos, Timed Get Up and Go, os Testes de Velocidade Média da Marcha e Subida e Descida de Escadas e o Questionário de Qualidade de Vida SF-36. RESULTADOS: Após 8 semanas de tratamento foram encontrados os seguintes resultados: aumento dos valores da Pimáx e Pemáx. Houve também aumento na distância percorrida no teste de caminhada de 6 minutos, na pontuação atingida no questionário SF-36, na velocidade média da marcha e nos tempos de realização dos testes de subida e descida de escadas. Houve diminuição no tempo de realização do timed get up and go. CONCLUSÃO: O treino de força muscular respiratória e o treinamento aeróbio associados à fisioterapia convencional neurológica podem potencializar a funcionalidade motora e respiratória e prevenir a atrofia muscular e a fadiga em pacientes com doenças do sistema nervoso periférico.

Palavras-Chave: Treino muscular respiratório, condicionamento aeróbio, pressão inspiratória máxima.

C.04.003

IgG-NMO antibody detection in NMO spectrum disorder. A relationship to clinical data.

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Optic neuromyelitis (NMO) is an inflammatory demyelinating disease, affecting selectively optic nerves and spinal cord and frequently misdiagnosed as multiple sclerosis (MS). Sera of patients with NMO spectrum disorders (NMO-SD): relapsing-NMO (R-NMO), recurrent optic neuritis (r-ON), recurrent longitudinally extensive transverse myelitis (r-LETM), and r-LETM-CNS were evaluated to NMO-IgG autoantibody. Peculiarities of optic events and attacks numbers in R-NMO patients with immunoreactivity to IgG-NMO are described being more relevant those regarding to optic (100%) and spinal events (87.5%) as well as to the spinal fatality index (75 %) relative to . Brain magnetic resonance (MRI, 1.5 Testa) was also performed according to the protocol from consortium MS centre. The findings of three cases with MRI lesions, particularly different to those found in MS are also showed and discussed in the context of morphological, spatial distribution of lesions, and lesion duration. Our results, suggest an autoimmune humoral component in NMO-SD, although with a probably different pattern from MS. From these findings, seropositivity of IgG-NMO can be considered as a useful complement to differentiate acute lesions of NMO/MS in addition to the consortium MS centre criteria.

Palavras-Chave: Demyelinating disease, IgG-NMO

Apoio Financeiro: CIREN and Hospital Clinica de Barcelona

C.04.004

Comparação entre treinamento com exercícios funcionais e não funcionais na recuperação de pacientes com seqüela motora por Acidente Vascular Encefálico.

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Objetivo: Avaliar e comparar a recuperação funcional e a reorganização cerebral de doze pacientes após infarto cerebral crônico reabilitados com dois tipos de seqüências: uma baseada em exercícios funcionais (SF) e outra baseada em exercícios não funcionais (SNF), através de escalas clínicas e de Ressonância Magnética Funcional (RMf).

Métodos: Dois treinamentos foram comparados: uma seqüência não funcional (grupo A, n=6) e outra funcional (grupo B, n=6). Todos os pacientes foram avaliados por escalas clínicas; Fugl-Meyer, ARA teste e Barthel modificado em quatro períodos: o pré-treino (P1), o pós-treino (P2), após um mês (P3) e após três meses (P4) do término do tratamento. Os pacientes foram tratados com trinta sessões de 90 minutos cada. Todos os pacientes foram submetidos aos exames de RMf.

Resultados: O grupo A melhorou suas pontuações na escala de Fugl Meyer nos quatro períodos e na escala de Barthel Modificada no P4 quando comparado com o P3. O grupo B melhorou suas pontuações nas escalas de Fugl-Meyer e no ARA teste em todos os períodos de análise. Quando comparamos os índices dos dois grupos não encontramos diferenças significativas em nenhum momento tanto para as escalas de Fugl-Meyer, ARA e Barthel modificada. A RMf mostrou uma ativação circunscrita em áreas vizinhas ao infarto e áreas motoras primárias ipsilaterais à lesão após a reabilitação. Diminuição da ativação de áreas motoras primárias do hemisfério contralateral à lesão e ativação da área motora suplementar pós-treino. Foi verificado, ainda, que quando os pacientes ficaram sem o treinamento (P3 e P4) a ativação voltou a ter o mesmo padrão de antes da reabilitação.

Conclusão: Os dois treinamentos melhoraram os pacientes, principalmente nas escalas de Fugl-Meyer e ARA. Além disso, a análise entre os dois grupos mostrou que as mudanças na pontuação das escalas parecem não ser dependentes do tratamento aplicado. Este trabalho ainda nos proporcionou visualizar os padrões de ativação encefálica presentes dos pacientes do estudo antes e depois da reabilitação, como também, o melhor conhecimento das dificuldades metodológicas e limitações da RMf, fornecendo informações para futuras investigações.

Palavras-Chave: 1. AVE. 2. reabilitação. 3. RMf. 4. escalas funcionais. 5. exercícios funcionais.

C.04.005

***Caenorhabditis elegans*: organismo modelo de síndromes miasténicos congénitos humanos**

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Caenorhabditis elegans es un organismo modelo para el estudio del sistema nervioso y sus alteraciones. El receptor nicotínico de acetilcolina (AChR) juega un rol clave en la contracción muscular tanto en el hombre como en *C. elegans*. La subunidad UNC-63 es un componente esencial del AChR muscular de nematodo. El alelo *unc-63(x26)* presenta una mutación en la región *Cys-loop* (UNC-63C151Y) que imita la reportada en un síndrome miasténico congénito (CMS) humano asociado a pérdida de función.

Objetivos: Establecer un modelo animal para el estudio de CMSs humanos.

Metodología: Utilizamos ensayos de motilidad para evaluar el movimiento de las cepas salvaje y mutante y aplicamos la técnica de *patch clamp* a nivel de canal único en células musculares de ambas cepas de *C. elegans* en el estadio larval L1.

Resultados: Los ensayos de motilidad demostraron que la cepa *unc-63(x26)*, en forma semejante al paciente, presenta severos trastornos motores. Registros de *patch clamp* muestran una marcada reducción en el número de sellos activos en presencia de ACh, una disminución de aproximadamente 100 veces en la frecuencia de aperturas y una reducción del tiempo de apertura del canal. Estas observaciones pueden explicarse mediante una disminución de la expresión del AChR junto con una reducción en su eficacia de activación. Dichos cambios llevan a una importante alteración en la transmisión neuromuscular, similar a la observada en pacientes. El tratamiento conjunto con piridostigmina y 3,4-diaminopiridina, utilizado en pacientes con este tipo de síndrome, revierte parcialmente el fenotipo de estos nematodos.

Conclusiones: Demostramos la validez de *C. elegans unc-63(x26)* como modelo para el estudio de CMSs asociados a pérdida de función de AChRs. Esta cepa constituye el primer modelo animal para la investigación de terapias farmacológicas para el tratamiento de este tipo de patologías.

Palavras-Chave: receptor nicotínico, síndrome miasténico, *C. elegans*

Apoio Financeiro: CONICET, UNS, FONCYT

Efectos de la apotransferrina en la remielinización: participación de la vía de señalización mediada por el receptor Notch.

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Estudios previos demostraron que una dosis de apotransferrina (aTf) inyectada intracranealmente a ratas de 3 días de edad produce un incremento en los componentes de la mielina. Hemos demostrado este efecto promielinizante de la aTf en un modelo de desmielinización experimental inducido por cuprizona (CPZ). El objetivo del presente trabajo es estudiar la posible participación de la vía de señalización mediada por el receptor Notch en la reversión de la desmielinización producida por la inyección de aTf. Ratas Wistar fueron alimentadas con una dieta conteniendo 0.6% de CPZ durante las dos semanas siguientes al destete. Al retirar la CPZ de la dieta, los animales recibieron estereotáxicamente una inyección de aTf o solución salina en uno de los ventrículos laterales y sacrificados a distintos tiempos. Se disecciona el cerebro para los estudios de inmunohistoquímica o la zona subventricular (SVZ) y el cuerpo calloso (CC) para evaluar el nivel de distintas proteínas involucradas en esta vía de señalización por Western blot o la expresión de genes específicos por RT-PCR. Resultados previos demostraron que el nivel de Notch es menor en la SVZ de los animales controles y aumenta 6 hs después de la inyección de aTf mientras que parte de un nivel mayor en los animales desmielinizados pero disminuye después de la inyección de aTf. La inyección de aTf induce una significativa disminución en la activación de la vía de Notch en los precursores de OLGs NG2+ presentes en la SVZ de ratas desmielinizadas concomitantemente con una disminución de los niveles de Jagged en las células GFAP+. Sin embargo, en el CC de los animales desmielinizados, observamos por ensayos de inmunoprecipitación usando un anticuerpo anti-Deltex que la inyección de aTf induce un incremento de los niveles de Deltex asociado al fragmento intracelular de Notch. Resultados preliminares obtenidos luego de inyectar conjuntamente la aTf con un inhibidor de la γ -secretasa en animales C y CPZ mostraron un nivel de remielinización similar al obtenido al inyectar sólo la aTf. Estos resultados sugieren la participación de la vía de señalización mediada por Notch en el efecto de la aTf sobre la remielinización. **Palabras-Chave:** Desmielinización; Oligodendrocitos; Zona Subventricular; Remielinización

Apoio Financeiro: Subsidio otorgado por la Agencia Nacional de Promoción Científica y Tecnológica (PICT 2004 N° 25408)

C.04.007

Bone marrow mononuclear cells characterization and participation in the Wallerian degeneration process

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We have previously described the spontaneous migration or after an intraorticly injection of fresh bone marrow mononuclear cells (BMMC) CD34⁺ to the distal stump of a ligated sciatic nerve, an irreversible model of Wallerian degeneration (WD). The aim of the present work is to demonstrate in a reversible model of Wallerian degeneration such as the crush of the sciatic nerve whether BMMCs migrate to the distal area in order to participate in the degeneration - regeneration process, and to characterize the BMMCs and the mesenchymal stem cells in order to know which is the population that migrate to the injured nerve during the WD process.

Adult Wistar rats were submitted to the crush of the right sciatic nerve and were sacrificed at different survival times. The nerve was dissected into proximal and distal areas; the left sciatic nerve, contralateral nerve, was taken as a control. BMMC were isolated from the bone marrow extruded from tibia and femur bones. A group was immediately processed and the other was seeded. After 24 and 48hs the non-adherent cells were separated and the adherent cells were grown to confluence. Both groups were analyzed by Western blot. Fresh isolated or cultured BMMC were dyed with a fluorescent probe and injected intravenously immediately after crushing the sciatic nerve in order to evaluate the migration of these cells to the injured area.

The characterisation of cultured BMMCs shows a heterogeneous population of small-rounded, spindle-shaped and large-flattened morphology; they showed fibroblast-like morphology at reaching confluence.

After 24 and 48hs in culture the adherent and non-adherent cells are CD34⁺, while adherent cells are CD45⁻, CD11b⁻, vimentin⁺, Thy 1.1⁺.

We demonstrated the migration of fresh isolated BMMC to the crushed area and to the distal area of the sciatic nerve during the first week post injury.

Our results demonstrate the existence of a progenitor population in BMMC that spontaneously migrates to the injured nerve to participate in the degeneration-regeneration process.

Palavras-Chave: demyelination; bone marrow mononuclear cells; mesenchymal stem cell; Wallerian Degeneration

Apoio Financeiro: ANPCYT, PICT 05-32975

C.04.008

ESTUDO DA CONDUÇÃO NERVOSA DO NERVO CAUDAL EM RATOS DIABÉTICOS

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Objetivo: Avaliar uma técnica recentemente desenvolvida no nosso serviço utilizando garras “jacarés” comuns no estudo da condução nervosa do nervo caudal de ratos diabéticos.

Métodos: 40 Ratos *Wistar*, machos, com 60 dias foram utilizados, sendo o diabetes induzido em 20 ratos por dose única intraperitoneal (60 mg/kg de peso) de Streptozotocina (Sigma, tampão citrato de sódio 10mM, pH 4,5). Os outros 20 ratos constituíram o grupo controle. A velocidade de condução (VC) e a amplitude do potencial de nervo sensitivo foram determinadas 5 dias antes da indução e semanalmente durante 6 semanas consecutivas 10 dias após a indução. Após ser anestesiado, o animal era posicionado ventralmente, de forma que sua cauda ficasse livre. O estudo da condução nervosa foi registrada na cauda do animal com técnica ortodrômica. A inserção da distância foi mantida constante em 8cm entre os eletrodos. Para a estimulação utilizaram-se dois eletrodos de superfície do tipo garras “jacaré”. Durante todo o procedimento a temperatura da cauda foi mantida em uma média de 31,5°. A análise estatística foi realizada por uma análise de variância (ANOVA). As comparações *post-hoc* foram feitas com teste de Tukey. O *p* crítico para ambos os testes foi de 0,05.

Resultados: Antes da indução tanto a VC (diabéticos 36±2m/s versus controles 34±3m/s) como a amplitude (33±7µV versus 29±6µV) não foi significativamente diferente. Após a indução a VC foi menor (39±3m/s versus 44±4m/s na 6ª semana pós-indução) e a amplitude maior (107±23µV versus 85±13µV) nos ratos diabéticos. A menor VC nos ratos diabéticos era esperada e é sugestiva de uma disfunção da bainha de mielina. A maior amplitude nos ratos diabéticos foi um achado inesperado. Isto provavelmente se deveu ao menor diâmetro da cauda dos ratos diabéticos, fazendo com que o nervo caudal ficasse mais próximo ao eletrodo de captação de superfície.

Conclusão: Nossos achados sugerem que quando se utiliza eletrodo de captação de superfície para o estudo da condução nervosa do nervo caudal em ratos diabéticos, a velocidade de condução é mais útil que a amplitude, pois não é influenciada pelo diâmetro da cauda. **Palavras-Chave:** Diabetes experimental, Condução nervosa, Nervo caudal

MORPHOLOGICAL AND FUNCTIONAL CHARACTERIZATIONS OF SCHWANN CELLS STIMULATED WITH *Mycobacterium leprae*.

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The major complication observed in leprosy is deformities development during the disease. In the present study, we used a human malignant Schwann cell (SC) lineage (ST88-14) to characterize the phenotype modulation and cell death when these cells were infected with *Mycobacterium leprae* (ML) and your glycolipid (PGL-I) *in vitro*. We also investigated the physiologic role of SC and the comprehension of the mechanisms by which ML and its components may modulate these functions. The SCs, established from malignant Schwannoma (neurofibrosarcoma) of patients with neurofibromatosis type 1, were cultured (7×10^4 cells/well) on 24-well plates for *in vitro* stimulations (ML and PGL-I). For morphological evaluation, control cells were seeded onto glass coverslips during 24h incubation, after which the cells were immunolabeled (antibodies anti-laminin, S-100, myelin basic protein (MBP), glial fibrillary acidic protein (GFAP), ninjurin, CD44 or CD68). Analysis of NGF-R (nerve growth factor receptor), MBP, S-100, GFAP, and Bcl-2 expression were also assessed by flow cytometry. Apoptosis levels were accessed by two distinct methods: 1) observation of phosphatidylserine externalization stained with annexinV-FITC conjugate; and 2) hypodiploid fraction of cells stained with PI by cell-cycle analysis. We observed that ST88-14 constitutively express markers that are compatible with SC, and both ML and PGL-I did not modulate the expression of these marked. We also observed that SC can be susceptible to death by ML and PGL-I. Our data suggest that ML may induce death in this lineage, by a mechanism that does not involve the intrinsic pathway of apoptosis. It is possible that apoptosis induction by ML in SC might be directly related to nerve damage in leprosy. **Palavras-Chave:** Schwann cell, ML, Apoptosis

Apoio Financeiro: Fiocruz, FAPERJ

ATIVACÃO MUSCULAR DO TIBIAL ANTERIOR DE PORTADORES DA DOENÇA DE CHARCOT-MARIE-TOOTH ATRAVÉS DE DIAGONAIS DE TRONCO DA TÉCNICA DE FACILITAÇÃO NEUROMUSCULAR PROPRIOCEPTIVA

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Objetivo: Verificar se as diagonais de tronco, da técnica de Facilitação Neuromuscular Proprioceptiva (PNF), podem modificar a atividade elétrica do músculo Tibial Anterior (TA) de sujeitos portadores da doença de Charcot-Marie-Tooth tipo1 A (CMT1A). **Materiais e Métodos:** Participaram deste estudo 8 sujeitos de ambos os sexos, acometidos pela doença CMT1A, com neuropatia periférica acometendo ambos os membros inferiores (MMII). Estes foram submetidos a dez sessões de tratamento com as seguintes diagonais de tronco: estabilização de tronco (EST), flexão, rotação e inclinação lateral para a direita (FRID) e flexão, rotação e inclinação lateral para a esquerda (FRIE), duas vezes por semana. Em cada sessão, a ativação muscular do TA, de ambos os MMII, foi mensurada com um equipamento portátil de Eletromiografia de Superfície (EMG System do Brasil, Modelo 400c-200c) com 6 canais de leitura. Foram utilizados eletrodos de superfície, duplos gelatinosos prata-prata auto-adesivos com diâmetro e distância entre eletrodos de 10 mm. Os dados foram coletados durante a realização de cada diagonal de maneira individualizada. Cada diagonal de PNF foi repetida quatro vezes. Os dados foram analisados comparando-se os valores iniciais da média do sinal retificado (RMS) com os valores encontrados após 10 sessões de PNF. Os dados iniciais (DI) e finais (DF) foram normalizados e submetidos ao teste em t de Student com valores de p significativos <0,05. **Resultados:** A EST produziu aumento nos valores de RMS do TA esquerdo e direito ($t=-3,11$; $p<0,005$) e ($t=-2,39$; $p<0,05$), respectivamente. Já a FRIE aumentou de maneira significativa apenas o valor do RMS do TA esquerdo ($t=-2,67$; $p<0,05$) e não do direito ($t=-1,57$; $p=0,14$). Na mesma direção a FRID modificou significativamente os valores de RMS do TA direito ($t=-2,58$; $p<0,05$) e esquerdo ($t=-2,36$; $p<0,05$). **Conclusões:** As diagonais de PNF, utilizadas nesse trabalho, foram efetivas em produzir modificações na ativação muscular do TA de sujeitos portadores da doença de CMT1A. Ainda, os aumentos da atividade RMS aconteceram dentro dos padrões de irradiação de força descritos na técnica de PNF.

Palavras-Chave: Doença de Charcot – Marie – Tooth tipo 1 A, neuropatia hereditária desmielinizante sensitivo-motora, Eletromiografia de superfície, Facilitação Neuromuscular Proprioceptiva

Apoio Financeiro: CNPq

C.04.011

Estudio de la participación de la familia de p53 en la diferenciación oligodendroglial a través de la regulación del promotor de MBP.

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Recientemente se ha adjudicado la participación de las proteínas de la familia de p53 en desarrollo y diferenciación oligodendroglial. Los objetivos que nos planteamos son: 1) El estudio de los niveles de expresión de p53, p73 y su dominante negativo ($\Delta Np73$), durante la diferenciación oligodendroglial y 2) La evaluación de las consecuencias de la sobreexpresión de las proteínas: p53, p73 y su dominante negativo ($\Delta Np73$) en células oligodendrogliales. La metodología utilizada fue la evaluación de los niveles de expresión de mRNA y de las proteínas de esta familia en cultivos primarios de células oligodendrogliales y en una línea oligodendroglial inmortalizada N20.1. Esto se realizó durante la diferenciación de las células y la sobreexpresión de las diferentes isoformas de la familia de p53. La línea celular N20.1 fue co-transfectada con el promotor de MBP unido a genes reporteros (luciferasa y b-galactosidasa) y los diferentes miembros de la familia de p53-FLAG. Nuestros resultados muestran importantes cambios en los niveles de los mRNA de estas proteínas, así como en su expresión y localización celular durante la diferenciación oligodendroglial indicando una relevante participación de estas proteínas en este proceso. Además la activación del promotor de MBP debida a la sobreexpresión de todos los miembros de la familia de p53 muestra una activa participación principalmente de p73 en dicha activación. Esto sugeriría un importante papel de esta familia de proteínas en la decisión de la expresión de una de las proteínas más importante de la mielina, que permite la diferenciación definitiva de las células oligodendrogliales.

Palavras-Chave: oligodendroglial differentiation, p53 family, MBP promoter

Apoio Financeiro: Dr. Laura Pasquini PhD

C.04.012

Treatment with bone marrow mononuclear cells induces functional recovery and decreases neurodegeneration after sensorimotor cortical ischemia in rats

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Objectives: In this work the beneficial effect of the treatment with bone marrow mononuclear cells (BMMC) in a model of cortical ischemia was evaluated. Functional recovery and neurodegeneration were analyzed. Structural plasticity was analyzed by quantification of plasticity-related proteins.

Methods: Ischemic lesion was induced by thermocoagulation in the left sensorimotor cortex. BMMC were obtained from donor rats and injected into the femoral vein one day after the ischemia. In the BMMC group, animals received 3×10^7 cells. In the control group, animals received PBS. Animals were tested for behavior at post-ischemic days (PID) 1, 7, 14, 21, 28 and 35. The course of the neurodegeneration was analyzed in animals of both groups by the reaction for Fluoro-Jade (FJ). Animals sacrificed at PID 7 or 28 were used for quantification of the proteins GAP-43 or synaptophysin, respectively, by immunoblotting.

Results: In the cylinder test, only the BMMC group presented recovery of function, with significant effect of the treatment ($F = 29.02$; $p < 0.0001$ - two-way ANOVA). The difference in the asymmetry score between BMMC and control groups was significant at PIDs 14, 21 and 28 ($p < 0.05$ - Bonferroni posthoc). In the beam test, both groups showed improvement, although a tendency of faster recovery was observed in the BMMC group. In the adhesive test, both groups did not show significant recovery of function. FJ⁺ cell counting revealed significant decrease in the neurodegeneration in the periphery of the lesion in the BMMC group ($p < 0.05$ - t test). Immunoblotting analysis revealed no significant difference in the expression of the studied proteins between the groups.

Conclusions: The treatment with the BMMC induced functional recovery, at least in the cylinder test. It might be the result of the decreased neurodegeneration observed in the periphery of the lesion after treatment. However, the recovery did not seem to be caused by plastic changes in cortical connections, since no significant change in the expression of plasticity-related proteins was seen. These results suggest that the therapy for stroke with BMMC is effective and might be a protocol of treatment for humans.

Palavras-Chave: Cell therapy; neuropasticity; stroke

Apoio Financeiro: CNPq; CAPES

C.04.013

Participación de la vía de señalización de Notch y Nrg-1 en la diferenciación oligodendroglial

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Notch es un receptor de membrana que parece estar implicado en la diferenciación oligodendroglial. Con su activación genera una cascada de señalización que puede determinar el destino celular, según el ligando que se une al receptor. En oligodendrocitos, se describió que la unión de Jagged inhibe la diferenciación mientras que F3-contactina la induce. Neuregulina-1 (Nrg-1) pertenece a proteínas reguladoras del desarrollo y del destino celular de precursores neuronales multipotentes.

Para estudiar la participación de la activación de estos receptores y la red de señales que desencadenan la diferenciación oligodendroglial, trabajamos con cultivos primarios de células oligodendrogliales (OLGs) y con una línea celular, N20.1, que posee su ciclo celular artificialmente alterado (expresa la proteína viral Tag(ts), que captura p53). A 39°C Tag(ts) se inactiva y el ciclo celular se regulariza, permitiendo la diferenciación. Estas células fueron transfectadas con los vectores de expresión de los dominios intracelulares, N1ICD (por activación de Notch1), N1ICD mutado (DTAD y DRA) y Nrg-1-ICD (back-signaling), además de promotores *target* de estas vías de señalización ligados a luciferasa. Evaluamos la diferenciación oligodendroglial de las situaciones experimentales a través de la activación del promotor de MBP.

En N20 se observa activación del promotor de MBP sobreexpresando tanto N1ICD como DRA, pero este aumento es independiente de Nrg-1-ICD. A 39°C la actividad basal del promotor es 4 veces superior que a 36°C, y no se observa inducción con N1ICD pero sí con DRA, atenuada levemente por NRG.

Se ha descrito una interacción directa entre NICD y Tap73, además de la participación de miembros de la familia de p53, en especial p73, en la diferenciación oligodendroglial. En OLGs se observa un aumento de MBP con DRA como en las N20.1 cultivadas a 39°C.

Parecería haber relación entre las vías de señalización de Notch y Nrg en células oligodendrogliales aunque sus efectos sobre la diferenciación no parecen estar mediados por rutas conocidas sino por medio de otras vías, siendo p73 un candidato posible aunque futuros experimentos son necesarios para confirmar esta hipótesis. **Palavras-Chave:** Oligodendrocito, Notch, Mbp, p73

Apoio Financeiro: Ph D Pasquini, L. A.

Caracterização morfológica do músculo esquelético de ratos *Wistar* submetidos à isquemia focal induzida por endotelina-1

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Patologias que afetam neurônios motores superiores são uma das principais causas de prejuízo motor na vida adulta. Os achados a cerca das modificações teciduais sofridas pelos órgãos efetores (músculos) após isquemia encefálica focal são controversos. Desta forma, objetiva-se com este estudo caracterizar morfológicamente o músculo sóleo de ratos *Wistar* submetidos à isquemia focal induzida por endotelina-1 (ET-1), no hemisfério ipsilateral e contralateral ao hemisfério lesado. Para este fim, foram utilizados 10 ratos *Wistar*, os quais foram divididos nos grupos (a) Sham e (b) Isquemia. A isquemia focal foi induzida por meio da administração de 2 µL de 800 pmol de ET-1 de acordo com as coordenadas estereotáticas: (1) AP 0, ML ±2.0, DV -2.0; (2) AP +2.3, ML ±2.0, DV -2.0; (3) AP +0.7, ML ± 3.8, DV -6.0. Decorridas 6 semanas da isquemia o músculo sóleo do membro posterior ipsilateral e contralateral ao hemisfério lesado foram retirados, fixados em paraformaldeído 4%, glutaraldeído 2,5% e tampão fosfato 0.1 M por 24 h e incluídos em resina DURCUPAN. Cortes semi-finos (800 nm) serão realizados, corados com azul de toluidina e examinados ao microscópio óptico a fim de determinar: (a) densidade de fibras musculares (número de fibras/mm²) e (b) área média de fibras musculares (µm²). A média (gramas ± DP) do peso muscular no hemisfério contralateral ao hemisfério lesado foi de 0,29 ± 0.02 e 0,31 ± 0.02 para os grupos sham e isquemia, respectivamente. No hemisfério ipsilateral ao hemisfério lesado as médias de peso muscular para os grupos sham e isquemia foram de 0,30 ± 0.01 e 0,30 ± 0.01. A média de peso corporal (gramas ± DP) no grupo sham foi de 336,4 ± 18.4 e no grupo isquemia foi de 336,0 ± 46.9. Não houve diferença significativa entre peso corporal; peso muscular no hemisfério afetado e não afetado entre os grupos, assim como, não houve correlação entre peso muscular e peso corporal.

Palavras-Chave: isquemia focal, músculo esquelético

Apoio Financeiro: CNPq

C.04.015

Análise morfológica do músculo sóleo de ratos submetidos à hipóxia-isquemia neonatal

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A hipóxia-isquemia perinatal é importante causa de mortalidade e morbidade em humanos, resultando em prejuízos cognitivos e motores. O modelo desenvolvido por Levine (1960) e modificado por Rice et al. (1981) induz encefalopatia hipóxico-isquêmica unilateral, a qual acomete principalmente hipocampo, estriado e córtices sensorial e motor. Objetiva-se com este estudo investigar as possíveis modificações morfológicas sofridas pelo músculo esquelético de ratos adolescentes submetidos à hipóxia-isquemia neonatal. Foram utilizados 8 ratos Wistar machos aleatoriamente distribuídos nos grupos (a) sham (5) e (b) hipóxia-isquemia (3) (HI). No 7º dia pós-natal (PN), os ratos foram submetidos à isquemia, com oclusão permanente da artéria carótida comum direita. Em seguida, os animais foram expostos à ambiente hipóxico, composto por 2% de O₂ e 92% de N₂ durante 90 minutos. No 45º dia PN o músculo sóleo do membro posterior direito e esquerdo foi retirado mediante anestesia, embebido em solução fixadora composta por paraformaldeído 4%, glutaraldeído 2,5% e tampão fosfato 0.1 M por 24 h e incluído em resina DURCUPAN. Cortes semi-finos (800 nm) serão realizados, corados com azul de toluidina e examinados ao microscópio óptico a fim de determinar: (a) densidade de fibras musculares (número de fibras/mm²) e (b) área média de fibras musculares (µm²). Os resultados parciais referentes a peso muscular e peso corporal (gramas ± D.P.) indicam: peso muscular no grupo HI de 0,050 ± 0.003 e 0,052 ± 0.012 para sóleo direito e esquerdo; 0,059 ± 0.006 e 0,060 ± 0.006 para sóleo direito e esquerdo no grupo sham. A média de peso corporal foi de 125,5 ± 36.0 e 142,36 ± 15.9 para grupos HI e sham, respectivamente. Os resultados parciais não indicam diferenças entre os pesos muscular e corporal entre os animais HI e sham. **Palavras-Chave:** hipóxia-isquemia, músculo esquelético

EFEITOS DE UM PROTOCOLO DE CINCO SEMANAS DE TRATAMENTO ATRAVÉS DA TÉCNICA DE FACILITAÇÃO NEUROMUSCULAR PROPRIOCEPTIVA NA ATIVAÇÃO VOLUNTÁRIA DO MÚSCULO TIBIAL ANTERIOR EM PORTADORES DA DOENÇA DE CHARCOT-MARIE-TOOTH

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Objetivo: O objetivo deste estudo foi verificar o efeito de 5 semanas de tratamento, através de um protocolo de Facilitação Neuromuscular Proprioceptiva (PNF), sobre músculo Tibial Anterior (TA) de sujeitos portadores da doença de Charcot-Marie-Tooth tipo 1 A (CMT1A). **Metodologia:** Participaram desse estudo, 8 sujeitos de ambos os sexos, com neuropatia periférica associada a CMT1A, acometendo ambos os membros inferiores. Estes Foram submetidos a 10 sessões de tratamento através de padrões de PNF. Os padrões foram: Tronco (Estabilização e Flexão-Inclinação Lateral com Rotação), Membro Superior (*Chopping* Bilateral Assimétrico e Simétrico) e Membro Inferior (Flexão-Abdução-Rotação Externa e Extensão-Adução-Rotação Interna). A avaliação foi realizada através de um equipamento de eletromiografia de Superfície (EMG) (EMG System do Brasil, Modelo 400c-200c). No início de cada sessão, foi registrada a média do sinal retificado (RMS) da contração muscular ativa de dorsiflexão em posição sentada e deitada. Os dados foram normalizados para a comparação dos efeitos obtidos pré e pós-tratamento. Os dados iniciais e finais foram submetidos ao teste em t de *Student* com valores de p significativos <0,05. **Resultados:** Não existem diferenças significativas entre os valores de RMS nas contrações ativas para o TA direito (D) e esquerdo (E) na avaliação inicial nas posições sentado e deitado ($t=-0,02$; $p=0,98$) e ($t=0,11$; $p=0,91$), respectivamente. Já as comparações pré e pós-tratamento, para a contração ativa com o paciente na posição sentada, evidenciaram um aumento significativo nos valores de RMS para o Músculo TA D ($t=-2,45$; $p<0,05$), entretanto o mesmo não aconteceu para o músculo TA E ($t=-1,52$; $p=0,15$). Na mesma direção, as comparações da contração ativa com o paciente na posição deitada demonstraram um aumento significativo para o TA D ($t=-3,79$; $p<0,005$) e não para o TA E ($t=-1,34$; $p=0,19$). **Conclusões:** O protocolo de PNF, utilizado nesse trabalho, foi efetivo em aumentar a contração muscular voluntária do TA D de sujeitos portadores da doença de CMT1A. Entretanto, o mesmo não acontece para o músculo TA E. Análises adicionais são necessárias para o entendimento desse fenômeno.

Palavras-Chave: Eletromiografia de superfície, Doença de Charcot – Marie – Tooth tipo 1 A , neuropatia hereditária desmielinizante, Facilitação Neuromuscular Proprioceptiva

Apoio Financeiro: CNPq

ATIVACÃO MUSCULAR IPSI-LATERAL DO TIBIAL ANTERIOR DE PORTADORES DA DOENÇA DE CHARCOT-MARIE-TOOTH ATRAVÉS DE DIAGONAIS DE MEMBRO SUPERIOR E INFERIOR DA TÉCNICA DE FACILITAÇÃO NEUROMUSCULAR PROPRIOCEPTIVA

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Objetivo: Avaliar a resposta da média do sinal retificado (RMS) produzida pela irradiação muscular ipsilateral ao Tibial Anterior (TA), pré e pós-tratamento de Facilitação Neuromuscular Proprioceptiva (PNF), em pacientes com polineuropatia desmielinizante associada à doença de Charcot-Marie-Tooth do tipo 1A (CMT1A). **Materiais e Métodos:** Participaram deste estudo 6 pacientes, de ambos os sexos, portadores da doença de CMT1A. Eles foram tratados em uma frequência de duas vezes por semana, durante cinco semanas, totalizando dez sessões. Em cada sessão foram aplicados os seguintes padrões de PNF: Membro Superior (*Chopping* à direita e à esquerda) e Membro Inferior (MI) (Flexão-Abdução-Rotação Externa e Flexão-Adução-Rotação Interna, ambas realizadas no MI direito e esquerdo). Esses padrões foram repetidos quatro vezes, em ambos os lados, estimulando a irradiação muscular e assim evocando a contração do músculo TA. Durante as repetições dos padrões, a resposta muscular dorsiflexora de cada paciente foi obtida por um eletromiógrafo (EMG) de superfície (EMG System do Brasil, Modelo 400c-200c). A média dos valores de RMS, dentre as quatro repetições coletadas, foram obtidas e normalizadas. Os dados iniciais e finais foram submetidos ao teste em t de *Student* com valores de p significativos <0,05. **Resultados:** A diagonal de *Chopping* à direita produziu aumento nos valores de RMS do TA direito ($t=-2,32$; $p<0,05$), entretanto a diagonal de *Chopping* à esquerda não produziu modificações significativas ($t=-0,44$; $p=0,67$). Para as diagonais de MI, somente a diagonal de Flexão-Abdução com rotação externa à direita aumentou significativamente os valores de RMS ($t=-2,51$; $p<0,05$). As demais diagonais, Flexão-Abdução com rotação externa à esquerda, Flexão- adução com rotação interna à Direita e Esquerda, não produziram modificações na mesma direção ($t=-0,95$; $p=0,36$), ($t=-1,17$; $p=0,28$) e ($t=-1,47$; $p=0,19$), respectivamente. **Conclusões:** As diagonais de PNF, utilizadas nesse trabalho, que se direcionaram para o membro inferior direito foram efetivas em produzir modificações na ativação muscular do TA direito de sujeitos portadores da doença de Charcot-Marie-Tooth tipo1 A (CMT1A).

Palavras-Chave: Doença de Charcot – Marie – Tooth tipo 1 A , neuropatia hereditária desmielinizante, Eletromiografia de superfície, facilitação neuromuscular proprioceptiva

Apoio Financeiro: CNPq e FAPESP

ATIVACÃO MUSCULAR CONTRALATERAL DO TIBIAL ANTERIOR DE PORTADORES DA DOENÇA DE CHARCOT-MARIE-TOOTH ATRAVÉS DE DIAGONAIS DE MEMBRO SUPERIOR E INFERIOR DA TÉCNICA DE FACILITAÇÃO NEUROMUSCULAR PROPRIOCEPTIVA

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Objetivo: Avaliar a resposta da média do sinal retificado (RMS) produzida pela irradiação muscular contralateral ao Tibial Anterior (TA), pré e pós-tratamento de Facilitação Neuromuscular Proprioceptiva (PNF), em pacientes com polineuropatia desmielinizante associada à doença de Charcot-Marie-Tooth do tipo 1A (CMT1A). **Materiais e Métodos:** Participaram deste estudo 6 pacientes, de ambos os sexos, portadores da doença de CMT1A. Eles foram tratados em uma frequência de duas vezes por semana, durante cinco semanas, totalizando dez sessões. Em cada sessão foram aplicados os seguintes padrões de PNF: Membro Superior (*Chopping* à direita e a esquerda) e Membro Inferior (MI) (Flexão-Abdução-Rotação Externa e Flexão-Adução-Rotação Interna, ambas realizadas no MI direito e esquerdo). Esses padrões foram repetidos quatro vezes, em ambos os lados, estimulando a irradiação muscular e assim evocando a contração do músculo TA. Durante as repetições dos padrões, a resposta muscular dorsiflexora de cada paciente foi obtida por um eletromiógrafo (EMG) de superfície (EMG System do Brasil, Modelo 400c-200c). A média dos valores de RMS, dentre as quatro repetições coletadas, foram obtidas e normalizadas. Os dados iniciais e finais foram submetidos ao teste em t de *Student* com valores de p significativos <0,05. **Resultados:** Ambas diagonais de *Chopping* à direita e a esquerda produziram aumento nos valores de RMS do TA esquerdo (t=-3,94; p<0,05) e direito (t=-3,87; p=0<0,05), respectivamente. Para as diagonais de MI, somente a diagonal de Flexão-Abdução com rotação externa a direita não aumentou significativamente os valores de RMS do TA contralateral (t=-1,55; p=0,17). As demais diagonais, Flexão-Abdução com rotação externa a esquerda, Flexão- adução com rotação interna a Direita e Esquerda, aumentaram significativamente os valores de RMS do TA contralateral a diagonal (t=-3,33; p<0,05), (t=-3,31; p<0,05) e (t=-4,58; p<0,05), respectivamente. **Conclusões:** As diagonais de PNF utilizadas nesse trabalho produziram potente irradiação de força contralateral, visualizadas pela elevação dos valores de RMS, em ambos TA de sujeitos portadores da doença de Charcot-Marie-Tooth tipo1 A (CMT1A).

Palavras-Chave: Doença de Charcot – Marie – Tooth, neuropatia hereditária desmielinizante, Eletromiografia de superfície, facilitação neuromuscular proprioceptiva

Apoio Financeiro: FAPESP e CNPq

C.04.019

AUTOFLUORESCENT VARIETY OF α -quartz (SiO₂) in electric TISSUE

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The electric organs (EO) of *Psammobatis extenta* produce weak electrical discharges to the surrounding environment. Its majority cells -electrocytes- derived from myoblasts have evolved and differentiated independently, losing the contractile ability. In previous works we employed EDS-SEM and documented the presence of aluminium and silicon in significant concentrations. Zinc, oxygen and copper were also localized. For Polarized Light Microscopy we could also identify specifically a microcrystalline variety of SiO₂: chalcedony. Now, using Epifluorescence Microscopy (Epi-fl) and Confocal Laser Scanning Microscopy (LCSM) on EO subcellular extracts of *P. extenta* obtained for differential centrifugation we identify that the chalcedony autofluorescent is distributed in all the subcellular fractions. Neuronal cell death and synaptic terminal degeneration have been noted in the EO of this fishes (Fox *et al.*, 1990 and our observations). The crystalline form of this mineral of quartz could interact with the DNA of the nucleus of the electrocyte and to intervene in processes of neuromuscular disorders. There is this the first time that is demonstrated the presence of the autofluorescent chalcedony in EO using Epi-fl and LCSM.

Palavras-Chave: Electrocytes, Neurodegeneration, Silica

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IMPAIRMENT IN MAGNO AND PARVOCELLULAR PATHWAY RESPONSES IN MULTIPLE SCLEROSIS

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Purpose: To assess magnocellular (M) and parvocellular (P) responses in patients with multiple sclerosis (MS), with and without history of optic neuritis. **Methods:** We evaluated 21 patients with diagnosis of MS (16F; 5M; mean age = 34.8±10.34 years). All patients had visual acuity between 0 and 0.1 logMAR and had no significant findings in an ophthalmologic exam performed at the time of testing. Nine patients had optic neuritis episodes between 1 and 26 years prior to the test. One patient had an episode 1 month before the test. We used Pokorny & Smith's (1997) Steady-Pedestal (SP) and Pulsed-Pedestal (PP) paradigms as adapted recently by Gualtieri *et al.* (2006). For both paradigms, a pedestal of four 1°x1° squares was presented on a 2.0 log Ped (Td) surround, and observers had to detect a brief (17 or 133-ms) increment of luminance of one of these (the test square, TS). For the PP paradigm, the pedestal is pulsed along with the TS, which is modulated relative to the other 3 squares. For the SP paradigm, the pedestal is on continuously, and only the TS is modulated. Pedestal luminances were 1.8, 2.0 and 2.2 log Ped (Td). Thresholds were measured using a double-interleaved staircase, in 4-alternative forced-choice task. Prior research indicates that the SP and PP paradigms reflect M- and P-dominated responses, respectively.

Results: For the PP paradigm, patients' contrast thresholds were significantly elevated relative to controls at 2.0 and 2.2 log Ped (Td) for the 17-ms TS duration. For the 133-ms TS, their thresholds were different for the 1.8 and 2.0 log Ped (Td). For the SP paradigm, patient's thresholds were significantly elevated for 17 ms in 2.0 log Ped (Td). In 133 ms, all luminances were significantly higher (Mann-Whitney U-test, $p < 0.01$). Threshold data did not correlate with time of disease. No significant differences between MS patients with and without a previous neuritis episode. **Conclusion:** MS patients evaluated in this study presented reduced contrast sensitivity in the PP and SP paradigm. This suggests impairment in both P and M visual pathways.

Palavras-Chave: Multiple sclerosis; parvocellular pathway; magnocellular pathway; luminance contrast sensitivity

Apoio Financeiro: FAPESP; CNPq; CAPES

C.05.001

Selective estrogen receptor modulators decrease reactive gliosis after brain injury

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Estradiol is a neuroprotective hormone that reduces reactive gliosis and brain inflammation. However, the peripheral effects of estradiol limit its potential therapeutic use as a neuroprotectant. We have explored whether two selective estrogen receptor modulators (SERMs), tamoxifen and raloxifene, that are used in human clinics and that do not have the pro-oncogenic effects of estradiol, may regulate reactive gliosis after brain injury. In addition, we assessed the effect of PPT and DPN, selective agonists of estrogen receptors alpha and beta, respectively. Wistar ovariectomized rats received a penetrating injury in the left cerebral hemisphere and were treated with daily sc injections of estradiol, tamoxifen, raloxifene PPT or DPN at doses of 1 mg/Kg on days 0-2 after injury. The number of vimentin immunoreactive astrocytes and the volume fraction of major histocompatibility complex-II (MHC-II) immunoreactive microglia were estimated one week after injury in the hippocampus, in the lateral border of the wound. All estrogenic compounds tested resulted in a significant decrease in the number of vimentin-immunoreactive astrocytes. In addition, the volume fraction of MHC-II immunoreactive microglia was significantly decreased in the animals that received estradiol, raloxifene or PPT. These findings suggest that SERMs may have neuroprotective effects by the reduction of reactive gliosis. In addition, both alpha and beta estrogen receptors appear to be involved in the regulation of reactive astroglia while only the alpha form of estrogen receptors is involved in the regulation of reactive microglia.

Palavras-Chave: Brain injury; estradiol; SERMs; glia; neuroprotection

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Testosterone regulates the proliferation of glial cells after brain injury

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In addition to its effects on reproduction, testosterone affects cognition, regulates brain plasticity and exerts neuroprotective actions. In this study we have assessed the effect of testosterone and its metabolites, estradiol and dihydrotestosterone (DHT), on glial proliferation after a penetrating injury in the left cerebral hemisphere of Wistar orchidectomized rats. Animals received daily s.c. injections of testosterone (5 mg/Kg), estradiol (1 mg/Kg), DHT (5 mg/Kg) or vehicle on days 0-2 after injury. BrdU was injected i.p. on days 1-3 after injury. The number of cells showing colocalization of BrdU and vimentin (BrdU+/Vim+), BrdU and major histocompatibility complex-II (BrdU+/MHC-II+) and BrdU and NG2 (BrdU+/NG2+) was estimated one week after injury in the CA1 layer of the hippocampus using the optical disector method. Administration of testosterone resulted in a significant decrease in the number of BrdU+/Vim+, BrdU+/MHC-II+ and BrdU+/NG2+ cells. Estradiol reduced the number of BrdU+/Vim+ cells, but did not significantly affect the number of BrdU+/MHC-II+ and BrdU+/NG2+ cells. DHT did not affect the number of glial cells labelled with BrdU. These findings indicate that testosterone may decrease the proliferation of reactive astroglia, reactive microglia and NG2 cells and suggest that its effect on the proliferation of reactive astroglia may be in part mediated by its metabolite estrad **Palavras-Chave:** Brain injury; estradiol; glia; neuroprotection

Apoio Financeiro: Supported by Ministerio de Educación y Ciencia, Spain (SAF 2005-00272) and the European Union (EWA project: LSHM-CT-2005-518245).

AÇÃO NEUROPROTETORA DO NEUROPEPTÍDEO ÁCIDO GLUTÂMICO-IOLEUCINA SOBRE NEURÔNIOS SENSITIVOS DE RATOS NEONATOS.

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Objetivos: O neuropeptídeo ácido glutâmico-isoleucina (NEI) é um peptídeo de 14 aminoácidos derivado do pré-pró-hormônio concentrador de melanina. O NEI possui similaridade como o alfa-hormônio estimulante de melanócito e tem sido considerado como um possível ligante de receptores de melanocortinas. O receptor de melanocortina 4 (MC4), bem como a pró-ópiomelanocortina, estão presentes na medula espinhal e gânglios da raiz dorsal (GRD) de ratos. Melanocortinas possuem efeito neuroprotetor sobre neurônios sensoriais e motores. O presente estudo teve como objetivo investigar a possível ação neuroprotetora do NEI sobre neurônios sensitivos após esmagamento do nervo ciático de ratos com 2 dias de vida (P2).

Métodos e Resultados: Ratos Wistar (P2; n=5/grupo) tiveram o nervo ciático esquerdo (NCE) esmagado e foram tratados por 5 dias (d) com NEI (1,2mg/g) ou PBS. Após 20, 30 e 45d a recuperação funcional sensitiva (RFS) foi avaliada através da medida da intensidade do estímulo limiar para a resposta de retirada da pata (LRR), provocada pela estimulação elétrica na região plantar. Após a última avaliação, o NCE foi seccionado e o coto proximal foi exposto a uma solução de Amina Dextrano Biotinilado (BDA). Após 7d os GRD L4 e L5 foram dissecados e processados para revelação histoquímica dos neurônios sensitivos contendo BDA (NS+). O número total de NS+ foi obtido a partir da sua contagem corrigida pela fórmula de Abercrombie. Os valores do LRR registrados nos ratos tratados com NEI foram significativamente inferiores (20d: 3,46±0,35 mA; 30d: 2,12±0,30 mA; 45d: 1,94±0,18 mA) aos do grupo controle (20d: 4,80±0,20 mA; 30d: 3,81±0,14 mA; 45d: 2,88±0,26 mA). Este dado foi corroborado pelo maior número de NS+ computados no grupo NEI (L4: 951±32; L5: 2512±267) relativamente ao grupo PBS (L4: 669±24; L5: 1038±41; p<0,001).

Conclusões: Nossos dados evidenciaram que o NEI é capaz de proteger neurônios sensitivos axotomizados em ratos neonatos e que este peptídeo pode ter estimulado a regeneração axonal dos neurônios dos GRD, contribuindo assim para a recuperação funcional sensitiva. A investigação dos mecanismos moleculares envolvidos nestes efeitos do NEI encontra-se em andamento.

Palavras-Chave: NEI, neuropeptídeo ácido glutâmico-isoleucina, ganglio da raiz dorsal, neuroproteção, nervo ciático

Apoio Financeiro: CAPES/SPU-CAPG-BA 06/02, FAPESP (04/13849-5), CNPq

FOLIC ACID PREVENTS OXIDATIVE STRESS IN CORTEX AND BLOOD OF HYPERHOMOCYSTEINEMIC RATS

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Objectives: We initially investigated the effect of chronic hyperhomocysteinemia on some parameters of oxidative stress, named non-enzymatic antioxidant potential and antioxidant enzymes activities, as well as DNA damage in parietal cortex and blood of rats. We also evaluated the effect of folic acid on biochemical alterations elicited by hyperhomocysteinemia.

Methods: Wistar rats received daily injections of Hcy (0.3-0.6 µmol/g body weight), and/or folic acid (0.011 µmol/g body weight) from the 6th to the 28th day of life. Twelve hours after the last injection the rats were sacrificed, parietal cortex and total blood was collected. We evaluated total antioxidant potential (Free Radic Res Commun. 17:299, 1992), superoxide dismutase (Handbook for oxygen radical research. 243, 1985 in cortex and J Biol Chem. 247:3170, 1972 in blood), catalase (Meth Enzymol. 105:121, 1984), and glutathione peroxidase activities (Meth Enzymol. 77:325, 1981 in cortex and J Lab Clin Med. 70:158, 1967 in blood); and DNA damage by comet assay (Environ Mol Mutagen. 35:206, 2000) or by micronucleus test (Mutagenesis. 16:323, 2001) in parietal cortex and blood of rats.

Results: Our results showed that homocysteine administration increased DNA damage, evaluated by comet assay, and disrupted antioxidant defenses in parietal cortex and blood of rats. Folic acid concurrent administration prevented homocysteine effects, possibly by its antioxidant and DNA stability maintenance properties.

Conclusions: Homocysteine induced oxidative stress and folic acid prevented such effects. If confirmed in humans, we could propose the supplementation of folic acid as an adjuvant therapy in disorders that accumulate homocysteine, such as homocystinuria, neurodegenerative and vascular diseases.

Palavras-Chave: homocysteine; folic acid; oxidative stress

Apoio Financeiro: CNPq, FAPERGS, PROPESQ/UFRGS, FINEP (IBN-Net 01.06.0842-00)

EVIDENCE THAT ISOVALERIC ACID AND ISOVALERYLGLYCINE THAT ACCUMULATE IN ISOVALERIC ACIDEMIA INDUCE OXIDATIVE STRESS IN BRAIN CORTEX OF RATS

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OBJECTIVES: Patients affected by isovaleric acidemia (IVA) suffer from acute episodes of encephalopathy whose underlying mechanisms are poorly known. The present study investigated the *in vitro* effects of isovaleric acid (IVA) and isovalerylglycine (IVG), which accumulate in IVA, on important parameters of oxidative stress in supernatants and mitochondrial preparations from brain of 30-day-old rats.

METHODS: Cortical supernatants or mitochondrial fractions were incubated for 1 hour at 37°C in the presence of IVA or IVG. Immediately after incubation, aliquots were taken to measure chemiluminescence, thiobarbituric acid-reactive substances (TBA-RS) levels, glutathione (GSH) concentrations and protein carbonyl formation (PCF).

RESULTS: With respect to supernatants, IVG significantly increased chemiluminescence (30%; Control: 3318±764 cpm/mg of protein) and TBA-RS levels (41%; Control: 3.7±0.7 nmol/mg of protein) and decreased GSH concentrations (20%; Control: 5.8±1.0 nmol/mg of protein). IVA significantly induced PCF in cortical supernatants (90%; Control: 0.4±0.2 nmol/mg of protein) and mitochondrial preparations (60%; Control: 2.7±0.3 nmol/mg of protein). We also observed that the addition of free radical scavengers fully prevented IVG-induced increase of TBA-RS. Furthermore, IVG did not alter all examined parameters in mitochondrial preparations, indicating that the generation of oxidants by IVG was dependent on cytosolic mechanisms.

CONCLUSIONS: The data indicate that oxidative damage mainly induced by IVG may underlie the pathophysiology of the neurological dysfunction of IVA.

Palavras-Chave: isovaleric acidemia, oxidative stress, rat brain

Apoio Financeiro: Financial support: CNPq, FAPERGS, PRONEX and the FINEP research grant Rede Instituto Brasileiro de Neurociência (IBN-Net) # 01.06.0842-00.

SELENO COMPOUNDS PREVENTS THE BRAIN ENERGY IMPAIRMENT INDUCED BY METHYLMERCURY POISONING IN ADULT MICE

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Methylmercury (MeHg), an organic form of mercury, has been shown to cause severe and irreversible neurobehavioral and neuropsychological disorders in both humans and animals. The objective of the present work was to investigate the potential protective effect of chronic subcutaneous administration of sodium selenite - Na₂SeO₃ - and diphenyl diselenide - (PhSe)₂ - on the respiratory chain function in adult Swiss albino male mice exposed to MeHg poisoning. Adult male mice were treated with MeHg dose of 40 mg.L⁻¹ diluted in drinking water during 21 days and the seleno compounds were daily subcutaneously injected (5 µmol.kg⁻¹). Control animals received drinking water *ad libitum*. The activities of complexes I, II, II-III and IV were assessed in mitochondrial preparations from mice cerebral cortex 24h after the last subcutaneous administration. Enzyme activities were measured spectrophotometrically. It was initially demonstrated that MeHg administration provoked a significant inhibition of the electron transport chain in mitochondrial preparations by diminishing the activities of complexes I, II, II-III and IV. Next, we observed that (PhSe)₂ chronic co-administration significantly prevented the reducing MeHg effect on the activities of complexes I to IV of the respiratory chain in cortical mitochondrial preparations. On the other hand, the inorganic seleno compound showed a differential effect; while it prevented the inhibition of the activity of complexes II-III and IV, it potentiated or not modified the inhibitory effect of MeHg on complexes I and II, respectively [Complex I: $F_{(5,26)}=40.93$; $P < 0.0001$; Complex II: $F_{(5,25)}=13.16$; $P < 0.0001$; Complex II-III: $F_{(5,21)}=5.55$; $P < 0.01$; Complex IV: $F_{(5,21)}=5.55$; $P < 0.01$]. Altogether, these data provide evidence for the first time, that the inhibitory effect of MeHg poisoning of the electron transfer chain in mice cerebral cortex might be prevented by using seleno compounds. This strongly suggests that impairment of brain energy metabolism might be involved in the MeHg deleterious mechanisms leading to neurotoxicity and that, particularly, (PhSe)₂ could be considered as a neuroprotective agent in MeHg-induced brain poisoning.

Palavras-Chave: methylmercury, respiratory chain complex activities, neurodegeneration

Apoio Financeiro: CNPq, FAPESC and FINEP research grant “Rede Instituto Brasileiro de Neurociência (IBN-Net)” #01.06.0842-00.

Metabolic Biomarkers found in Pediatric Brain Tumors by High Field Magic Angle Spectroscopy

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The study of pediatric brain tumor metabolic profiles may help in the understanding of the changes in brain development pathways. Subtle biochemical changes can be detected in intact tissues by high resolution magic angle spectroscopy (¹H HR-MAS) revealing the status of tumor micro-heterogeneity, observing tumor metabolic alterations before they are morphologically detectable, and correlating them to histopathological features and diagnosis.

The aim of this study was to obtain metabolic profiles of medulloblastomas, ependymomas and pilocytic astrocytomas, which are the most common tumors in pediatric population by using ¹H HR-MAS, to further characterize possible differences among these three types of tumors and to identify additional potential diagnosis biomarkers. Twenty pediatric brain tumor samples underwent ¹H HR-MAS spectroscopy measurements. Principal component analysis (PCA) performed over the ¹H HR-MAS spectra region between 0.5-4.5 ppm allowed to identify differences among those tumors and provided a set of relevant metabolites, which are proposed as potential childhood brain tumor markers. Medulloblastomas were characterized by the highest levels of Choline compounds and Taurine and lowest levels of Creatine. Ependymomas were characterized by the lowest values of Glutamate, GABA and NAA. Pilocytic Astrocytomas had the highest levels of fatty acids despite they are the less aggressive kind of tumor included in the study. Summarizing, our results suggest that the additional information given by HR-MAS spectroscopy can be useful in the identification of potential biomarkers of childhood brain tumors.

Palavras-Chave: pediatric brain tumors, medulloblastoma, ependymoma, pilocytic astrocytoma, biochemical profiling, ¹H HR-MAS, metabolomics.

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Relevancia clínica del índice Haptoglobina / IgG en el diagnóstico diferencial de las meningoencefalitis virales y bacterianas

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Resumen:

Introducción. La haptoglobina es una proteína de transporte que protege al organismo contra la pérdida de hierro y podría estar involucrada en procesos inflamatorios en el sistema nervioso central. **Pacientes y métodos.** Se examinaron el líquido ceforraquídeo y el suero obtenido de 39 pacientes pediátricos, 14 que sufrían meningoencefalitis bacterianas, 25 con meningoencefalitis vírica y 5 casos controles. La haptoglobina, la IgG y la albúmina fueron cuantificadas en ambos fluidos por inmunodifusión radial. Se calculó la razón líquido ceforraquídeo/suero de haptoglobina, el índice haptoglobina y el índice haptoglobina/IgG. La síntesis intratecal de IgG se determinó por reibergrama. Se aplicó la escala de Boyer para conocer su utilidad en el diagnóstico diferencial de las meningoencefalitis. La relevancia del índice haptoglobina/IgG y la escala de Boyer fueron comparada a través de las curvas ROC. **Resultados.** El índice de haptoglobina fue mayor, aunque no de manera estadísticamente significativa, en las meningoencefalitis vírica en comparación con las de origen bacteriano; pero ambos lo fueron con respecto a los controles. El incremento de los niveles del índice haptoglobina/IgG fue estadísticamente significativo en meningoencefalitis bacterianas en relación con las víricas. No se encontró asociación entre el recuento celular de polimorfonucleares y la velocidad de sedimentación globular. La escala de Boyer no presentó asociación estadísticamente significativa con el origen de las meningoencefalitis. **Conclusión.** El índice Haptoglobina/IgG fue útil para establecer el origen diferencial de las meningoencefalitis. El haptoglobina/IgG mostró su superioridad diagnóstica con relación a la escala de Boyer para establecer el origen de las meningoencefalitis.

Cell Therapy with Bone Marrow Mononuclear Cells (BMMCs) Modulates Molecules Involved in Optic Nerve Regeneration in Adult Rats

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Objectives and Methods: The expression of Glial Fibrillary Acidic Protein (GFAP) and the expression of the ganglioside 9-O-acetyl GD3 (9OacGD3) were evaluated in retinas of adult rats submitted to optic nerve crush and injected with saline or with bone marrow mononuclear cells (BMMCs) into the vitreous body. BMMCs were pre-labeled with Cell Trace to analyse their homing into the retina and the animals were sacrificed 1 or 14 days after the lesion. Retinas were fixed with 4% paraformaldehyde, frozen sectioned and immunostained for GFAP and 9OacGD3.

Results: Numerous BMMCs were found in the vitreous body, in the retina 1 day after injection and are still present although in a reduced number in the animals that survive for 14 days. The expression of 9OacGD3 in the retinas of the control group is very low and restricted to the outer plexiform layer (OPL). Optic nerve crush results in a great increase in the expression of 9OacGD3 mainly in the OPL and nuclear layers. The expression of this ganglioside was down-regulated to levels similar to the control group in the animals that received BMMCs transplantation after optic nerve crush. A similar pattern was observed with the expression of GFAP by Müller cells. The expression of GFAP is low in the control group, increases with the optic nerve crush and is down-regulated in the animal that received BMMCs. This changes were quantified by counting the number of GFAP⁺ Muller processes that transverse the inner plexiform layer (IPL). In control animals these processes never cross the IPL (0, n=3). After optic nerve crush there was an increase in the number of GFAP⁺ processes (35.18±1.31, n=3) transversing the IPL and this number is reduced in the treated group (28.86±2.15, n=5; p < 0, 0001).

Conclusion: Our results show an increase in the expression of 9OacGD3 and GFAP in retinas after optic nerve crush and that the expression of these molecules is greatly reduced in animals treated with BMMCs. Since activation of Müller cells is considered deleterious for nerve regeneration our observations suggest that the therapeutical effect of BMMCs in this model might be due to the modulation of the response of Muller cells to injury.

Palavras-Chave: Cell Therapy, Optic Nerve Crush, Müller cells

Apoio Financeiro: CNPq, FAPERJ

C.05.010

Vitamin A supplementation increases α -synuclein and oxidative stress markers levels in rat cerebral cortex and induces behavioral disturbances in adult Wistar rats

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Excessive vitamin A intake is associated with behavioral disturbances in animals and humans. Vitamin A is therapeutically used at high doses to treat several diseases, for instance some types of cancer and dermatological impairments, to cite a few. Furthermore, vitamin A and its derivatives – the retinoids – are redox active molecules that exhibit a prooxidative role depending on its concentration. The aim of this study was to investigate the effects of vitamin A supplementation at 1000, 2500, 4500, and 9000 IU/kg/day (intragastrically - gavage treatment) for 28 days on the redox state, mitochondrial electron transfer chain (METC), superoxide anion ($O_2^{\cdot-}$) production, and α -synuclein levels of the adult (90 days old) rat cerebral cortex. Furthermore, we have investigated depressive and anxiety-like behavior in a tail suspension test, forced swimming test, sucrose intake test and open field apparatus, respectively. We have found increased levels of oxidative stress markers and α -synuclein in cerebral cortex of vitamin A-treated animals. In addition, increased METC activity and $O_2^{\cdot-}$ production were found. Interestingly, we did not observe depressive-like behavior in animals that were treated with vitamin A. However, vitamin A supplementation induced anxiety-like behavior in vitamin A-treated animals, which was represented by decreased activity in the center of an open field apparatus and increased time spent in freezing behavior in the open field. Supported by: CNPq, PROPESQ-UFRGS, FAPERGS. **Palavras-Chave:** vitamin A, oxidative stress, α -synuclein, anxiety-like behavior

Apoio Financeiro: CNPq, PROPESQ-UFRGS, FAPERGS

C.05.011

Influence of dietary omega-3 polyunsaturated fatty acids in glutamatergic system in rat hippocampus: ontogenetic parameters and neuroprotection.

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Introduction and objective: In this study we investigate the effect of dietary essential omega-3 polyunsaturated fatty acids (w3 PUFAs) on parameters of the glutamatergic system related to rat hippocampus ontogeny and neuroprotection.

Methods: Rats were fed, since its conception, with two different diets: omega-3 diet (w3 group) and omega-6 diet (w6 group), modeled after a Japanese and Western diets, respectively. In ontogeny experiments, it was evaluated the hippocampal immunocontent of subunits of the ionotropic glutamatergic receptor NMDA and AMPA (NR2 A\B and GluR1, respectively), the alfa isoform of the calcium-calmodulin protein kinase type II (aCaMKII), as well as, the binding and uptake of [³H] glutamate at 2 (P2), 21 (P21) and 60 (P60) days of life. To assess the influence of w3 PUFAs on brain responses to ischemic insult, hippocampal slices were submitted to 1h of oxygen and glucose deprivation (OGD, a model of *in vitro* ischemia) following 3h of recovery. After the recovery, it was measured cellular viability by lactate dehydrogenase (LDH) and neuron-specific enolase (NSE) liberation in the incubation medium, MTT reduction and Trypan blue incorporation in the hippocampal slices. The immunocontent of p-Akt, p-GKK3b and p-ERK 1/2 and were also assessed after OGD.

Results: The w6 group showed lower immunocontent of all proteins analyzed at P2 than the w3 group, and it was normalized at P21 (except for aCaMKII) and P60. The same pattern was found for [³H] glutamate binding, while [³H]glutamate uptake was not affected. In the OGD studies, the w6 group showed higher cell damage and stronger decrease in the [³H] glutamate uptake. Moreover, w3 group increased anti-apoptotic cell response after OGD, affecting GSK-3beta and ERK1/2, but not Akt phosphorylation.

Conclusion: Taken together, these results suggest that w3 PUFAs are important for the glutamatergic system development and cell protection after ischemia. In addition, w3 PUFAs seem to play an important role on activation of anti-apoptotic signaling pathways and consequent cell survival.

Palavras-Chave: Omega-3, glutamatergic system, ischemia, neuroprotection, development

Apoio Financeiro: CNPq/Brazil, CAPES, IBNnet, FAPERGS

C.05.012

Protective effect of tumor necrosis factor in an animal model of Parkinson's disease

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Parkinson's disease (PD) is a slowly progressive disorder, mainly characterized by the selective loss of dopaminergic neurons of the substantia nigra (SN). Overexpression of tumor necrosis factor alpha (TNF) has been associated with neurotoxicity in brain parenchyma and it is upregulated in neurodegenerative disorders. On the other hand, TNF can have also beneficial effects in models of stroke, Multiple Sclerosis or retinal ischemia. Because of its diverse bioactivities, the functional role of TNF on neuronal tissues remains unclear.

Objectives: To study the functional role of the chronic TNF expression on dopaminergic neurons and understand the mechanism mediating TNF protection.

Methods: We used knock-in mice to express constitutive levels of TNF in the SN of adult mice. Animals were treated with 4ug 6-hydroxydopamine in the striatum. We performed tyrosin-hydroxylase-positive cell and area counting, Nissl staining, GFAP and Tomato lectin immunohistochemistry in wt and k-in animals either naïve or after 6-OHDA treatment. Besides, we analyzed genes related to TNF mechanism of action such as the Manganese Superoxide Dismutase (MnSOD), the neuronal nitric oxide synthase (nNOS) and insulin-like growth factor 1 (IGF-1) by Western blot or real-time PCR.

Results: The k-in mice increased the TNF mRNA levels in the SN TNF reduced the nigral and striatal neurodegeneration after 20 days of 6-hydroxydopamine (6-OHDA) in k-in mice vs wt animals. There was no evidence for inflammatory response in naïve animals; k-in animals had astrogliosis in the SN *pars reticulata*.

In the SN of naïve animals, the IGF-1 and MnSOD expression and nNOS activity were increased in k-in vs wt animals. After 20 days of 6-OHDA, both SOD and nNOS activities were increased in k-in treated animals vs. wt, with similar MnSOD protein levels.

Conclusions: These results suggest that TNF might produce a protective effect by upregulation of the survival pathway such as IGF-1 or astrocyte activation and activation of the antioxidant capacity by the modulation of the MnSOD and nNOS activities, supporting the concept of the beneficial role of cytokines on the central nervous system under certain conditions. **Palavras-Chave:** Parkinson, TNF, neuroprotection, 6-OHDA

Apoio Financeiro: CONICET, ANCYT, Fundación Barón

REGRESSÃO DE LINFOMA CEREBRAL NÃO-HODGKIN PELO USO DE SULFASSALAZINA

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Objetivos: Abordar o recém descoberto mecanismo de ação da sulfassalazina (SLZ) na terapia de inibição de desenvolvimento tumoral.

Métodos: Acompanhamento da terapêutica com SLZ na evolução de linfoma cerebral não-Hodgkin (confirmado por biópsia estereotáxica e histoquímica) por ressonância magnética nuclear de crânio (rmn-c) e as técnicas SE T1, antes e após a administração de Gadolínio, TSE T2, GRE T2* e FLAIR em paciente feminino.

Resultaldo: Conhecidamente salicilatos, como a SLZ, bloqueiam a ativação do NF-kB. Este bloqueio promove inibição do ciclo celular, redução da expressão de citocinas e indução de apoptose em glioblastomas. A SLZ tem sido ampla e seguramente utilizada no tratamento de doenças inflamatórias como a doença de Crohn. Um novo mecanismo associado à SLZ é a inibição do sistema antiporte Xc⁻, um conhecido trocador de glutamato/cist(e)ina. O crescimento e viabilidade das células linfóides é dependente da captação de cist(e)ina extracelular e sua inibição ocasiona morte celular em linhagens de linfoma em cultura ou em modelos de camundongos implantados com gliomas humanos. Nosso trabalho investiga o potencial da SLZ na terapia antineoplásica.

Em 08/11/2006, ICS 77 anos realizou rmn-c apresentando formações expansivas nodulares com sinal isointenso e realce importante pelo Gadolínio. Localização subependimária junto aos ventrículos laterais, bem como no tálamo e corpo caloso, com extensa área com hipersinal em T2 e FLAIR e hipossinal em T1 circunjacente, correspondendo a infiltração neoplásica. Em 18/12/2006 o resultado histopatológico da biópsia estereotáxica e da imunohistoquímica revelou um linfoma não-Hodgkin difuso de grandes células. Após um mês de tratamento com 3g de sulfassalazina/dia, a rmn-c (antes e após a injeção de contraste) apresentou importante redução das lesões, e ausência de efeito compressivo sobre o ventrículo lateral. Um mês após, em outra rmn-c, não foram registrados qualquer tipo de nódulos. Após 18 meses e administração de 1g diário de SLZ, o exame continua sem alterações e a paciente encontra-se levando vida normal.

Conclusão: A sulfassalazina aparece como um novo agente potencial na terapia antineoplásica.

Palavras-Chave: Linfoma, glioma, glutatona, cisteína

BIOENERGETICS IS DISTURBED BY HOMOCITRULLINE AND ORNITHINE IN CEREBRAL CORTEX OF YOUNG RATS

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Objective: Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome is biochemically characterized by tissue accumulation of ornithine (Orn), ammonia and homocitrulline (HCit). Affected patients present lethargy, vomiting, ataxia, choreoathetosis, delayed development and severe cerebral retardation. Considering that the pathophysiology of the brain alterations in this disorder is poorly known, the aim of the present work was to investigate the *in vitro* effects of HCit and Orn (0.1 - 5mM) on various parameters of energy metabolism in cerebral cortex of young rats.

Methods: The parameters investigated were: ¹⁴CO₂ production from glucose or acetate, as well as the activities of the respiratory chain (RC) complexes II, II-III, IV, succinate dehydrogenase and creatine kinase (CK).

Results: Orn significantly reduced aerobic glycolysis (15%) and citric acid cycle activity (31%) [Glucose (control: 54,911±1327 nmol ¹⁴CO₂. h⁻¹. g tissue⁻¹) and acetate (control: 5776±197 nmol ¹⁴CO₂. h⁻¹. g tissue⁻¹)]. HCit similarly reduced aerobic glycolysis (29%) and citric acid cycle activity (28%) [Glucose (control: 68,431±3185 nmol ¹⁴CO₂. h⁻¹. g tissue⁻¹) and acetate (control: 6376±574 nmol ¹⁴CO₂. h⁻¹. g tissue⁻¹)]. In contrast, Orn and HCit did not alter the activities of the RC complexes. Moreover, HCit, but not Orn, markedly reduced (30%) CK activity [HCit (control: 1.13±0.09 μmol creatine. min⁻¹.mg protein⁻¹)], indicating a disturbance on intracellular ATP transfer.

Conclusions: It is presumed that energy metabolism impairment may be involved in the pathophysiology of HHH syndrome.

Palavras-Chave: HHH syndrome, ornithine, homocitrulline, bioenergetics

Apoio Financeiro: Research grants from CNPq, PROPESq/UFRGS, FAPERGS, PRONEX, FINEP Rede Instituto Brasileiro de Neurociência (IBN-Net) # 01.06.0842-00.

Oligômeros solúveis da proteína prion: Caracterização e investigação da ligação a culturas primárias de neurônios hipocâmpais

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Introdução: As encefalopatias espongiformes transmissíveis (EETs) são doenças neurodegenerativas fatais. Nas EETs, o evento chave corresponde a uma mudança conformacional da proteína prion celular. Até recentemente, se acreditava que as fibras amilóides fossem as principais espécies envolvidas na patogênese das doenças conformacionais. No entanto, um conjunto crescente de evidências sugere que as principais espécies tóxicas nas diversas doenças conformacionais podem ser, na realidade, oligômeros solúveis formados em estágios iniciais de agregação dos peptídeos ou proteínas amiloidogênicas.

Objetivo: Caracterizar a formação *in vitro* de oligômeros solúveis da proteína prion e investigar a interação destes oligômeros com neurônios hipocâmpais. **Metodologia:** Foram utilizados métodos bioquímicos e biofísicos para caracterizar a formação dos oligômeros de PrP-(23-230) recombinante *in vitro*. Culturas primárias de neurônios hipocâmpais foram empregadas como modelo experimental para avaliar, através de imunocitoquímica, a interação destes oligômeros com neurônios hipocâmpais.

Resultados e conclusões: A incubação da mPrP-(23-230) murina recombinante, sob condições parcialmente desnaturantes, promove a formação de oligômeros solúveis da proteína. Os oligômeros são resistentes a SDS, possuem um peso molecular aparente em torno de 250 kDa (correspondendo a ~ 12 monômeros de PrP-(23-230)), apresentam uma estrutura secundária rica em folhas-beta e resistência parcial à digestão por proteinase K. A incubação de culturas primárias de neurônios hipocâmpais com oligômeros solúveis de PrP-(23-230), seguida de imunocitoquímica com anticorpo anti-PrP, revela ligação predominantemente intensa nos processos celulares. Este estudo pode contribuir para uma melhor compreensão das bases moleculares e celulares da neurodegeneração nas doenças do prion.

Palavras-Chave: Encefalopatias espongiformes transmissíveis; proteína prion; mudança conformacional; oligômero solúvel de prion; neurônios hipocâmpais

Apoio Financeiro: CAPES, CNPq e FAPERJ

Brazilian marine sponge *Polymastia janeirensis* induces apoptotic cell death in human U138MG glioma cell line, but not in a normal cell culture.

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Marine sponges have been prominently featured in the area of cancer research. Here, we examined the anti-proliferative effects of crude extracts (aqueous and organic) of the Brazilian marine sponge *Polymastia janeirensis* in the U138MG human glioma cell line. Moreover, we examined the effects of extracts on selective cytotoxicity in the glioma cells in comparison with a normal cell culture. Exposure of glioma cells to treatments (24 h) resulted in cell number decrease at all doses tested, with both aqueous and organic extracts (IC₅₀ <20 and <30 µg/ml, respectively). Parallel to this result, sponge extracts reduced glioma cell viability (IC₅₀ <15 µg/ml for both extracts). However, higher doses (50 and 100 µg/ml) induced a stronger cytotoxic effect when compared to the lower dose tested (10 µg/ml), inhibiting more than 80% of cellular growth and viability. Propidium iodide uptake and flow cytometry analysis further showed that sponge extracts caused necrosis in the glioma cell line at higher doses, while a high percentage of apoptotic glioma cells were observed at 10 µg/ml. Moreover, apoptosis was prevented by the pan-caspase inhibitor Z-VAD, suggesting that marine sponge extracts, at lower doses, induce caspase-dependent apoptosis in U138MG glioma cells. Surprisingly the extracts herein tested were more effective than temozolomide, a potent inductor of apoptosis used for the treatment of malignant gliomas. Furthermore, our results suggested a selectivity cytotoxic effect on glioma cell line in comparison with a normal cell culture, since the effect on viability found in glioma cells was not observed in astrocyte cultures with the lower dose (10 µg/ml). Thus, this marine sponge may be considered a good candidate for development of new cancer medicines with antitumor activity against gliomas.

Palavras-Chave: marine sponges, apoptosis, glioma, astrocyte

Apoio Financeiro: CNPq, FAPERGS, PROPESQ-UFRGS, FUNDAÇÃO ZOOBOTANICA

Group II metabotropic glutamate receptor activation prevents apoptotic-like death of cultured astrocytes induced by nitric oxide.

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We investigated the effect of (-)-2-oxa-4-aminocyclo-[3.1.0] hexane-4,6-dicarboxylic acid (Ly379268) and (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (L-CCG-I), selective agonists of group II metabotropic glutamate receptors (mGluRs), on nitric oxide (NO)-induced cell death of rat astrocytes in culture.

Astrocytes were incubated for 48 h. with diethylenetriamine nitric oxide adduct (DETA/NO) 1 mM in presence or absence of Ly 379268 100 mM or L-CCG-I 1 mM. Receptor selectivity was verified with a specific group II mGluR antagonist, (2S)- α -ethylglutamic acid (EGLU) at 300 mM.

Astrocytes viability was reduced significantly by DETA/NO by 35% ($p < 0.01$ vs. control), as measured by MTT method, while both Ly 379268 and L-CCG-I prevented this effect ($p < 0.05$ vs. DETA/NO). DETA/NO increased the percentage of TUNEL positive cells by 5 fold ($p < 0.01$) and both Ly 379268 and L-CCG-I decreased this effect by 35% ($p < 0.01$ vs. DETA/NO). Preincubation with EGLU blocked the protective effects of the agonists. However, neither DETA/NO nor Ly 379268 modified Caspase-3 activity (Assay Designs Inc.). Moreover, a broad spectrum caspases inhibitor, Z-VAD-FMK, 50 mM did not revert the decrease in cell viability induced by NO.

Both p53 and phospho-p53 protein levels (detected by Western-blot) were increased in presence of DETA/NO by 3.5 fold and this increase was reduced by Ly 379268 by 12% and 20% ($p < 0.05$), respectively.

DETA/NO increased Bax (10 fold, $p < 0.05$) and decreased Bcl-2 (by 85%, $p < 0.001$) protein levels, while Ly 379268 had no effect on Bax levels but slightly incremented Bcl-2 protein (by 18%, $p < 0.05$ vs. DETA/NO). However, we detected by Western-blot an additional band at 100 kDa, which may correspond to active Bax oligomers as described previously¹. Putative Bax oligomers were induced by DETA/NO by 2.5 fold ($p < 0.05$) and Ly 379268 completely reverted this effect. Moreover, we observed by immunocytochemistry a transition from a punctuate to a diffuse staining pattern of cytochrome c when astrocytes were incubated with DETA/NO. This was partially reverted by Ly 379268, indicating a decrease of the NO-induced-release of cytochrome c from the mitochondria.

In summary, group II mGluR activation prevents astrocytic death triggered by NO, by modulating p53 activation, Bcl-2 protein levels, Bax oligomerization, and cytochrome c release from mitochondria.

¹) The EMBO Journal 17:3878, 1998. **Palavras-Chave:** group II mGluR - NO - apoptosis - astrocytes

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Reacción astrogial temprana como posible mecanismo de neuroprotección del ambiente enriquecido en un modelo de Enfermedad de Parkinson

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En nuestro laboratorio hemos demostrado que el Ambiente Enriquecido (estímulos+ejercicio físico+interacciones sociales, AE) es capaz de proteger el sistema dopaminérgico nigroestriatal funcional y estructuralmente de la lesión con el tóxico 6-Hidroxidopamina (6-OHDA). En animales mantenidos en condiciones estándar la degeneración neuronal por el tóxico es evidente a los 4 días después de su administración donde se observan numerosas neuronas dañadas en la substantia nigra (con el marcador FluoroJadeB). Por el contrario en animales inyectados con el tóxico y mantenidos en AE se observa una disminución significativa en el número de células FluoroJadeB+.

Para comenzar a elucidar el mecanismo de neuroprotección se determinó en la substantia nigra y el estriado (mediante Western blot) si el AE modifica los niveles del Factor Neurotrófico Derivado del Cerebro (BDNF), Factor Neurotrófico Derivado de la Glia (GDNF) y del Factor de Crecimiento de Fibroblastos de tipo 2 (FGF2). También se estudió la reacción astrogial mediante inmunohistoquímica contra proteína fibrilar ácido glial (GFAP) la cual se conoce que está implicada en muchos fenómenos de neuroprotección.

La administración de 6-OHDA y/o el ambiente enriquecido per se no modificaron significativamente los niveles de los 3 factores tróficos estudiados por Western Blot en la substantia nigra y el estriado. Sin embargo en animales lesionados y mantenidos en AE se observó un notable incremento en la reactividad de astrocitos tanto en la substantia nigra (control=100±9,8% ; 6-OHDA+AE=141±13,1 ; p=0,02) como en el estriado (control=112±2,6% ; 6-OHDA+AE=132±3,4 ; p=0,03).

Los resultados sugieren que el AE podría prevenir la muerte neuronal temprana inducida por 6-OHDA por medio de una reacción astrogial a tiempos muy tempranos en las regiones comprometidas en la lesión. **Palabras-Chave:** Astrocitos, 6-OHDA, Neuroprotección, Factores Tróficos

Apoio Financeiro: Conicet, Secyt UNC, Foncyt

Evaluation of Activity and Body Temperature after Seizures Induced by Organophosphorous Compounds in Sprague-Dawley Rats.

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The understanding of the mechanisms of propagation of status epilepticus (SE) induced by organophosphorus (OP) compounds and the search for neuroprotectants are essential for the advance of the knowledge in this field and to avoid casualties in an eventual incident. The exposure to toxic levels of OP nerve agents inhibits cholinesterase and induces SE and death. Control of seizures induced by OP and means to mitigate central nervous system damage continue to be major military and civilian research goals. Exposure to soman causes brain damage as a result of SE. Our laboratory has been assessing the neuroprotective effects of novel therapeutics post soman exposure in rodents by characterizing electrophysiological and behavioral seizures, motor performance, and neuropathology. In the present study, we characterized long-term EEG, activity and temperature changes after SE induced by OP. Male rats were implanted with telemetry transmitters and after 7 days of recovery were pretreated with the oxime HI-6 (125 mg/kg, i.p.) 30 min before the injection of soman (110 µg/kg, s.c.). One min after soman administration, rats were injected with atropine SO₄ (2 mg/kg, i.m.). At 30 min post soman exposure, diazepam (DZP, 10 mg/kg, s.c.) was injected for treatment of SE. Following the injection of DZP, a group of animals received a potential neuroprotectant or its vehicle. EEG, activity and temperature were recorded continuously for at least 2 days of baseline and fifteen days after soman exposure. The average latency for soman-induced seizures was (8.29 ± 1.82 min; STD; n=235 rats). The core body temperature decreased after the exposure, but returned to basal levels 24 hours after exposure. A disruption of the circadian cycle both on temperature and activity was noticed during up to 3 days after SE. Also, the increase on activity observed during this period was usually followed by electrographic seizures. At the same time, a marked increase on the power spectra on the EEG delta band (0.1 – 4 Hz) was also noticed. In a long term window the activity increased both during the day and night time among the rats that developed SE (79.6 %). These persistent changes in the activity pattern are suggestive of long term modifications in the exploratory behavior. Finally, rats that developed SE had marked brain damage. Continuous seizure activity persisted even after the injection of DZP + potential neuroprotectant and appears to be the main cause of brain damage. Rats that did not develop SE (20.4 %) did not show long term alterations in the activity.

Palavras-Chave: seizure, EEG, activity, temperature

Apoio Financeiro: Defense Threat Reduction Agency under Grant # 1.E0042_08_WR_C and National Academies of Sciences

Identification of genes with altered expression after transplant of bone marrow derived cells in rat model of optic nerve crush.

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Objectives: In the adult mammalian central nervous system, regeneration is very limited. When axons from optic nerve are injured, they grow inside the retina until they reach the glial ambient of the nerve and, from then, there is no further regeneration. The section or crush of the optic nerve leads to the death of most ganglion cells, mainly by apoptosis. Bone marrow stem cells have been used with considerable success improving regeneration and/or functional recovery in animal models of several neurological diseases. One hypothesis for its beneficial effect is the release of cytokines and trophic factors modulated by the lesion process. In this work, we intend to identify genes with altered expression in a rat model of optic nerve crush lesion and treatment with bone marrow derived cells.

Methods: Male rats (3 months-old) had the left optic nerve crushed and received an intravitreal injection of 5ul of 0,9% saline solution (untreated group) or syngeneic bone marrow derived cells (5×10^6 cells, treated group). Animals were sacrificed fourteen days after lesion and the retinal RNAs were extracted following a Trizol Reagent protocol. Using the Differential Display method, we obtained a screening of gene expression in the retinas of treated, untreated and control groups. DNA band pattern alterations among them were analyzed by sequencing and bioinformatics.

Results and Conclusions: We identified four genes, and two of them, Tax1-binding protein 1 and Synaptotagmin IV (which are respectively involved in apoptosis and neurotransmission) were considered relevant for posterior studies in order to confirm its differential expression by other techniques and contribute to the comprehension of bone marrow derived cells' therapeutical effect (previously demonstrated by our group) in this model. Preliminary experiments suggest a tendency of these two genes to be less expressed in the retinas of the untreated group as compared with the control and treated animals. Further studies are needed in order to investigate the possible role of these genes in the neuroprotection and regeneration present in the bone marrow treated animals which has already been described by our group.

Palavras-Chave: optic nerve crush, cell therapy, regeneration, differential display

Apoio Financeiro: CNPq, FAPERJ

EVIDENCE THAT β -HYDROXYBUTYRATE AND ACETOACETATE DO NOT PRESENT ANTIOXIDANT OR PRO-OXIDANT PROPERTIES IN RAT BRAIN

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Objective: Ketone bodies (KB) accumulation occurs during fasting, in diabetes and during metabolic decompensation in inherited neurometabolic disorders, especially in acetoacetyl-CoA thiolase and succinyl CoA: 3-oxoacid CoA transferase deficiencies. Pro-oxidant and antioxidant activities have been reported for these compounds in peripheral tissues, but virtually nothing is described in the brain. The present study investigated the effect of acetoacetate (AcAc) and β -hydroxybutyrate (BHB) on several oxidative stress parameters in cerebral cortex of young rats.

Methods: The following parameters were analyzed: thiobarbituric acid-reactive substances (TBA-RS), chemiluminescence, carbonyl formation, sulfhydryl oxidation, total antioxidant reactivity (TAR) and glutathione (GSH).

Results: We observed that lipid peroxidation [chemiluminescence (Control: 3632 ± 706 cpm/mg protein) and TBA-RS levels (Control: 4.2 ± 0.2 nmol/mg protein)], protein oxidative damage [carbonyl formation (Control: 0.7 ± 0.1 nmol/mg protein)], as well as the non enzymatic [TAR (Control: 66.7 ± 6.3 nmol/mg protein) and GSH levels (Control: 8.8 ± 0.9 nmol/mg protein)] antioxidant defenses were not changed by AcAc and BHB. We also verified that lipid peroxidation induced by the Fenton reaction (55%, Control: 3.7 ± 0.4 nmol/mg protein) was not altered by BHB. Finally, these KB were not able to oxidize a commercial GSH solution in a cell free system (Control: 2154 ± 18 fluorescence units).

Conclusion: The data do not support KB-induced pro-oxidant or antioxidant effects in the CNS, as previously reported in the liver, heart, erythrocytes and endothelial cells. **Palavras-Chave:** ketone bodies; oxidative stress; cerebral cortex

Apoio Financeiro: Research grants from CNPq, PROPESQ/UFRGS, FAPERGS, PRONEX, FINEP Rede Instituto Brasileiro de Neurociência (IBN-Net)#01.06.0842-00.

EVIDENCE THAT α -KETOISOCAPROIC ACID BEHAVES AS AN UNCOUPLER OF OXIDATIVE PHOSPHORYLATION IN RAT BRAIN

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Objective: Maple Syrup Urine Disease (MSUD) is caused by deficiency of branched-chain α -keto acid dehydrogenase complex activity. Leucine (LEU), α -ketoisocaproic acid (KIC) and α -hydroxyisovaleric acid (HIV) accumulate at the highest concentrations in MSUD. The objective of the present work was to investigate the in vitro effect of LEU, KIC and HIV on respiratory parameters measured by oxygen consumption in purified mitochondria prepared from brain of young rats.

Methods: Mitochondrial preparations were obtained from brain of 30-day-old rats and the respiratory parameters state III, state IV and the respiratory control ratio (RCR) were measured by using the Clark electrode. Glutamate/Malate, succinate and α -ketoglutarate were used as the respiratory substrates.

Results: KIC increased state IV and decreased the RCR when the oxygen consumption was stimulated by glutamate/malate or succinate [(substrate glutamate/malate: state IV: 56%, Control: 6.0 ± 1.1 nmol O₂ / min / mg protein; RCR: 39%, Control: 13.7 ± 1.7 nmol O₂ / min / mg protein) (succinate: state IV: 20%, Control 16.9 ± 1.4 nmol O₂ / min / mg protein; RCR: 16%, Control: 5.4 ± 0.4 nmol O₂ / min / mg protein)]. State III was also decreased by KIC when α -ketoglutarate was the substrate (state III: 15%, Control: 34.5 ± 1.0 nmol O₂ / min / mg protein; state IV: 36%, Control: 8.8 ± 0.6 nmol O₂ / min / mg protein; RCR: 37%, Control: 3.9 ± 0.4 nmol O₂ / min / mg protein). In addition, LEU and HIV did not change the tested respiratory parameters.

Conclusions: The data indicate that KIC behaves as an uncoupler of oxidative phosphorylation and that cellular respiration is compromised by this metabolite.

Palavras-Chave: Maple Syrup Urine Disease; Leucine; α -ketoisocaproic acid ; α -hydroxyisovaleric acid; oxygen consumption

Apoio Financeiro: Research grants from CNPq, PROPESq/UFRGS, FINEP Rede Instituto Brasileiro de Neurociência (IBN-Net) # 01.06.0842-00.

Profile of glutamate uptake in human glioma slices from patients submitted to surgery.

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Objectives: Experimental studies demonstrate that glioma-associated brain damage is related to disruption of glutamate (Glu) homeostasis, including transporter mislocalization, increased glutamate release and deficient uptake. However, there are no studies evaluating glutamate uptake profile in human gliomas. The aim of this study is to evaluate the glutamate uptake in gliomas resected from human patients. **Methods:** Glioma-like and subjacent grossly normal brain (cortex) tissues were obtained during surgical procedures. Gliomas and normal brain slices were incubated during 7 minutes at 37° C in Hank's balanced salt solution with [3H] glutamate. The radioactivity was quantified by scintillation and protein determination was assessed using method described by Lowry. **Results:** Four histological types of glioma were used in this study (anaplastic astrocytoma, low-grade astrocytoma, oligoastrocytoma and glioblastoma, the most harmful of these gliomas). In glioblastomas slices were observed a significant decrease in glutamate uptake (0.29 ± 0.024 nmol Glu/mg protein/min; n=6; $p=0.018$, T-test) when compared to cortical slices (0.50 ± 0.19 nmol Glu/mg protein/min; n=6). The glutamate uptake levels found in anaplastic astrocytoma, low-grade astrocytoma and oligoastrocytoma were 0.72, 0.90 and 0.58 nmol Glu/mg protein/min, respectively. These results are previous and more data are needed to analyze them. **Conclusions:** Decrease in glutamate uptake was statistically significant in glioblastomas slices when compared to cortical slices, an expected result according to literature. This effect could explain, at least in part, the mechanism involved in the seriousness and capability of invasion of glioblastomas. The less aggressive gliomas tend to increase the glutamate uptake or maintain cortex levels when compared to glioblastomas. However, further studies need to be performed in order to elucidate this parameter. **Palavras-Chave:** Glutamate uptake, human gliomas

Apoio Financeiro: FAPERGS, Propesq/UFRGS, CNPq, IBNNet

EFFECTS OF Neuropeptide S and D-Amphetamine in the activity of FREE RADICAL-SCAVENGING enzymes in the mouse brain

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Objetives: Neuropeptide S (NPS) is a 20-amino acid peptide, and it is the endogenous ligand of a G protein-coupled receptor. In vivo studies revealed that NPS increases locomotion similar to amphetamine (AMPH). Studies have shown that AMPH administration causes oxidative stress in the rat brain. The present study aimed to investigate the effects of NPS (0.01, 0.1 and 1 nmol, ICV, 5 min) and AMPH (2 mg/kg, IP, 30 min) on locomotion and antioxidant enzymes activity (catalase, CAT, and superoxide dismutase, SOD) in distinct mouse brain structures (i.e. cortex, cerebellum and striatum).

Methods: Male CF-1 mice (3 months) were used in this study. Infrared beam array cages connected to a PC were used for assessing locomotor activity in mice. This assay was done for each mouse individually, during a 30-min period. After behavioral tests mice were sacrificed by decapitation and brain was dissected (striatum, cerebellum and cortex). CAT and SOD activity were measured in brain structures homogenates using a spectrophotometric assay.

Results: The administration of 0.1 nmol NPS and AMPH increased distance traveled compared to control group assessed for 30 min (Sal ICV: 5191±707, NPS 0.1: 8105±702*; Sal IP: 3927±481; AMPH IP: 7977±1898*, *P<0.05 vs. respective control). Biochemical analyzes revealed that AMPH significantly increased SOD activity in the striatum (Sal: 0.00029±0.00073; AMPH: 0.052±0.010*; *P<0.05), while no alterations were observed in CAT activity. The treatment with NPS at low doses reduced SOD activity in the cortex (Sal: 0.016±0.0051; NPS 0.01: 0.0047±0.0042*; NPS 0.1: 0.0029±0.0038*; NPS 1: 0.007±0.004*, *P<0.05) and cerebellum (Sal: 0.0387±0.0189; NPS 0.1: 0.0025±0.0028*; NPS 1: 0.0049±0.0012; *P<0.05) compared to control group. By contrast, an increase in CAT activity in the cortex (Sal: 0.0003±0.0002; NPS 0.01: 0.0034±0.0026*, *P<0.05) was observed compared to control.

Conclusions: In conclusion, these data suggest that despite behavioral similarities between NPS and AMPH, NPS significantly reduces SOD activity, while AMPH induced an opposite effect. These actions of NPS on SOD activity can cause oxidative stress if any other repair mechanisms were activated.

Palavras-Chave: Neuropeptides; D-Amphetamine; Diazepam; Superoxide dismutase; Catalase; oxidative stress

Apoio Financeiro: CNPq (Brazil) and IBRO (Return Home Fellowship).

D-SERINE PROVOKES LIPID AND PROTEIN OXIDATIVE DAMAGE AND REDUCES THE ANTIOXIDANT DEFENSES IN BRAIN OF YOUNG RATS

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Objectives: D-serine (D-Ser), an endogenous co-agonist of the N-methyl-D-aspartate glutamate receptor, is found at high concentrations in the mammalian central nervous system. More recently, D-Ser has been proposed to play a prominent role in the neurotoxicity of some common neurodegenerative diseases.

Methods: We investigated the *in vitro* effects of D-Ser on various parameters of oxidative stress in cerebral cortex from thirty-day-old rats.

Results: D-Ser significantly increased chemiluminescence (29%, Control: 2553 ± 453 cpm/mg protein) and thiobarbituric acid-reactive substances (TBA-RS) levels (45%, Control: 3.4 ± 0.4 nmol/mg protein), indicators of lipid peroxidation. Moreover, D-Ser decreased GSH (37%, Control: 4.7 ± 0.5 nmol/mg protein), the major non-enzymatic antioxidant defense in the brain. Furthermore, this neuromodulator provoked protein damage, as verified by the induction of carbonyl formation (50%, Control: 0.8 ± 0.2 nmol/mg protein).

Conclusions: It may be therefore presumed that D-Ser induces selective lipid and protein oxidative damage and compromises the antioxidant defenses in the brain *in vitro*, which may be associated with the pathophysiology of the neurodegenerative diseases in which D-Ser accumulates.

Palavras-Chave: D-serine and oxidative stress

Apoio Financeiro: CNPq, PROPESq/UFRGS, FAPERGS, PRONEX, FINEP research grant Rede Instituto Brasileiro de Neurociência (IBN-Net) # 01.06.0842-00.

A prooxidant effect of vitamin A supplementation at therapeutic doses on the adult rat cerebellum

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Vitamin A is a micronutrient that participates in the maintenance of the mammalian cells homeostasis. However, excess of vitamin A, which may be achieved through increased intake of the vitamin either therapeutically or inadvertently, induces several deleterious effects in a wide range of mammalian cells, including neuronal cells. Vitamin A is a redox-active molecule, and it was previously demonstrated that it induces oxidative stress in several cell types. In the present work, we decided to investigate the effects of vitamin A supplementation at therapeutic (1,000 or 2,500 I.U./kg/day) or excessive (4,500 or 9,000 I.U./kg/day) doses on the redox environment of adult rat cerebellum. In addition, we have investigated the effects of vitamin A supplementation on mitochondrial electron transfer chain (METC) activity. The animals were treated for 28 days with vitamin A as retinol palmitate. We found increased levels of molecular markers of oxidative damage in the rat cerebellum. Furthermore, we have found increased cerebellar METC activity. Increased METC activity may lead to increased superoxide anion ($O_2^{\cdot-}$) production, a by-product of METC function. Indeed, we found increased ($O_2^{\cdot-}$) production in cerebellar submitochondrial particles. In conclusion, our results suggest a potential ability of vitamin A also in inducing an imbalance in the redox environment of the adult rat cerebellum. **Palavras-Chave:** Vitamin A ; Cerebellum; oxidative stress

Apoio Financeiro: CNPq, PROPESQ-UFRGS, FAPERGS.

Conditioned medium from cultured rat mesenchymal stem cells induces cellular death in organotypic cultures of rat hippocampus and is not neuroprotective in a model of oxygen and glucose deprivation

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Objective: Stroke is the third cause of mortality and the leading cause of morbidity in the world and despite immense efforts, no efficient approach to the treatment of this disease is available. Cell therapy using bone marrow-derived mesenchymal stem cells (MSC) seems to be a new alternative for the treatment of stroke. The aim of this study was to investigate the response of hippocampal tissue conditioned medium from MSC and if this medium is neuroprotective in a model of oxygen and glucose deprivation (OGD).

Methods: Rat organotypic hippocampal cultures were submitted or not to oxygen and glucose deprivation and exposed to a medium conditioned by bone marrow-derived MSCs. The MSC in these experiments were seeded in a density of 30.000 cells/well and used between the 10th and 25th passages. Organotypic cultures recovered for 24 h after OGD and propidium iodide was used as a marker of cell death. Pictures were quantified and results are shown as % of cell death in hippocampus.

Results: Our results show that the conditioned medium obtained from bone marrow-derived rat and mice MSC is not neuroprotective after OGD (control: 0.2%±0.1; OGD: 18.1%±2.5 and OGD+MSC medium: 52.7%±7.2; n=9, p<0.05). Besides, the presence of conditioned medium was toxic mainly to cells from CA2 and CA3 regions of the hippocampal culture, even in basal conditions (control: 0.2%±0.1 and MSC medium: 17.9±4.5; n=9; p<0.05). Cell death decreased when the conditioned medium was diluted or boiled (control: 0.2%±0.1; MSC medium: 26.6%±5.6; MSC boiled medium: 7.3%±1.9 and MSC diluted medium: 5.3%±2.2, n=9, p<0.05), suggesting that some proteic factors secreted by MSC are being toxic to the hippocampal slices. The toxic effect was specific to MSC conditioned medium, once other cell types were not able to induce the same response.

Conclusion: Conditioned medium from bone marrow-derived rat and mice MSC is not neuroprotective after OGD. In addition, CA2 and CA3 areas of hippocampus die in contact with MSC conditioned medium. To our knowledge, this is the first study showing that factors secreted by MSC in culture could be toxic to the brain tissue.

Palavras-Chave: mesenchymal stem cells; oxygen glucose deprivation; neurotoxicity; organotypic hippocampal culture

Apoio Financeiro: Supported by CNPq and Propesq/UFRGS.

Rat bone marrow mesenchymal stem cells conditioned medium induces cell death in hippocampal cultures: involvement of NMDA and AMPA receptors and oxidative stress

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Objective: Cell therapy using bone marrow-derived mesenchymal stem cells (MSC) seems to be a new alternative for the treatment of neurodegenerative diseases. In spite of several good and promising results about the use of these cells, their side effects are still unknown. Previous results from our group have already shown that MSC conditioned medium is toxic to hippocampal slice cultures and that this toxicity seems to be selective to CA2 and CA3 areas. In an attempt to understand why hippocampus is dying in response to MSC, we decided to investigate whether classic receptors such as AMPA and NMDA and oxidative stress were involved in this toxic effect.

Methods: Rat organotypic hippocampal cultures from 6-8 old male Wistar rats were exposed to rat bone marrow isolated MSC conditioned medium for 24h. Cultures were treated with the conditioned medium from MSC and/or the channel antagonists (MK801, CNQX) or antioxidants (ascorbic acid, trolox and L-NAME). Propidium iodide was used as a marker of cell death and results are shown in % of cell death in hippocampus. DCF oxidation was measured to estimate free radical production.

Results: MK801 (30μM), a NMDA antagonist, and CNQX (100μM), an AMPA antagonist, were able to protect from cell death the hippocampus that was exposed to MSC conditioned medium (control: 1.3%±1.6; MSC medium: 34.1%±6.0; MSC medium+MK801: 4.5%±3.7; MSC medium+CNQX: 16.1%±8.3; n=6; p<0.05). In the same way ascorbic acid trolox and L-NAME (control: 1.3%±1.6; MSC medium: 34.1%±6.0; MSC medium+ascorbic acid: 10.2%±5.6; MSC medium+trolox: 12.7%±5.6; MSC medium+L-NAME: 6.1%±3.5; n=5, p<0,05) decreased cell death in hippocampus after exposure to conditioned medium. DCF oxidation also was higher in slices exposed to conditioned medium (control: 8.2±6.6; MSC medium: 46.7±27.8; n=5; p<0.05).

Conclusion: The results obtained in this work suggest that classic mechanisms such as calcium toxicity and oxidative stress induction are involved in cell death induced by MSC conditioned medium.

Palavras-Chave: mesenchymal stem cells; neurotoxicity; oxidative stress; AMPA; NMDA

Apoio Financeiro: CNPq and Propesq/UFRGS

Chronic and acute Hyperhomocysteinemia reduces glutamate uptake in slices FROM parietal cortex OF RATS

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Objectives: In the present study we investigated the effect of chronic and acute hyperhomocysteinemia on glutamate uptake, as well as on the activities of aspartate amino transferase and alanine amino transferase in parietal cortex of rats.

Methods: For chronic hyperhomocysteinemia, Wistar rats were treated with homocysteine solution (0.3-0.6 µmol/g) subcutaneously twice a day at 8 h intervals from 6th to 28th day of age. Control animals received saline solution (Neurochem Res. 27:1593, 2002). Twelve hours after the last injection, animals were sacrificed by decapitation, and the parietal cortex was isolated. For acute hyperhomocysteinemia, rats received a single homocysteine administration (0.6 µmol/g) at 29th day-old (Neurochem Res. 27:1593, 2002). One hour after that, animals were sacrificed and the parietal cortex was isolated. Glutamate uptake was realized in slices of parietal cortex, and glutamate radioactive was used to do the measure (Cell Mol Neurobiol. 25:913, 2005). Transaminases activities determination was accomplished in cytosolic and mitochondrial fractions with the utilization of a commercial kit (Labtest).

Results: Chronic and acute hyperhomocysteinemia significantly inhibited glutamate uptake ($p < 0.05$), but did not change the activities of alanine aminotransferase ($p > 0.05$) and aspartate aminotransferase ($p > 0.05$) in parietal cortex of rats.

Conclusions: The reduction of glutamate uptake caused by homocysteine observed in our study can be associated with the excitotoxicity. In this line, report from literature show that homocysteine provokes glutamatergic receptors activation (Neurochem Int. 43:481, 2003). We suggest that the reduction in the glutamate uptake could, at least in part, be related to neurological dysfunction characteristic of homocystinuria.

Palavras-Chave: homocysteine, glutamate, transaminases, parietal cortex

Apoio Financeiro: CNPq, PROPESQ/UFRGS, FAERGS, FINEP (IBN-Net 01.06.0842-00)

C.05.030

Cultured rat cortical neurons are protected by BDNF following NMDA-induced excitotoxicity.

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Particular brain structures display differential vulnerability to brain insults such as ischemia and status epilepticus, however, the underlying mechanisms are unknown. In the hippocampus, status epilepticus induces a synaptic and morphological reorganization accompanied by apoptosis that frequently leads to temporal lobe epilepsy (TLE). These changes are largely mediated by the neurotrophin BDNF. The objective of the present work was to compare the sensitivity of cultured neurons from cortical and hippocampal origin following continuous neuronal stimulation. Methods: Hippocampal and cortical neurons were cultured for 10 days *in vitro* and stimulated for 48 h with 10 μ M bicuculline as an *in vitro* model for status epilepticus. Neuronal viability following an excitotoxic stimulus (30 μ M NMDA for 1 h) was tested by the Trypan Blue exclusion test and the MTT reduction colorimetric assay. The subcellular localization of TrkB was determined by immunocytochemistry. Following bicuculline, BDNF reduced the viability in hippocampal cultures but increased it in cortical cultures, indicating that BDNF potentiates the excitotoxic damage induced by NMDA in hippocampal neurons but is protective in cortical cultures. The application of an inhibitor of nitric oxide synthase together with BDNF reversed cell death in hippocampal neurons but did not affect cortical neurons. Simultaneously, the localization of TrkB in dendrites from hippocampal neurons increased from $23,7 \pm 8,7$ % to $85,5 \pm 2,0$ %, whereas in cortical neurons a decrease from $94,1 \pm 3,2$ % to $24,4 \pm 2,6$ % was observed. Our results show that the same molecular players (i.e. BDNF, TrkB, NMDA and nitric oxide) have opposite actions on hippocampal and cortical neurons. The understanding of the mechanisms involved in BDNF-dependent neuroadaptive responses in the brain cortex would give us a clue for novel strategies to protect vulnerable populations of neurons against brain insults relevant to a variety of neurodegenerative disorders.

Palavras-Chave: Excitotoxicity; NMDAR; BDNF; TrkB.

Apoio Financeiro: Supported by BMBF (UW and KHS), and Programa Bicentenario para Ciencia y Tecnologia (project Anillo 09-2006 (UW)).

Inhibition of brain creatine kinase activity caused by lysine is prevented by reduced glutathione

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OBJECTIVES: Familiar hyperlysinemia is an inherited metabolic disease characterized by tissue accumulation and high urinary excretion of lysine (Lys). Although some affected patients are asymptomatic, a considerable number of them present neurological dysfunction, whose pathomechanisms are yet poorly known. Therefore, the present work investigated the *in vitro* effects of Lys on parameters of energy metabolism in cerebral cortex of young rats.

METHODS: Cerebral cortex homogenates from young rats were centrifuged and the supernatants were incubated in the presence of Lys (5mM) and used to measure ¹⁴CO₂ production from glucose, as well as the activities of creatine kinase and of the complexes I-V of respiratory chain.

RESULTS: Our results show that Lys inhibited total (20%, Control 9,69±0,68 μM creatine.m⁻¹.mg of protein⁻¹) and cytosolic creatine kinase activity (15%, Control 99,99%). On the other hand, this amino acid did not alter aerobic glycolysis (¹⁴CO₂ production from glucose), neither changed the activities of complexes I-V of the respiratory chain. Furthermore, the addition of glutathione (GSH) fully prevented Lys-induced creatine kinase inhibition (Control 10,4± 1,2; Lys 9,09±1,2; Lys+GSH 10,59±0,63), suggesting that free radical attack on critical cysteine groups at the active catalytic site of the enzyme was involved in these effects.

CONCLUSIONS: The present data indicate that Lys moderately compromises cellular high energy phosphate buffer and transfer in cerebral cortex. It is presumed that these deleterious effects may induce higher vulnerability of the central nervous system to insults such as systemic infections in symptomatic patients affected by familiar hyperlysinemia.

Palavras-Chave: Hyperlysinemia and energy metabolism

Apoio Financeiro: Research grants from CNPq, PROPESq/UFRGS, FAPERGS, PRONEX, FINEP Rede Instituto Brasileiro de Neurociência (IBN-Net) # 01.06.0842-00.

AÇÃO *IN VITRO* DO ZINCO EM FATIAS DE HIPOCAMPO SUBMETIDAS À PRIVAÇÃO DE GLICOSE E OXIGÊNIO

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Objetivos: O zinco é um dos micronutrientes mais abundantes em nosso organismo e um elemento essencial na estrutura de diversas proteínas. O presente trabalho visa verificar o efeito do tratamento com zinco em fatias de hipocampo submetidas a um modelo de isquemia *in vitro*, através da privação de glicose e oxigênio (PGO).

Métodos e resultados: Ratos Wistar de 90 dias foram utilizados para obtenção de fatias hipocâmpais (0,4mm), as quais foram submetidas à PGO por 15 ou 60 minutos. A viabilidade celular reduziu 28,2±6,5% ou 30,2±16,9% em 15 ou 60 min de PGO. A permeabilidade celular aumentou proporcionalmente ao período de PGO (+125±36; +230±34%). O tratamento com ZnCl₂ (1uM, 10uM e 100uM) foi capaz de proteger as fatias de hipocampo nas concentrações de 10uM e 100uM nos 15 min de PGO com 2h de reperusão, porém ZnCl₂ 100 µM demonstrou neurotoxicidade quando estas fatias foram expostas a 60 minutos de PGO seguido ou não por 2 horas de reperusão. Na avaliação da permeabilidade celular, o ZnCl₂ 100 µM demonstrou tendência à neuroproteção no período de reperusão, após 15 e 60 minutos de PGO. Ainda neste tratamento houve diminuição do conteúdo de PSH e GSH-t no período de 15 e 60 minutos de PGO, onde 100 mM de ZnCl₂ teve uma tendência a neurotoxicidade, especialmente na concentração mais elevada (100 mM). O ZnCl₂ também inibiu a atividade da enzima glutatona redutase.

Conclusões: O tratamento *in vitro* mostrou um efeito dual do zinco, ora demonstrando um efeito protetor contra a isquemia, ora promovendo neurotoxicidade. Os efeitos *in vitro* do ZnCl₂ contrastam com a neuroproteção observada *in vivo*. **Palavras-Chave:** zinco, isquemia *in vitro*, glutatona, estresse oxidativo

Apoio Financeiro: Apoio financeiro: IBN-Net - 01.06.0842-00, FAPESC, CNPq

EVALUATION OF ANTIOXIDANT ACTIVITY OF TARAXACUM OFFICINALE FRUIT IN RAT BRAIN

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Objectives

Some plants of the genus *Taraxacum*, know as dandelion, has been widely used in popular medicine as antioxidant, anti-inflammatory and hepatoprotector. However the antioxidant properties of dandelion in the brain as not well comprehend. Our study was designed to investigate the antioxidant effect of the fruit extract from the *Taraxacum officinale* in brain of the rats in vitro.

Methods

T. officinale fruits were separated from aerial parts and extracted with 70% ethanol. The antioxidant activity of the extract in vitro was evaluated against Sodium Nitroprusside (SNP) 5 μ M according to Ohkawa et al (1979), using 1- 20 μ g/mL of the fruit ethanolic extract. The brain of the rat dissected and the homogenized (1:10) in Tris-HCl 10 mM buffer, pH 7.4. The DPPH assay (Hu and Kitts, 2001) and the nitric oxide (NO) scavenger assay (Green et al,1991) was used. The phenolic content was measured according Shahidi and Naczk (1995). Statistical significance was assessed by one-way ANOVA, followed by SNK test for post hoc comparison. Results were considered statistically significant at $p < 0.05$.

Results

SNP increased TBARS level in cortex and cerebellum in 96% from basal levels ($p < 0.005$). Similarly, SNP induced an increase of 67% ($p < 0.005$) in striatum and hippocampus. The extract in concentrations of 5-20 μ g/mL significantly decreased SNP-induced TBARS formation in all structures homogenates to control level ($p < 0.005$) in a concentration dependent manner. In addition, the extract also exhibit scavenger activity against NO radical. The concentrations of the 1-100 μ g/mL inhibited the NO radical in 16.72-46.39%. Moreover, the extract (10- 75 μ g/mL) inhibited the DPPH radical in 6.66-37.76 %. The total phenolic content of *T. officinale* fruit was calculated as equivalent of the gallic acid $0.18 \pm 0.0157 \mu$ g gallic acid/ μ g *T. officinale* fruit extract.

Conclusions

Taken together, those results demonstrated for the first time that dandelion fruit extract present a potent antioxidant and scavenger activity in brain. Those activities occur because the extract posses significance content of the phenolic compounds response by antioxidant activity and scavenger capacity against free radicals.

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Palavras-Chave: Taraxacum Officinale, antioxidant activity, brain

Apoio Financeiro: FAPERGS, FIPE, CnPQ

MECANISMOS ENVOLVIDOS NO PRÉ-CONDICIONAMENTO COM NMDA E A NEUROTOXICIDADE INDUZIDA POR ÁCIDO QUINOLÍNICO NO HIPOCAMPO DE CAMUNDONGOS

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Objetivo: Este estudo teve como objetivo avaliar os níveis de glutatona total (GSH-t) e a expressão da caspase-3 no córtex e hipocampo de camundongos tratados com uma dose sub-convulsivante de NMDA antes de convulsões induzidas por AQ.

Métodos: Camundongos adultos Swiss machos foram submetidos à cirurgia esterotáxica para a implantação de uma cânula guia no ventrículo lateral, tratados com uma dose sub-convulsivante de NMDA 24h antes da infusão de AQ (4μL, 9.2mM, i.c.v.) e observada a ocorrência de convulsões por 10 minutos. Córtex e hipocampus dos camundongos foram dissecados 24h após a infusão de QA, para a avaliação de GSH-t e para a extração de amostras para separação de proteínas por eletroforese e identificação da caspase-3.

Resultados: O pré-condicionamento com NMDA e a infusão de AQ não alteraram o conteúdo de glutatona total (GSH-t), e a atividade das enzimas glutatona redutase e da glicose 6-fosfato desidrogenase. Contudo, um aumento (37,5±10,2) na atividade da glutatona S-transferase (GST) foi observado no córtex cerebral após o tratamento com NMDA e AQ. Além disso, o pré-condicionamento com NMDA preveniu a diminuição na atividade da glutatona peroxidase (GPx) induzida pelo AQ no hipocampo (27,45±5,2), mas não teve efeito sobre a diminuição da atividade da GPx cortical (31,4±7,6). Os resultados estão expressos em porcentagem do controle. A expressão da caspase-3 não foi alterada pelo pré-condicionamento com NMDA e nem pelo insulto com AQ.

Conclusões: Os resultados indicaram que, apesar do pré-condicionamento com NMDA e a toxicidade do AQ modular algumas enzimas relacionadas a GSH, o metabolismo da GSH não parece estar diretamente associado aos efeitos neuroprotetores do pré-condicionamento com NMDA. A partir dos resultados sugere-se que a via efetora de apoptose não está relacionada ao pré-condicionamento e à morte celular induzida pelo AQ.

Palavras-Chave: NMDA neuroproteção ácido quinolinico

Apoio Financeiro: CNPq

Astroglial S100B secretion is stimulated by IL-1 β : another piece in the “cytokine cycle” puzzle of Alzheimer’s disease.

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Background: S100B is an astrocyte-derived cytokine, which has been implicated in the IL-1 β -triggered cytokine cycle in Alzheimer’s disease. However, the secretion of S100B following stimulation by IL-1 β has not been directly demonstrated. We investigated S100B secretion in glial preparations and hippocampal slices exposed to IL-1 β .

Methods: Cortical primary astrocyte cultures, C6 glioma cells and acute hippocampal slices from the brain of Wistar rats exposed to IL-1 β (from 1 pg to 10 ng/mL) were utilized. Extracellular S100B was measured by ELISA. Nuclear migration of NF-kB was investigated by immunocytochemistry and immunoblotting. S100B secretion was also evaluated in the presence of specific inhibitors for NF-kB and MAPK. Statistical analyses were carried out by Student t test or one-way ANOVA, assuming $p < 0.05$.

Results: A fast response (at 15 min) of S100B secretion was observed in primary astrocyte cultures exposed to IL-1 β 10 pg/mL (178.5 ± 41.1 %) and 100 pg/mL (199.8 ± 38.1 %). On the other hand, in C6 glioma cells and hippocampal slices exhibited an increase in S100B secretion at 1 h after IL-1 β addition at concentrations of 1 ng/mL (147.8 ± 17.3 % and 132.9 ± 10.1 %, respectively) and 10 ng/mL (165.8 ± 12.8 % and 145.1 ± 8.6 %, respectively). Nuclear migration NF-kB was also observed under these experimental conditions. PD98059, inhibitor of MEK/ERK, and SB203580, inhibitor of p38 kinase, decreased IL-1 β -stimulated S100B secretion. The involvement of NF-kB was indicated by the inhibitors PDTC and SN-50.

Conclusions. Our data demonstrate IL-1 β -induced S100B secretion in three different *in vitro* preparations: cortical primary astrocytes, C6 glioma cells and hippocampal slices of rats; this secretion was apparently mediated by MAPK/NF-kB signalling. Primary astrocytes and C6 cells exhibited different sensitivities to IL-1 β concerning S100B expression and secretion. These results contribute to the idea that extracellular S100B could be involved in the inflammatory response in brain injury. The mechanism of IL-1 β -induced S100B secretion supports the hypothesis for a “cytokine cycle”, proposed in the genesis of Alzheimer disease.

Palavras-Chave: S100B; IL-1 β ; Neuroinflammation; Alzheimer’s disease

Apoio Financeiro: CNPq, CAPES, FINEP/ Rede IBN 01.06.0842-00, FAPERGS.

C.05.036

Beta-amyloid peptides A β 1-42 and A β 25-35 induces toxicity in organotypic hippocampal slice cultures in a similar pattern

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Objectives: Accumulation of the neurotoxic amyloid β -peptide (A β) in the brain is a hallmark of Alzheimer's disease (AD). There is a plethora of genetic, physiological and biochemical evidence that supports the idea that A β is at least one of the originating causes of the AD and several synthetic A β peptides have been used to study the mechanisms of toxicity. The aim of this study was to establish the comparison between two commonly used A β peptides A β 1-42 and A β 25-35 in an *in vitro* model of A β toxicity.

Methods: We used organotypic slice cultures of rat hippocampus exposed to 25 mM of both A β peptides for 48h. Cell death was quantified by measuring propidium iodide (PI) uptake. In an attempt to investigate whether the A β peptides modulated the cell signaling pathways in a similar manner we performed Western blot assay with specific antibodies.

Results: Our results show that both A β peptides caused about 39% of cell damage in hippocampus. There were no significant differences on cell death between A β 1-42 and A β 25-35 peptides (Control: 5.45% $\pm$ 2.83; n=15; A β 1-42: 39.58% $\pm$ 6.5; n=7; A β 25-35: 39.8% $\pm$ 8.06; n=15; p<0.001), indicating that both caused the similar toxic effect. Besides, we observed similar levels of the caspase-3 cleavage after 48h of exposure to 25 μ M of A β 1-42 or A β 25-35 peptides (Control: 1.69 $\pm$ 0.48; A β 1-42: 6.05 $\pm$ 0.97; A β 25-35: 5.54 $\pm$ 1.36; n=3; p<0.001). In addition, we did not observe any effect of both peptides on PTEN immunocontent (Control: 1.26 $\pm$ 0.31; A β 1-42: 0.87 $\pm$ 0.14; A β 25-35: 1.06 $\pm$ 0.52; n=3; p>0.05) and Akt immunocontent (Control: 1.31 $\pm$ 0.44; A β 1-42: 1.5 $\pm$ 0.73; A β 25-35: 1.14 $\pm$ 0.14; n=3; p>0.05) phosphorylation; otherwise the phosphorylation of GSK-3 β was increased by both A β peptides (Control: 0.88 $\pm$ 0.22; A β 1-42: 1.53 $\pm$ 0.24; A β 25-35: 1.88 $\pm$ 0.56; n=3; p<0.05).

Conclusions: Although further studies are necessary for understanding mechanisms underlying A β peptide toxicity, our results provide strong evidence that A β 1-42 and the A β 25-35 synthetic peptides induce neuronal injury in a similar pattern and that A β 25-35 is a convenient tool for the investigation of neurotoxic mechanisms involved in AD.

Palavras-Chave: Alzheimer's disease; amyloid β -peptide; organotypic culture; caspase-3

Apoio Financeiro: CNPq, CAPES and Propesq/UFRGS

MODULAÇÃO DA VIA DA Akt NO SISTEMA NERVOSO CENTRAL DE RATOS DIABÉTICOS TRATADOS COM DESIDROEPIANDROSTERONA (DHEA)

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Objetivos: A diabetes está associada com déficits cognitivos e com alterações neuropatológicas. A Desidroepiandrosterona (DHEA) é um esteróide neuroativo envolvido em diversas atividades no encéfalo, exercendo efeitos neuroprotetores em modelos de hiperglicemia. O objetivo deste estudo foi investigar se a DHEA modula a via da Akt no hipotálamo e no hipocampo de ratos diabéticos.

Metodologia: Ratos Wistar (230-250g) foram induzidos à diabetes com injeção intraperitoneal de estreptozotocina (STZ) (70 mg/kg) e foram subdivididos em 4 grupos (n=3-5/grupo). Os grupos tratados receberam uma injeção subcutânea de DHEA (10mg/kg) por semana durante 5 semanas. Os grupos controle receberam óleo. A glicemia dos animais foi avaliada no quarto dia após a indução da diabetes e no final da quinta semana. Os ratos foram decapitados e as estruturas foram dissecadas. As amostras foram preparadas para análise da expressão da proteína Akt total e fosforilada por Western blot. A imunoreatividade foi revelada por quimiluminescência (ECL) e os resultados foram apresentados como média $\pm$ desvio padrão da média em valor percentual referente ao grupo controle não-diabético. Foi realizado teste t de Student e ANOVA de duas vias seguida pelo teste post hoc de Student-Newman-Keuls.

Resultados: Após a primeira coleta, a glicemia foi maior no grupo que recebeu a STZ ($137,386 \pm 22,658$ mg/dL x $603,474 \pm 86,653$ mg/dL, $P < 0,0001$). O tratamento com DHEA não interferiu nos valores da glicemia. No hipocampo e no hipotálamo, a relação Aktfosforilada/Akttotal diminuiu no grupo controle diabético, 33,4% ($100 \pm 11,89\%$ x $66,62 \pm 14,95\%$) e 23,5% ($100 \pm 9,91\%$ x $76,5 \pm 10,02\%$), respectivamente, em relação ao grupo controle não-diabético ($P < 0,05$). O tratamento com DHEA aumentou a relação Aktfosforilada/Akttotal no hipotálamo dos ratos diabéticos ($76,5 \pm 10,02\%$ x $103,32 \pm 10,5\%$; $P < 0,05$).

Conclusão: Nos animais diabéticos, houve uma redução da fosforilação da Akt nas duas estruturas, indicando que a doença é um fator que interfere na ativação da via. Porém, o hipotálamo parece ser mais sensível ao efeito do tratamento com DHEA, pois apenas nesta estrutura a redução da ativação da via foi revertida pelo tratamento.

Palavras-Chave: DHEA, Akt, diabetes, sistema nervoso central

Apoio Financeiro: FAPERGS, CNPq, CAPES

EFFECT OF PROLINE ON GLUTAMATE UPTAKE AND Na⁺,K⁺-ATPase ACTIVITY: PREVENTION BY ANTIOXIDANTS

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OBJECTIVES: Hyperprolinemia type II (HPII) is a metabolic disease characterized by tissue accumulation of proline (Pro) and by severe neurological symptoms. Considering it has been proposed that Pro might potentiates glutamate transmission, the aim of the present study was to investigate the *in vivo* (acute) effect of Pro on glutamate uptake by cerebral cortex and hippocampal slices of rats. We also verified the *in vivo* effect of Pro on Na⁺,K⁺-ATPase activity in cerebral cortex of rats and the influence of α -tocopherol (vit E) and/or ascorbic acid (vit C) on the effects elicited by acute administration of Pro on glutamate uptake and Na⁺,K⁺-ATPase activity.

METHODS: For acute administration, 29-day-old rats received one subcutaneous injection of Pro (18.2 μ mol/g b.w.) or saline (controls) and were killed 1h later. After, 22-day-old rats were pretreated for 1 week with daily intraperitoneal administration of vit E (40 mg/kg) and/or vit C (100 mg/kg) or saline. Twelve hours after the last vitamins injection, rats received a single injection of Pro or saline and were killed 1h later.

RESULTS: Results showed that Pro *in vivo* reduces glutamate uptake in the cerebral cortex (C:0.52 $\pm$ 0.1;Pro:0.38 $\pm$ 0.06,p<0.01), but not in the hippocampal slices of rats (C:0.77 $\pm$ 0.2;Pro:0.69 $\pm$ 0.19,p>0.05). Pretreatment with vit E and/or vit C not prevent the effect of Pro administration on glutamate uptake. Furthermore, acute administration of Pro provoked a reduction on Na⁺,K⁺-ATPase activity (C:1041 $\pm$ 43;Pro:796 $\pm$ 50,p<0.01) and vit E plus vit C prevented this effect (C:1041 $\pm$ 50;Pro:811 $\pm$ 35;Vits:1073 $\pm$ 30;Pro+Vits:1115 $\pm$ 105,p<0.01). Glutamate uptake was expressed as nmol/min/mg protein and Na⁺,K⁺-ATPase activity as nmolPi/min.mg protein.

CONCLUSION: Data suggest that the effect of Pro on reduction of glutamate uptake and Na⁺,K⁺-ATPase activity may be, at least in part, involved in the brain dysfunction observed in HPII.

Palavras-Chave: Proline, hyperprolinemia type II, glutamate uptake, Na⁺,K⁺-ATPase, vitamins

Apoio Financeiro: Supported by CNPq, FAPERGS, PROPESQ/UFRGS

BRAIN OXIDATIVE DAMAGE CAUSED BY INTRACEREBROVENTRICULAR ARGININE ADMINISTRATION

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OBJECTIVES: Hyperargininemia is a metabolic disease characterized by tissue accumulation of arginine (Arg) and by severe neurological symptoms. In this study we evaluated the effect of intracerebroventricular (i.c.v.) infusion of Arg on Na⁺,K⁺-ATPase activity, thiobarbituric acid-reactive substances (TBA-RS) and total radical-trapping antioxidant parameter (TRAP) in hippocampus of rats. We also tested the influence of N^w-nitro-L-arginine methyl ester (L-NAME) on the effects elicited by Arg.

METHODS: First, the rats were divided as follows: Group (G)1 (naive), that did not suffer surgery; G2 (sham), that suffered surgery and received saline; and G3 (Arg-treated), that received 0.1, 0.5 and 1.5mM of Arg solution (5μL). Rats were killed by decapitation at 30min, 1h or 4h after injection. After, the animals were divided into five groups: G1 (naive); G2 (sham); G3 (Arg 1.5mM); G4 (L-NAME 250μg) and G5 (Arg 1.5mM plus L-NAME 250μg) and were killed at 1h after injection.

RESULTS: Results showed that 1.5mM Arg solution increases TBA-RS (N:1.99±0.1;S:1.85±0.3;Arg:2.76±0.2,p<0.01) and reduces Na⁺,K⁺-ATPase activity (N:1029±38;S:996±51;Arg:733±46,p<0.01) and TRAP (N:12.4±0.7;S:12.8±1.3;Arg:8.8±0.6,p<0.01) in the rat hippocampus at 1 h after injection. Furthermore, i.c.v. infusion of L-NAME prevented the inhibition caused by Arg on Na⁺,K⁺-ATPase activity (N:1051±41;S:1077±78;Arg:763±32;L-NAME:1116±97,Arg+L-NAME:1096±35,p<0.01) and the increased of TBA-RS (N:2.18±0.2;S:2.16±0.2;Arg:3.0±0.2;L-NAME:2.20±0.2;Arg+L-NAME:2.30±0.1,p<0.01). Na⁺,K⁺-ATPase activity was expressed as nmolPi/min.mg protein, TBARS as nmol of malonaldehyde per mg protein and TRAP as nmol of trolox per mg protein.

CONCLUSION: Data indicate that i.c.v. infusion of Arg induces oxidative stress in rat hippocampus and that the inhibition of Na⁺,K⁺-ATPase activity caused by Arg was probably mediated by NO and/or its derivatives ONOO⁻.

Palavras-Chave: Arginine, oxidative stress, Na⁺,K⁺-ATPase, intracerebroventricular, L-NAME

Apoio Financeiro: Supported by CNPq, FAPERGS, PROPESQ/UFRGS

C.05.040

Chronic GMP treatment decreases the immunocontent of glutamatergic system related proteins in cortex of mice.

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During the last 10 years, our group has been concerned in investigate the potential neuromodulatory role of the guanine based purines (GBPs) in the CNS. We already demonstrated that GBPs are able to antagonize some brain cellular response to glutamate in vitro (; i.e. GFAP phosphorylation [Neuroreport, 1995, 6:249], ROS [Neurochem Res, 1998, 23: 519], cAMP [Neurochem Res, 2000, 25: 181] and calcium influx [NeuroReport, 2000, 11:2303] evoked by glutamate). In vivo, some studies evidenced that acute and chronic GBPs administration promotes neuroprotective effects in events whose glutamate is involved (i.e. anticonvulsant against i.c.v. QA infusion [Brain Res 2000, 2001, 2003, 2004] and against neonatal hypoxic- ischemic insult [Moretto et al., Exp. Neurol. 2005, 195: 400]) in both mice and rats, however the exactly mechanism of GBPs actions is unknown. In the way to investigate the potential glutamatergic system modulation of GBPs, we administrated GMP (1,5mg/ml) orally for 21 days to mice with the aim to investigate the immunocontent of some glutamatergic receptors (NMDA, AMPA), transporter (EAAC1) and some synaptic proteins (PSD95 and SNAP 25) using Western blotting. Our results demonstrated that the GMP treatment statistically decreases the immunocontent of NMDA, AMPA, EAAC1 and PSD95 (decreases 42, 39, 40 and 25% comparing to control animals, $p < 0,05$) without modulate the SNAP25 in cortex of mice. In conclusion, our results could interesting demonstrated that GBPs has a great modulatory potential upon glutamatergic system and this interaction needs more attention since neuroprotective effects was already observed.

Palavras-Chave: Glutamate, GMP, Neuroprotection, Immunocontent

Apoio Financeiro: CNPq

EFFECT OF 2,4-DINITROPHENOL IN SCIATIC NERVE DEGENERATION

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Peripheral nerve injuries are used as a promising model for the understanding of neuronal regeneration, as well as axon growth, pathfinding and communication. These studies comprise nerve crush and sectioning, which triggers neuron degeneration stages. Although the peripheral nerves have the ability to grow by itself after an injury, it grows 1mm/day in the human body. Combined therapies and many techniques are been developed to decrease nerve degeneration, speed up the regeneration process, avoid misconnection of fibers, reconstruct the neuroarchitecture at the injury site and recover the functional ability of the limb. Previous works demonstrated that 2,4-dinitrophenol (DNP), a well known uncoupler of the cellular oxidative phosphorylation, is capable of increasing the neurite extension and number in neuro 2A cell cultures, besides considerably increase the intracellular cAMP level and protects cortical neurons from the deleterious effects of A β peptide. The aim of our work is to investigate the DNP effects in murine sciatic nerve and its respective dorsal root ganglia neurons after crushing injury. In this work we caused a mild injury in 2 month old swiss mice. Our injury model consisted in a sciatic nerve crushing in the left hindlimb at mid thigh. After injury each animal was injected with 0.5 ml of 20 μ M DNP right after surgery and during the following 48 h. Control animals were injured in the same way but did not receive DNP injections. After this period the animals were fixed by intracardiac perfusion with 4% paraformaldehyde. The ipsilateral and contralateral sciatic nerves and the L4 and L5 level dorsal root ganglia were collected and processed to light and electron microscopy. Our preliminary results demonstrated that DNP did not cause any harmful effect in the animals used in these experiments, although this compound is considered toxic. Ultrastructural and immunofluorescence analysis showed that DNP treated animals presented a reduction in peripheral neurodegeneration and an increase in protein expression related to axon growth and myelination compared to control. In conclusion, DNP can be a promising alternative for neuroregeneration treatment.

Palavras-Chave: neurodegeneration, dinitrophenol, electron microscopy, axon, myelin

Apoio Financeiro: CNPq, HHMI, Faperj, CAPES

**EFEITO ANSIOGÊNICO DA INOCULAÇÃO DO VENENO BRUTO DA SERPENTE
CROTALUS DURISSUS TERRIFICUS EM RATOS WISTAR.**

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OBJETIVOS: Acidentes ofídicos são problemas recorrentes de saúde pública, sobretudo em países tropicais. O acidente crotálico (cascavéis) ocupa o segundo lugar em número de ocorrências envolvendo humanos, no Brasil. A peçonha destas serpentes é composta por enzimas, peptídeos e toxinas. Tal veneno tem efeitos tóxicos aos músculos esqueléticos, rins, fígado, sangue e sistema nervoso. Este trabalho objetivou avaliar os efeitos ansiogênicos tardios da inoculação experimental de veneno bruto de *C.durissus terrificus* em ratos Wistar.

MÉTODOS: Foram testadas duas doses do veneno bruto de *C. durissus terrificus*, 90µg/100g e 120µg/100g, i.p. em ratos Wistar machos (200-250g). Vinte e um dias após a inoculação, os animais foram expostos aos testes, em duas sessões, no labirinto em cruz elevado (LCE); que constitui um paradigma amplamente utilizado para avaliação experimental de ansiedade. Os dados foram analisados por meio da ANOVA e *post hoc* Tukey HSD ($p<0,05$).

RESULTADOS: Os resultados apontaram que a inoculação da maior dose de veneno aumentou os níveis de ansiedade nos animais, demonstrado pela menor porcentagem de tempo despendida nos braços abertos do aparelho, pelo grupo 120µg/100g ($F_{(2,21)}=1,34$; $p<0,0283$), em relação ao grupo 90µg/100g e aos animais controle, bem como a menor frequência de entrada nos braços abertos ($F_{(2,21)}=1,80$; $p<0,0196$). Os animais tratados, nas duas doses, permaneceram maior porcentagem de tempo nos braços fechados do aparato ($F_{(2,21)}= 4,45$; $p<0,0244$), o que reforça tal efeito ansiogênico da peçonha. Não houve diferença na frequência de entrada nos braços fechados do LCE entre os grupos, o que demonstra que os animais não tiveram comprometimentos motores.

CONCLUSÕES: É possível concluir que o veneno bruto de *C. durissus terrificus* teve ação ansiogênica, mesmo em uma avaliação relativamente tardia. Os níveis de ansiedade apresentaram uma correlação com a dose inoculada, ou seja, tais efeitos podem ocorrer de forma dose-dependente, pois, mesmo sendo a dose 120µg/100g promotora do aumento mais evidente do nível de ansiedade, ambas apresentaram o efeito ansiogênico.

Palavras-Chave: Ansiedade, Labirinto em cruz elevado, *C. durissus terrificus*, veneno bruto.

Hyperhomocysteinemia increases damage on brain slices exposed to in vitro model of oxygen and glucose deprivation: prevention by folic acid.

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Objectives: In the present study we evaluated the effects of homocysteine (Hcy) on cellular damage using hippocampal slices from Wistar rats exposed to oxygen and glucose deprivation (OGD, followed by reoxygenation), an in vitro model of hypoxic–ischemic events. **Method and results:** For chronic treatment, we induced elevated levels of homocysteine in blood (500 mM), comparable to those of human homocystinuria, of young rats by subcutaneous injections of homocysteine (0.3–0.6 mmol/g of body weight), twice a day with 8 h intervals, from the 6th to the 28th postpartum day. Controls received saline. Rats were sacrificed 1h after the last injection. For acute treatment, 29-day-old rats received one single injection of homocysteine (0.6 mmol homocysteine/g body weight) or saline and were sacrificed 1 h later. In another set of experiments rats were pretreated with vitamins E (40 mg/kg) and C (100 mg/kg) or folic acid (5 mg/kg) during 1 week; 12 h after the last administration they received a single injection of homocysteine or saline and were sacrificed 1 h later. Results showed that both chronic (Control: 304±46 and Hcy: 432±116; n=4; p<0.001) and acute hyperhomocysteinemia (Control: 226±37 and Hcy: 269±30; n=4; p<0.001) increased the cellular damage measured by LDH released to de incubation medium, suggesting an increase of tissue damage caused by OGD. Pretreatment with folic acid prevented the damage caused by acute hyperhomocysteinemia, whereas vitamin E just partially prevented such effect. **Conclusion:** These findings may be relevant to explain, at least in part, the higher susceptibility of hyperhomocysteinemic patients to be susceptible to ischemic events and point to a possible preventive treatment. **Palavras-Chave:** Homocysteine, ischemia, brain, rat, folic acid, vitamins

Apoio Financeiro: CNPq, and PROPESQ/UFRGS

C.05.044

Depletion of reduced glutathione is involved in 1,2-dihydroxybenzene-induced apoptosis on human glioblastoma cells

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The exposition to benzene is a serious problem, mainly in the modern industrialized world. It is known that metabolites of this agent are responsible for its toxicity, but there are few informations about its activity in the central nervous system (CNS). It has been previously demonstrated that the cytotoxicity of catechol towards human glioblastoma cells occurs via superoxide and reactive quinones generation, but its mechanisms are not completely known. OBJECTIVES: Human glioblastoma GL-15 cells were used as a model in order to study the effects of 1,2-dihydroxybenzene (catechol), a metabolite of benzene, in human cells of glial origin. METHODS: MTT assay was used for cell viability measurement, the type of cell death was identified by flow cytometry and depletion of reduced glutathione (GSH) was analyzed by using monochlorobimane (MCB). RESULTS: Catechol was toxic to GL-15 cells by inducing apoptosis.

The depletion of GSH was noted after 24 h of exposition to 600 μ M catechol. GSH depletion occurred before the decrease in cell viability has become evident. The dose-response after expositions to 24, 48 and 72 h enabled to calculate IC₅₀ values for each time: 2368, 864, and 252 μ M, respectively.

CONCLUSIONS: The toxic effects of catechol in these cells were dose- and time-dependant. Catechol induced apoptosis, which was preceded by GSH depletion.

Palavras-Chave: Apoptosis, Catechol, Glioblastoma

Apoio Financeiro: Supported by FAPESB

EPICATECHIN GALLATE EXERTS GENOPROTECTIVE AND GENOTOXIC EFFECTS *IN VITRO*

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Introduction: Green tea contains polyphenolic compounds that have important biological properties, including strong antioxidant activity. Oxidative stress is recognized as a major contributing factor for the development of brain pathologies.

Objective: The present study was undertaken to evaluate the effect of one of the major green tea polyphenols, epicatechin-gallate (ECG), at 0.1, 1 and 10 μ M (1-24 h) on DNA damage and also on genotoxicity induced by hydrogen peroxide (H_2O_2) in C6 astroglial cells.

Methods: We have employed the comet assay to measure permanent DNA strand breaks in individual cells and the micronucleus assay to investigate DNA damage at the chromosome level in binucleated cells under oxidative stress induced by H_2O_2 . Data were analyzed by ANOVA for repeated measures, followed by a post hoc analysis (Tukey's test). Values of $P < 0.05$ were considered to be significant.

Results: The index of DNA damage under control conditions was 15 ± 1.3 . Higher concentrations of ECG showed genotoxic effects depending on time in both methods, comet ($F_{(3,9)} = 336,148$; $P < 0.001$) and micronucleus ($F_{(3,9)} = 23,228$; $P < 0.001$). Interestingly, smaller doses of ECG were able to partially prevent genotoxicity induced by H_2O_2 from 42.5 ± 3.9 to 27 ± 2.1 (0.1 μ M ECG) and 29 ± 3.1 (1 μ M ECG) ($F = 47,529$; $P < 0.001$).

Conclusions: In summary, this study demonstrates that different concentrations of ECG can exerts genoprotective and genotoxic effects in C6 cells. It indicates that small doses of polyphenols from diet could have beneficial effects on neural cells, contributing to a counteract brain pathologies associated with oxidative stress. Also, the consumption of large quantities of antioxidants in fortified foods or supplements needs caution and should not be encouraged.

Palavras-Chave: ECG, astrocytes, neuroprotection, oxidative stress

Apoio Financeiro: CNPq, PROPESq-UFRGS, FINEP/ Rede IBN 01.06.0842-00, FAPERGS.

CENTRAL EFFECTS OF THE TOXIN 4-V ISOLATED FROM *Tityus bahiensis* VENOM IN RATS

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Introduction: *Tityus bahiensis* is responsible for many scorpion accidents in Brazil. However, few studies on its venom were done, mainly on the central nervous system.

Objective: Evaluation of the behavioral, electrographic and histological effects after the intrahippocampal injection of Tb 4-V toxin as well as changes in the neurotransmitter amino acids levels and in the intracellular calcium concentration.

Method: Groups of Wistar 250g male rats were submitted to stereotactic surgery for cannula guide and/or electrode implantation in the hippocampus region. One group received ringer solution (1µl; n=6) and the other toxin 4-V (1µg/µl; n=6). Both groups were to carry out electrographic and behavioral analysis. Subsequently, the brains were processed for histological analysis. Another group received the toxin (1µg/µl; n=6) and the perfusates were collected by micriodialysis. The amino acids were measured by HPLC. In order to verify the release of intracellular calcium in confocal microscope, slices (200mm) of hippocampus of 8 animals were made in vibratome and stimulated with 10µl of the toxin. Statistical analysis was done by ANOVA test followed by Tukey Kramer, with $p < 0,05$.

Results: The toxin increased significantly the frequency (6.3 ± 2.3) and the time of light discharge (22.5 ± 9.2 min) and frequency of isolated spikes (14.5 ± 5.1). There was a significant decrease of cells number only in the region CA1 contralateral (48 ± 6.5) when compared to the control (63 ± 1.4). No significant difference were found in the release of amino acids. Regarding the calcium's mobilization, there was an increase of 90-110% of fluorescence in 4 slices, for the other ones there was no effect.

Conclusion: The toxin has a neurotoxic effect, but the decrease of cells found in contralateral CA1 region is not due to increased release of glutamate, but most probably by increasing the release of intracellular calcium caused by the toxin.

Palavras-Chave: *Tityus bahiensis*, toxin, hippocampus

Apoio Financeiro: FAPESP processo 03/13502-2, Fundação Butantan e Coordenadoria de Controle de Doenças - SES/SP.

c-FOS, A NOVEL TARGET TO CONTROL TUMOR DEVELOPMENT IN THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM.

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OBJECTIVES. As a member of the AP-1 family of transcription factors, c-Fos regulates the expression of genes involved in mitosis and differentiation. Previously, we found that in addition to its AP-1 activity, c-Fos associates to the endoplasmic reticulum (ER) and activates the synthesis of phospholipids for the genesis of membrane required for cell proliferation (FASEB J. 15:556, 2001) and differentiation (Mol Biol Cell. 15:1881, 2004). Herein it is determined if this novel activity is important for the development of CNS and PNS tumors. For this, tumors and matched benign specimens were examined for:

- Expression and localization of c-Fos
- Importance of c-Fos-activated phospholipid synthesis on tumor progression.
- Effect of the blockage of c-Fos expression on tumor progression

METHODS. The following systems were used:

- Human brain tumor biopsies and normal human brain tissue.
- Intracranial xeno-transplants in nude mice of glioblastoma multiforme-derived cells.
- CNS and PNS tumors from NPcis mice, a mouse model of the human disease Neurofibromatosis Type I that spontaneously develop CNS and PNS tumors.
- Brain samples from NPcis mice bred on a *fos* (-/-) background.
- Cultured cell lines derived from NPcis mice tumors.

RESULTS.

- High levels of c-Fos were observed co-localizing with ER markers in all CNS and PNS tumors examined, correlating with high rates of cell proliferation and activated phospholipid synthesis. No significant expression was found on normal tissue.
- Blocking c-Fos expression either with antisense oligonucleotides or in *fos* (-/-) NPcis mice significantly decreases phospholipid synthesis rates and tumor growth.

CONCLUSIONS. The c-Fos-dependent activation of the *de novo* synthesis of phospholipids required for membrane biogenesis is necessary to sustain the exacerbated growth of nervous system tumors; abrogating this activation by impeding its synthesis, tumor progression is blocked. This phenomenon is not related to its well-known transcriptional function. By contrast, the level of expression of c-Fos in normal CNS is under its limit of detection indicating that this protein should be considered as an important target to control this disease.

Palavras-Chave: c-Fos/Phospholipids/Nervous system tumors/Neurofibromatosis Type I

Apoio Financeiro: CONICET/SECyT-Universidad Nacional de Cordoba and JS McDonnell Foundation

Protective effect of some commonly used medicinal plants from Brazil against oxidative stress in rat brain

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Objectives: Reactive oxygen species have been demonstrated to be associated with a variety of diseases including neurodegenerative disorders. Plants among other functions are considered as free radical scavengers and antioxidants. The present study was aimed to investigate the antioxidant effect of some commonly used plants in Brazil against oxidative stress induced by iron in rat brain.

Methods: The extracts of five plants *Baccharis trimera*, *Calendula officinalis*, *Peumus boldo*, *Phyllanthus niruri*, *Solidago microglossa* and *Solanum fastigiatum* were investigated for their antioxidant activity using a lipid peroxidation assay (Anal Biochem. 95:351, 1979). The data were analyzed statistically by one way analysis of variance and different group means were compared by Duncan^s multiple range test; $P < 0.05$ was considered significant in all cases. The IC_{50} (Extract concentration that will cause 50% inhibition of lipid peroxidation) were determined.

Results: The results revealed that the incubation of brain tissue with 10 μM $FeSO_4$ caused a significant ($P < 0.05$) increase in MDA (malondialdehyde), a marker of lipid peroxidation compared to the basal. However, the treatment with plant extracts caused a significant ($P < 0.05$) decrease in Fe^{2+} -induced lipid peroxidation in brain homogenate. Except *Baccharis trimera* (inhibition less than 50% at 100 $\mu g/ml$) the other plant species showed excellent inhibition against lipid peroxidation with IC_{50} values less than 100 $\mu g/ml$. The most active plants examined were *Solidago microglossa* ($IC_{50} = 7 \pm 0.3 \mu g/ml$), *Phyllanthus niruri* ($IC_{50} = 8.23 \pm 0.7 \mu g/ml$), *Peumus boldo* ($IC_{50} = 15.2 \pm 0.5 \mu g/ml$), *Solanum fastigiatum* ($IC_{50} = 22.47 \pm 3.2 \mu g/ml$) and *Calendula officinalis* ($IC_{50} = 98.2 \pm 5.5 \mu g/ml$) respectively.

Conclusions: The present results suggest that the studied plants may protect the brain against oxidative stress for their ability to prevent lipid peroxidation and may be utilized against the neurotoxicity resulting from the overload of iron. Further studies are required to determine the detailed mechanism and neuroprotective role of these plants.

Palavras-Chave: Oxidative stress, rat brain, medicinal plants, antioxidant, iron

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THE ROLE OF PrP^c AND STI1 IN THE MICROGLIA-GLIOBLASTOMA INTERACTION

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There is evidence that microglial activation is controlled by tumor cells, supporting their progression and infiltration. Our group demonstrated previously that STI1, a PrP^c ligand, is secreted by glial cells and promotes glioblastoma (GBM) proliferation (J Neurochem 103: 2164, 2007; GLIA 55: 1690, 2007). In the context of microglia-GBM interaction, the present study aimed at investigating the influence of PrP^c and STI1 in the tumoral growth regulation.

Highly pure cultures of microglial cells from neonatal mice and tumor cells from GBM95 human cell line were obtained as described previously by us (J Neurosci. 21: 20-28, 2001; GLIA 55: 1690, 2007). We have verified by immunocytochemistry that microglial cells express STI1. In GBM cultures, the [³H]-thymidine incorporation assays showed that proliferation increased 100% when GBM cells were cultivated with microglial conditioned medium (MCM), compared to the control condition (fresh medium). However, this effect was reverted when anti-STI1 antibody was added to the MCM. In the migration assays by scratch, both MCM and MCM added of anti-STI1 antibody promoted cell migration in a similar manner. Besides, when microglial cells were activated by lipopolysaccharide (LPS), recombinant STI1 blocked the secretion of nitric oxide by microglia from wild-type mice, but not from PrP^c-null mice.

Our preliminary results suggest that STI1 is produced by microglia and favors growth but not tumoral invasion. In addition, PrP^c-STI1 interaction can be relevant to regulation of microglial activation. This question deserves further investigation.

Palavras-Chave: glioblastoma; microglia; interaction; STI1; PrP^c

Apoio Financeiro: CNPQ, FAPERJ and CAPES

C.05.050

Effect of the atypical neuroleptic risperidone on morphology and S100B secretion in C6 astroglial lineage cells.

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Objectives: Risperidone (Risperidal[®]), an atypical antipsychotic agent, is thought to act via dopamine D2 and serotonin 2A receptor antagonism. Autism spectrum disorder (ASD) represents a major mental health problem with estimates of prevalence ranging from 1/500 to 1/2000. Astrocytes are one of the most functionally diverse groups of cells in the nervous system and have many functions that could be relevant to abnormalities described in psychiatric disorders such as schizophrenia. The C6 lineage cells are widely used astrocyte-like. The aim of this study was to examine the effect of the atypical neuroleptic risperidone on morphology and S100B secretion in C6 cells, considering the putative involvement of astroglial cells in neuropsychiatric disorders.

Methods: C6 glial cells were seeded (5×10^3 cells/well) and cultured in DMEM (pH 7.4) supplemented with 5% fetal bovine serum (FBS), at 37°C/ 5 % CO₂/air. After 1, 6, 12 and 24 h the experiments were performed in absence or presence of risperidone in a range from 10 to 40 µM. Glial marker S100B was measured by ELISA.

Results: In the presence of high experimental doses of risperidone, C6 cells become stellate, with process bearing cells and partial retraction of the cell body followed by detachment from the adhesion surface with practically no cell death. These results indicate that risperidone is able to interfere with C6 cell adhesion without toxic effects. RhoA activator LPA prevented the effects of risperidone on cell morphology. From 6 h risperidone induced a statistically significant increment of about 80% in S100B secretion (0.157 ± 0.032 to 0.283 ± 0.035 ng/ml).

Conclusions: These data contribute to the proposal that glial cells are targets of risperidone, which could be involved in the therapeutic response of risperidone to improve autism symptoms. **Palavras-**

Chave: Risperidone, Autism, C6 astroglial cells, S100B

Apoio Financeiro: CNPq, PROPESq-UFRGS, FINEP/ Rede IBN 01.06.0842-00, FAPERGS.

C.05.051

Curso temporal de las alteraciones conductuales, histológicas y de marcadores de estrés oxidativo inducidas en el hipocampo de ratas irradiadas al nacimiento.

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Objetivos: El objetivo de este trabajo fue estudiar el efecto de las radiaciones ionizantes sobre el hipocampo (Hip), desde el punto de vista conductual, histológico y bioquímico.

Métodos: Ratas Wistar macho neonatas fueron irradiadas con radiación X en la región cefálica. Luego de 30 y 90 días, se testó la conducta de evitación pasiva (EP) y de reconocimiento de objetos (RO), así como los niveles de especies reactivas de oxígeno (ROS), la actividad de las enzimas antioxidantes catalasa (CAT) y superóxido dismutasa (SOD) y de la proteína kinasa C (PKC). Finalmente, el Hip se evaluó histológicamente.

Resultados: Los resultados mostraron un aumento en las ROS a los 30 días en los Hip de los animales irradiados (Rx) con respecto a los controles (C) (pmol DCF/mg/min, C: 0.21 ± 0.02 , Rx: 0.31 ± 0.01 , $p < 0.05$) y una disminución a los 90 días (pmol DCF/mg/min, C: 0.15 ± 0.02 , Rx: 0.06 ± 0.01 , $p < 0.05$). No se encontraron cambios en CAT, mientras que la SOD se encontró disminuida a los 30 días (U/mg, C: 0.38 ± 0.01 ; Rx: 0.33 ± 0.01 , $p < 0.05$) y sin cambios a los 90. La actividad de PKC se encontró aumentada en los animales irradiados a los 30 días (pmol/mg, C= 39.2 ± 1.7 ; Rx= 96.9 ± 10.9 , $p < 0.05$). La histología del Hip irradiado se encontró afectada, tanto a nivel morfológico como de la citoarquitectura, la cual empeoró a los 90 días. Finalmente, se observó una mejor performance en EP de animales de 30 días (T2/T1, C: 28.9 ± 6.7 ; Rx: 51.6 ± 8.2 $p < 0.05$), sin cambios a los 90. El test de RO mostró en los animales Rx un empeoramiento en la memoria de reconocimiento sólo a los 90 días (N/T, C: 0.57 ± 0.05 ; Rx: 0.42 ± 0.03 , $p < 0.05$).

Conclusiones: Si bien se observó un daño histológico en el Hip, los resultados sugieren que el aumento de ROS y la concomitante caída de SOD a los 30 días podrían estar induciendo el aumento de PKC, favoreciendo una mejor performance en el test de EP. Estos resultados, junto con el hallazgo de un daño histológico en el Hip y un empeoramiento de la memoria de RO, y una caída de las ROS con recuperación de los niveles de SOD observados a los 90 días, sugieren la existencia de un mecanismo plástico compensatorio para contrarrestar el ataque de ROS.

Palabras-Chave: Radiaciones ionizantes - hipocampo - SOD - ROS - evitación pasiva

Apoio Financeiro: UBACYT M083

Heterogeneous effects in the central nervous system of intraperitoneal injection of *Tityus serrulatus* and *Tityus bahiensis* scorpion venoms in rats

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The great number of studies on scorpion venoms associates their effects to the autonomic nervous system and there are few data about their action on the central nervous system (CNS).

The aim of this work was to evaluate some central effect after intraperitoneal injection of *Tityus serrulatus* (0.3 mg/kg) or *T. bahiensis* (0.5 mg/kg) scorpion venom.

After the venoms injection, several neurotransmitters and their metabolites (glutamate, glycine, GABA, dopamine, DOPAC, HVA, 5-HT and 5-HIAA) were collected by microdialysis from hippocampus and dosed by HPLC. In other animals electroencephalographic and behavioral observations were performed, and all the brains were removed for histopathological analysis of hippocampal areas.

Not any neurotransmitter or metabolite level showed alteration unless HVA, which was enhanced until 10 times in approximately 1/3 of animals of both groups (from *T. serrulatus*: basal line 2.85 ± 0.83 microgram/ml, maximal alteration 29.6 ± 7.6 microgram/ml; from *T. bahiensis*: basal line 2.35 ± 0.99 microgram/ml, maximal alteration 27.7 ± 3.9 microgram/ml; data expressed as mean $\pm$ SD). Both venoms produced an increase in salivation, in lachrymal and bronchial secretions and respiratory disorders. Some animals yawned frequently (data not quantified). *T. serrulatus* venom injection modified electrographic recordings displaying epileptic-like discharges. Otherwise, *T. bahiensis* venom didn't induce some electrographic alteration. There was no damage to the hippocampal pyramidal neurons in animals of both groups (control group CA1 61.8 ± 2.8 , CA3 29.6 ± 2.2 , CA4 30.1 ± 1.3 ; *T. serrulatus* group CA1 59.3 ± 1.6 , CA3 30.6 ± 2.9 , CA4 29.9 ± 2.7 ; *T. bahiensis* group CA1 59.4 ± 2.9 , CA3 30.6 ± 1.8 , CA4 29.4 ± 1.9 ; data expressed as mean $\pm$ SD).

Our results suggest that some effects of scorpion venoms may be due, at least in part, to their direct action in the CNS. Otherwise venoms from *T. serrulatus* and *T. bahiensis* seem to act differently in the CNS.

Palavras-Chave: scorpion venom, central nervous system, neurotransmitters, neurotoxicity

Apoio Financeiro: Fundação Butantan

The Janus face of resveratrol in C6 lineage cells.

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Objectives: Resveratrol (3, 5, 4',- trihydroxy stilbene), a redox active compound present in grapes and wine, have been shown to play a wide range of biological effects. The glial cells have emerged as key modulators of neuropathology associated with oxygen-derived free radical species. The aim of the present study was to investigate whether resveratrol is able to prevent hydrogen peroxide (H₂O₂)-induced oxidative damage in C6 astroglial cells.

Methods: C6 glial cells were seeded (5 x 10³ cells/well) and cultured in DMEM 5% fetal bovine serum at 37°C/ 5 % CO₂/95 % air. The cells were treated of resveratrol (100 µM) and two models of oxidative insult were used: model I (1 mM H₂O₂/30 min) and model II (0.1 mM H₂O₂/6 h). Glial marker S100B was measured by ELISA. Glutamate uptake was measured during 10 min by addition of 0.1 mM L-glutamate and 0.33 mCi/mL L-[2,3-³H] glutamate in HBSS. Glutamine synthetase (GS) activity was measured by a colorimetric assay, glutathione (GSH) levels were measured by a fluorimetric assay and NF-kB was determined by Electrophoretic Mobility Shift Assay.

Results: In model I of oxidative insult, resveratrol increased glutamate uptake (0.25±0.02 to 0.34±0.02 nmol/mg protein/min, P<0.001), (GS) (0.38±0.02 to 0.72±0.032 µmol/mg protein/h, P<0.001), (GSH) (5.7±0.18 to 9.1±0.18 nmol/mg protein, P<0.001) and S100B (0.100±0.0016 to 0.120±0.0023 ng/ml, P<0.001) compared of control. But in model II, resveratrol had an opposite effect, potentiating the H₂O₂-induced decrease in glutamate uptake (0.38±0.02 to 0.14±0.03 nmol/mg protein/min, P<0.001), in GS (0.08±0.004 to 0.40±0.026 µmol/mg protein/h, P<0.001) and in GSH (20.3±0.29 to 9.3±0.14 nmol/mg protein, P<0.001) compared of control. The transcription factor NF-kB was activated in both models.

Conclusions: In summary, these findings showed that resveratrol, a redox active compound, was able to modulate important functions of astrocytes, under different oxidant conditions.

Palavras-Chave: Resveratrol, hydrogen peroxide, C6 astroglial cells, glutamate

Apoio Financeiro: CNPq, PROPESq-UFRGS, FINEP/ Rede IBN 01.06.0842-00, FAPERGS.

Antiepileptic drugs and glutamate antagonists block behavioral, electroencephalographic, and neuropathological outcomes induced by TsII isolated from *Tityus serrulatus* scorpion venom

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Introduction: TsII, a β -toxin that binds to site 4 of Na⁺ channels, induces an increase in the neurotransmitters release. After hippocampal injection of TsII behavioral and electrographic seizures and neuronal loss are observed. **Objectives:** To investigate the possible blockade of the toxic actions of TsII using anticonvulsive and glutamate antagonists drugs. **Methods:** Cannulas and electrodes were implanted into the hippocampus of Wistar rats (250g). The TsII (2 μ g/ μ l) or phosphate buffer injection was preceded by one of the following drugs (1) Diazepam Dz (7.5 mg/kg), Phenobarbital Pb (12, 25, 50 or 60 mg/kg), Carbamazepine Cz (50 mg/kg), Diphenylhydantoin DPH (200 mg/kg), Valproic acid VPA (400 mg/kg), Ethoxusimide ETX (300, 500 mg/kg) or NaCl 0.9%; (2) MK-801 (0.5 mg/kg), CNQX (2.0 μ g/ μ l), AP-3 (0.1 μ g/ μ l) or MCPG (0.2 μ g/ μ l). EEG and animal behavior were recorded for 8 hours. After 7 days, the brains were processed for morphological analyses. Statistical analyses: Analysis of Variance followed by Dunnett test ($p < 0,05$). **Results:** Antiepileptic drugs Dz 28,6 $\pm$ 0,32; Pb 37,92 $\pm$ 2,3; Cz 25,21 $\pm$ 2,66; ETX 21,5 $\pm$ 2,66; VPA 18 $\pm$ 1,32; DPH 35,64 $\pm$ 1,00 were not able to protect the rats from the hippocampal lesion induced by TsII (Control 54,57 $\pm$ 3,0; TsII 3,75 $\pm$ 0,55;). Dz, Pb and VPA blocked convulsions and epileptic discharges. Cz and ETX induced neuronal loss “*per se*” (Cz 29,65 $\pm$ 2,01 and ETX 26,42 $\pm$ 1,92). Glutamate (Glu) antagonists, MK-801 and CNQX protected the rats from the neuronal loss (31 $\pm$ 8 and 21,2 $\pm$ 18,51 respectively) induced by TsII. These drugs were able to block seizures and decrease the epileptic discharges and spikes. The Glu receptor antagonists, AP-3 and MCPG (25,36 $\pm$ 3,51 and 9,11 $\pm$ 1,18) were not able to protect the rats from the TsII-induced neurotoxicity. **Discussion:** The mainly neurotoxic effects induced by TsII were blocked by MK-801 (non-competitive NMDA receptor antagonist), CNQX (AMPA receptor antagonist) and the antiepileptic drugs, Dz, PB and VPA. It is known that scorpion neurotoxins evoke glutamate release in the brain. The present results show that glutamate antagonists and drugs that increase GABA system activity (Pb and Dz) can reduce or block the outcomes induced by TsII.

Palavras-Chave: glutamate antagonists and antiepileptic drugs

Apoio Financeiro: Supported by: FAPESP; CNPq and Fundação Butantan.

The inhibition of p38^{MAPK} by SB203580 increases the cadmium toxicity in immature hippocampus *in vitro*

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The developing brain is very sensitive to damage by toxic agents. Cadmium (Cd²⁺) is an environmental pollutant which is widely used in industry and is a constituent of tobacco smoke. Cd²⁺ causes severe toxicity for several tissues and recently the central nervous system has been recognized as an important target for Cd²⁺ actions. The aim of this study was investigate the neurotoxicity of Cd²⁺ on immature hippocampus by assessing cell viability and modulation of AKT/PKB and MAPK family members including ERK1/2, p38^{MAPK} and JNK. Hippocampal slices from immature rats (post natal day 14; PN14) were incubated with Cd²⁺ (5-200 µM) for 3 h. The effects on protein phosphorylation were analyzed by western blotting and cell viability was assessed by the MTT reduction assay. Phosphorylation of p38^{MAPK} was enhanced by Cd²⁺ at all concentrations tested (approximately 65%, p<0.001). Cd²⁺ also stimulated the phosphorylation of ERK1/2 in a concentration-dependent manner (up to 150%, p<0.01). However, the phosphorylation of JNK and AKT were not altered by the metal. Moreover, Cd²⁺ reduced cell viability (about 50% at 200µM, p<0.001). We tested the participation of p38^{MAPK} and ERK in the mechanism of neurotoxicity. The inhibition of p38^{MAPK} by SB203580 aggravated the acute Cd²⁺-induced impairment of cell viability (about 26% at 200µM, p<0.001), whereas inhibition of MEK by PD98059 did not alter the effect of Cd²⁺. The present data suggest that in immature hippocampal cells p38^{MAPK} may be part of a signaling pathway that counteracts acute Cd²⁺ neurotoxicity. In conclusion, our results showed that Cd²⁺ impairs cell viability and disturbs MAPKs pathways in an important developmental stage for synaptic organization. **Palavras-Chave:** Cadmium, neurotoxicity, hippocampus, ERK1/2, p38MAPK

Apoio Financeiro: CNPq, FAPESC and FINEP (IBN-Net #01.06.0842-00)

Effects of ethanol in a neuron-like cell line.

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Ethanol (EtOH) is a diffusible molecule with access to the CNS tissue. It directly acts on neurons. Our previous "*in vivo*" results showed neuronal changes in EtOH-exposed rats. In this work, we have studied the "*in vitro*" response of a neuron-like cell type to EtOH exposure. Neuro-2a (an immortalized murine neuroblastoma) is a suitable model to study the effects of EtOH in the morphology and survival of these cells in a short-term bioassay. Cells were plated at 2×10^4 cells/well in 35mm Nunclon™Δ dishes containing 80μl DMEM/High Glucose supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, penicillin G and streptomycin 1% and fetal bovine serum to a final concentration of 10% at 37°C in an atmosphere of 95% air and 5% CO₂. 24 h after plating the medium was replaced by OPTIMEM, a reduced serum medium, and exposed to 50mM or 75mM EtOH for 72 h. Control were cells incubated in OPTIMEM only. Caspase-3 immunoreactivity (-ir) showed that a 72-hour treatment with 50 mM or 75 mM EtOH caused an increase in the apoptotic cells percentage (enolase neuron specific-ir) (control: 1.044 ± 0.173 vs 50 mM EtOH: 3.48 ± 0.216 , $P < 0.0001$ vs 75 mM EtOH: 2.188 ± 0.2703 , $P = 0.0242$). 5HT1A-ir cells were increased (control: 27.09 ± 1.957 vs 50 mM EtOH: 41.88 ± 2.053 , $P < 0.0001$ vs 75 mM EtOH: 41.15 ± 2.166 , $P < 0.0001$). Binucleated cells increased in EtOH- treated cells (control: 9.983 ± 1.016 vs 50 mM EtOH: 13.75 ± 0.9739 , $P = 0.016$ vs 75 mM EtOH: 15.36 ± 1.502 , $P = 0.0091$). *In vivo* previous works showed that EtOH-altered neuronal development could be partially reversed by a 5HT1A agonists treatment. The present results suggest that EtOH strongly affects neuronal survival and that the 5HT1A receptor is overexpressed in EtOH-treated Neuro-2a cells. Further studies are needed to elucidate the mechanisms involved in the functional relationship between EtOH and 5HT1A receptor. **Palavras-Chave:** Neuro2A, Ethanol, 5-HT1A,, Caspasa 3,

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EFFECTS OF IFN- γ AND TNF- α TREATMENT ON GLIAL CELLS INFECTED BY *Neospora caninum*

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Neospora caninum is an intracellular protozoan which causes abortion in cattle and neurological disorders in dogs. The immunological response to this parasite was predominantly Th1-type, but in Central Nervous System we showed that it released IL-10 and IL-6, which are anti-inflammatory cytokines. Furthermore, it has been showed that glial cells did not release the pro-inflammatory cytokine INF- γ . This profile suggests a protective response. The goal of this study was to investigate the effects of pro-inflammatory cytokines on mixed rat astrocytes and microglia primary cultures infected with *N. caninum*. Cells were treated with different concentrations of IFN- γ or TNF- α , and 24h after, they were infected with parasite tachyzoites. Lactate dehydrogenase (LDH) activity was measured to study cell viability. Infected cultures, 72h p.i., revealed a significant increase of LDH activity; the same effect was observed when TNF- α (50, 150 and 300 IU/mL) and IFN- γ (300 UI/mL) were used. Cytokine treatments have reduced significantly the number of tachyzoites in 26.6 and 31.7% when treated with 50 and 150 IU/mL of IFN- γ and 30.2, 38.8 and 31.7% when treated with 50 to 300 IU/mL of TNF- α . In order to investigate if nitric oxide (NO) plays a role on parasite control, we measured nitrite levels in culture supernatants by Griess method. TNF- α treatment did not influence nitrite levels while IFN- γ treatment (150 and 300 UI/mL) increased significantly the nitrite released by non-infected cells and reduced levels in infected cells. Meanwhile, in infected cells INF- γ treatment reduced significantly nitrite levels, showing an inhibitory effect on NO production. These results demonstrate that IFN- γ triggers some parasite "escape mechanisms". Both INF- γ and TNF- α can control parasite multiplication, but they could also cause a synergic toxic effect on infected glial cells. **Palavras-Chave:** *Neospora caninum*, glial cells, neuroprotection, inflammation

Apoio Financeiro: CNPq (PIBIC), CAPES (PPV e PROAP), FAPESB

EFEITOS DO ÁCIDO GLUTÁRICO NA CAPTAÇÃO DE $[^3\text{H}]$ -GLUTAMATO E NA FORMAÇÃO DE ESPÉCIES REATIVAS EM SINAPTOSSOMAS

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Objetivos: Acidemia glutárica tipo 1 é uma doença metabólica autossômica recessiva ocasionada por uma deficiência da enzima glutaril-CoA desidrogenase, resultando no acúmulo de ácido glutárico (AG) no cérebro dos pacientes afetados. Como o envolvimento da excitotoxicidade na degeneração estriatal nesta doença, não está totalmente elucidado, foi investigado o efeito do AG (10 nM) na captação de $\text{l-}[^3\text{H}]$ glutamato e produção de espécies reativas (ER) em sinaptossomas de estriados de ratos.

Métodos: Foram preparados sinaptossomas de estriado de ratos Wistar machos de 270-300 g (Brain Res. 441:59, 1988). Para a determinação da captação de $\text{l-}[^3\text{H}]$ glutamato (Brain Res. 1185:336, 2007) e produção de ER (Neurotoxicol Teratol. 20:317, 1998), os sinaptossomas foram incubados nos tempos de 0, 1, 5, 10, 15 e 30 minutos a 37°C com $\text{l-}[^3\text{H}]$ glutamato (5 μM) ou DCFH-DA (10 μM) na ausência e presença de AG (10 nM). No tempo de 10 minutos (37°C), foi medida a captação de $\text{l-}[^3\text{H}]$ glutamato e a produção de ER na presença do antioxidante Trolox (0,3; 0,6 and 6 μM).

Resultados: AG reduziu significativamente a captação de $\text{l-}[^3\text{H}]$ glutamato em sinaptossomas de estriados quando comparado ao controle [$F(5,60)=23.56$; $P<0.001$] em todos os tempos testados. Em relação à formação de ER, o AG aumentou a taxa de oxidação da DCFH nos sinaptossomas de maneira tempo dependente [$F(5,30)=80.06$; $P<0.001$]. O envolvimento do estresse oxidativo na inibição da captação de $\text{l-}[^3\text{H}]$ glutamato em sinaptossomas foi investigado pela adição de Trolox que não alterou significativamente a redução da captação de $\text{l-}[^3\text{H}]$ glutamato induzida pelo AG [$F(3,56)=0.76$; $p>0.05$]. No entanto, o Trolox, em todas as concentrações testadas, preveniu o aumento da produção de ER induzida pelo AG em sinaptossomas [$F(3,24)=8.37$; $p<0.001$].

Conclusão: O AG reduziu a captação de $\text{l-}[^3\text{H}]$ glutamato e aumentou a produção de ER em sinaptossomas de estriado de ratos. Entretanto, enquanto o Trolox preveniu o aumento da produção de ER, ele não protegeu contra a redução da captação de $\text{l-}[^3\text{H}]$ glutamato induzida pelo AG. Esses dados sugerem que a produção de ER e a inibição da captação de $\text{l-}[^3\text{H}]$ glutamato pelo AG são fenômenos independentes.

Palavras-Chave: Glutamato; Espécies reativas; sinaptossomas

Apoio Financeiro: CAPES/CNPq

Minocycline as a treatment for experimental cerebellar ataxia: effects on microglial activation and motor recovery

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Many works show that, beyond conventionally antibiotic action, minocycline is capable of acting in other mechanisms such as neuroprotection. These mechanisms include modulation of microglial activation and of changes in other cell types intrinsic and extrinsic to the central nervous system. Cerebellar ataxia induced by the neurotoxin 3-acetylpyridine (3-AP) is followed by severe alterations in motor behavior and by microglial morphological changes. In the present work, we verified the effect of minocycline on the microglial response and motor behavior in this model. Rats of 30 – 35 days of age were treated by 3-AP and afterward euthanased at 24h, 4, 10 e 21 days post-injection. Minocycline (50mg/kg) diluted in saline solution was given by intraperitoneal injection in animals untreated and treated with 3AP. Twelve hours after treatment started, a second minocycline dose (25mg/Kg) was given, followed by daily doses in the same concentration. After submitted to a walking test, animals were perfusion-fixed with 4% paraformaldehyde, the brains dissected out and criosectioned in the coronal plane. Histochemical analyses for microglia identification were performed using the *Griffonia simplicifolia* isolectin (I-B4). Walking test revealed the recovery of the normal pattern in animals treated by 3AP. I-B4 reaction showed strongly tagged cells with few or no processes 24h after minocycline treatment. After 4 days minocycline treatment, reactive microglia, commonly observed in the animals treated with 3AP only, were virtually absent. We conclude that minocycline induce two microglial response: first, an early response, related with possible beneficial effects on motor recovery, and a second, by the 4th day, characterized by impairment of microglial activation. Since this activation appears to worsen 3AP induced damage, minocycline prevents this late phase of toxicity.

Palavras-Chave: minocycline, microglia, ataxia

Apoio Financeiro: CNPq / FAPERJ

Risperidona aumenta a captação de glutamato, a atividade da enzima glutamina sintetase e os níveis de glutathione em células astrogliais.

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Introdução: Risperidona é um antipsicótico atípico, freqüentemente utilizado na terapêutica de psicoses como a esquizofrenia. Recentemente, está sendo utilizada para tratamento sintomático de irritabilidade em crianças e adolescentes autistas.

Objetivos: O objetivo do presente estudo foi avaliar se risperidona é capaz de alterar parâmetros gliais como captação de glutamato, atividade da enzima glutamina sintetase (GS) e os níveis de glutathione (GSH) em células da linhagem celular C6.

Metodologia: Células de glioma C6 foram cultivadas em DMEM 5 % de soro fetal bovino, pH 7,4, em incubadora de CO₂. Após atingirem a confluência, as células foram tratadas com risperidona na concentração de 10 µM por 6 horas e avaliou-se a captação de glutamato utilizando L-[2,3-³H]glutamato, a atividade da enzima (GS) foi avaliada através de ensaio colorimétrico e os níveis de (GSH) por um ensaio fluorimétrico.

Resultados: Observamos que a risperidona aumenta a captação de glutamato ($0,37 \pm 0,006$ para $0,49 \pm 0,008$ nmol/mg proteína/min, $P < 0,005$), a atividade da GS ($0,63 \pm 0,02$ para $0,77 \pm 0,023$ µmol/mg proteína/h, $P < 0,005$), e os níveis de glutathione ($17 \pm 0,4$ para $33 \pm 1,3$ nmol/mg proteína).

Conclusões: Considerando o importante papel das células astrogliais em diversas desordens psiquiátricas, e a utilização de risperidona como agente no tratamento do autismo, uma patologia que altera o desenvolvimento neurológico, podemos inferir que a ação benéfica da risperidona nestes pacientes pode envolver resposta glial.

Palavras-Chave: Risperidona, Glutamato, Células Astrogliais C6, Autismo.

Apoio Financeiro: CNPq, PROPESq-UFRGS, FINEP/ Rede IBN 01.06.0842-00, FAPERGS.

Late Erythropoietin receptor (Epo-R) and Multidrug Resistance (MDR-1) overexpression in an experimental stroke model. A novel long-term therapeutic opportunity?

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Stroke is a major human health problem inducing long-term disability without therapeutic options, reducing brain aerobic metabolism, loss of function and/or cell death. In focal ischemia, neurons from the penumbra area surrounding infarct core could be rescued with early therapeutics within first 3-6h after stroke. Under hypoxia transient hypoxia-inducible factor-1 (HIF-1a) activation induces Epo/Epo-R system, related with O₂ supply and/or cell survival, and neuronal MDR-1 gene expression related with potential impairment to neuroprotective therapeutics. We recently reported high neuronal MDR-1 gene expression 5 days after experimental focal hypoxia induced by direct CoCl₂ brain cortical injection, and we have asked if it could be a new obstacle for neuroprotection. Now we ask if other cell-markers as HIF-1 or EPO-R could remain expressed 5 days after injury, offering novel therapeutic stratagems irrespective to the closed therapeutic windows and/or MDR-1 mediated multidrug refractoriness. Our aim was to evaluate the MDR-1, HIF-1a and EPO-R brain expression in a focal cortical brain hypoxia-ischemia model. In normal adult male Wistar rats (~250g; n=18) saline or sterile CoCl₂ (2µl-50mM) were injected (frontoparietal cortex at Bregma-1.30mm-right hemisphere) by stereotaxic surgical procedure. After 5 days of recovery, animals were anaesthetized (300mg/kg chloral hydrate) and perfused with paraformaldehyde (4% w/v). Coronal 40 µm thick brain sections corresponding to the nearby area to the saline or CoCl₂ injection sites were cut. Immunostaining for MDR-1, HIF-1a and EPO-R were performed in brain slides with specific antibodies. After 5 days of stroke, high expression of HIF-1a and MDR-1 was observed in neurons, astrocytes and vessel with lower EPO-R immunostaining intensity in neurons from penumbra area but several cells shared MDR-1 and EPO-R expression. In controls only MDR-1 was lightly expressed in vessels. We found persistent expression of HIF-1a, MDR-1 and EPO-R (day 5). In spite the potential farmacoresistance due to MDR-1, EPO-R expression could give a long term therapeutic opportunity in stroke by using Epo.

Palavras-Chave: Hypoxia; Stroke; MDR-1; HIF-1; Epo-R

Apoio Financeiro: Support: UBACYT M072

Antiproliferative effect of boldine, an aporphine alkaloid of *Peumus boldus*, in U138-MG human glioma cell line

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Objective: Malignant gliomas, tumors that usually arise from astrocytic lineage, are the most common and devastating primary tumors of the central nervous system. The aim of this study was to investigate the effect of boldine, an aporphine alkaloid of *Peumus boldus*, in U138-MG glioma cell line.

Methods: Cells were grown and maintained in DMEM medium supplemented with 10% (v/v) fetal bovine serum and incubated at 37°C with 5% of CO₂. Cells were seeded in a 24-well plates and treated with different boldine concentrations (80, 250 e 500 mM) for 24 hours or 72 hours. The medium was changed once a day with replacement of the drug. Sulforhodamine assay, based on a protein staining method, was used to provide a quantitative measure of the percentage of cells after 72 hours of treatment. Propidium iodide (PI - 5 mM) incorporation after 72 hours of treatment and Western blot to detect caspase-3 and caspase-9 (key executioners of apoptosis) after 24 hours of treatment, were used to determine glioma cell death. Mitotic index, analyzed by fluorescence microscopy, was used to assess cell proliferation.

Results: Cell percentage after boldine treatment, analyzed by Sulforhodamine assay, was significantly lower at concentrations (mM, %control±SEM, n=5, p<0.01) 80 (42.5±4.8), 250 (46.2±4.4) and 500 (24.5±4.3). PI uptake was observed only at 500 mM, indicating cellular death in three independent experiments. Boldine treatment (80 mM) did not activate caspase-3 and caspase-9, assessed by Western blot assay in four independent experiments. We observed reduction in mitotic index, in all concentrations tested: (mM, %control±SEM, n=3, p<0.01): 80 (25.0±4.4), 250 (18.6±12.0), 500 (26.8±13.7).

Conclusion: Our results showed that boldine reduce cell percentage in a concentration range of 80 – 500 mM, but we did not observe cellular death by classic apoptosis. Necrosis was observed only at 500 mM boldine concentration. Considering this, we suggest that boldine treatment could reduce cellular proliferation once we observed a reduction on mitotic index in boldine treated cells.

Palavras-Chave: boldine, glioma, cell proliferation

Apoio Financeiro: CAPES and CNPq

MELATONINA REDUZ A PERDA DE NEURÔNIOS IMUNORREATIVOS A TIROSINA HIDROXILASE NA SUBSTÂNCIA NEGRA DE RATOS LESADOS COM MPTP.

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Objetivos: avaliar o efeito da administração aguda de melatonina (50 mg/kg) sobre a morte de neurônios dopaminérgicos da substância negra (SN) induzida por MPTP.

Métodos e Resultados: 60 ratos machos foram divididos nos seguintes grupos: controle+veículo (n=10), controle+melatonina (n=10), SHAM+veículo (n=10); SHAM+melatonina (n=10); MPTP+veículo (n=10) e MPTP+melatonina (n=10). Os grupos MPTP receberam infusão bilateral (1mmol MPTP em 2,1mL de salina, 0,35mL/min) na SN parte compacta, enquanto os grupos SHAM receberam a infusão de 1mL de salina bilateralmente. Trinta minutos antes do procedimento cirúrgico cada animal recebeu a administração via intraperitoneal (i.p.) de veículo (1 mg/kg) ou melatonina (50 mg/kg). Vinte e quatro horas e sete dias após a cirurgia estereotáxica, todos os animais foram anestesiados profundamente e perfundidos intracardialmente, primeiramente com salina e após, com solução fixadora formaldeído 4% para realização da técnica de imunohistoquímica. A perda de neurônios imunorreativos a enzima tirosina hidroxilase (TH) foi quantificada (ANOVA seguida de Newman-Keuls). Os resultados mostram que o grupo MPTP+veículo exibiu significativa redução na quantidade de neurônios imunorreativos a TH 24h (42.0±7.3) e sete dias após cirurgia (45.3±2.6), em comparação ao grupo controle+veículo (100±4.5). A administração prévia e única de melatonina impediu a morte de neurônios dopaminérgicos imunorreativos a TH na SN dos animais lesados com MPTP e tratados com melatonina 24h após a infusão de MPTP (87±8.5) em comparação aos grupos controles. Entretanto, tal efeito não foi observado 7 dias após a cirurgia estereotáxica (MPTP+melatonina - 46.35±2.6).

Conclusão: A administração aguda de melatonina (50 mg/kg) previamente a infusão de MPTP impediu a perda de neurônios imunorreativos a TH na SN de ratos lesados com MPTP 24h após a cirurgia estereotáxica. Entretanto, essa administração única e prévia de melatonina não foi capaz de prevenir a perda destes neurônios quando avaliados 7 dias após a lesão.

Palavras-Chave: Doença de Parkinson, MPTP, melatonina e tirosina hidroxilase

Apoio Financeiro: CAPES, CNPq.

Effects of chronic cyclosporine administration in rat behavior and brain oxidative stress

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Objectives: Neurotoxicity is one of the most significant clinical side effects of the immunosuppressive treatment with cyclosporine (CsA). Minor symptoms include tremor, agitation, insomnia, anxiety, amnesia, headache, and paraesthesia. Herein, we examined the effects of CsA upon brain oxidative stress, exploratory activity and anxiety-related behavior in rats submitted to chronic CsA administration.

Methods: Male Wistar rats were divided in control and treated group, and treated with vehicle and CsA 15 mg/kg, respectively, by once daily gastric gavage during 8 weeks. The blood CsA concentration was measured. Brain oxidative stress was evaluated through TBARS assay (to quantify lipid peroxidation), total sulfhydryl content (an index of redox balance) and total antioxidant potential by TRAP technique. Behavioral effects were evaluated by Elevated Plus Maze (EPM) test that analyze entries in the open arms (EOA) and time in the open arms (TOA) and Open Field (OF) test for locomotor activity.

Results: Brain lipid peroxidation and total sulfhydryl content were altered in hippocampus of CsA-treated animals when compared to controls: 0.53 ± 0.04 and 0.42 ± 0.02 , $P < 0.01$ (MDA equivalents/mg protein); and 15.5 ± 0.58 and 21.1 ± 1.17 , $P < 0.0002$ (-SH μ mol/mg protein), respectively. Furthermore, the total antioxidant defences of hippocampus were decreased in CsA-treated animals as well. CsA reduced locomotor activity in the OF test when compared to control (3316 ± 329 and 2065 ± 152 , respectively $P < 0.001$) and increased anxiety in both parameters of the EPM: entries in the OA = 3.23 ± 0.580 and 7.9 ± 1.21 ($P < 0.001$) and time in the OA = 51 ± 11.57 and 109 ± 18.4 ($P < 0.05$).

Conclusions: These data suggest that CsA-treated animals had a significant increase in hippocampal oxidative stress, which are supported by a remarkably reduction of antioxidant potential. Anxiety, a well-known cognitive disorder associated with hippocampal dysfunction was increased by CsA treatment whereas locomotor activity decreased. We propose that CsA chronic treatment might promote behavioral disturbances through hippocampus oxidative stress.

Palavras-Chave: Cyclosporine, neurotoxicity, oxidative stress, behavior

Apoio Financeiro: IBNnet, CNPq, Finep

PROTECTIVE EFFECTS OF DISCRETAMINE AND A DIPLotropin DERIVATIVE ON CATECHOL-INDUCED CYTOTOXICITY

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Catechol (CAT) oxidizes in physiological conditions consuming oxygen and forming reactive oxygen species (ROS) and quinones. Discretamine (DSC) is an alkaloid isolated from *Duguetia gardneriana*. 5,6-Dimethoxy-7-phenyl-6,7-dihydro-5H-furo[3,2-g]cromen-4,9-dione (DPTN) is a synthetic derivative of diplotropin, which was obtained from the stem bark of *Diplotropis ferruginea*. OBJECTIVES: The aim of this study was to evaluate the cytotoxicity of these compounds to human neuroblastoma SH-SY5Y cells *in vitro*. The protective effects of DSC and DPTN against CAT-induced cytotoxicity were also investigated. METHODOLOGY: Cell viability was assayed by the MTT test. The mode of catechol-induced cell death was determined by flow cytometry using annexin V and propidium iodide staining. RESULTS: CAT was cytotoxic to SH-SY5Y cells after 72 hours ($EC_{50} = 26.6 \mu M$). There was a correlation between the formation of quinones and CAT-induced cytotoxicity. However, superoxide dismutase (300 U) and catalase (6000 and 10000 IU) did not protect cells against the toxicity induced by 60 μM CAT after 72 hours. Flow cytometry showed that catechol kills cells by apoptosis. DSC showed no toxicity up to 20 μM , and DPTN presented an EC_{50} of 61 μM . DSC in concentrations between 3 - 20 μM and DPTN between 20 and 60 μM protected cells against CAT-induced cytotoxicity. CONCLUSIONS: These results indicate that CAT is toxic to SH-SY5Y cells, although it does not seem to be associated with the extracellular production of ROS. DSC and DPTN protected them, but additional studies are needed to elucidate mechanisms that are involved in these phenomena. **Palavras-Chave:** Alkaloids, Catechol, Neuroblastoma

Apoio Financeiro: Supported by FAPESB, CNPq.

Resveratrol prevents the inhibition of $\text{Na}^+\text{K}^+\text{-ATPase}$ activity after global cerebral ischemia.

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Objective: Cerebral ischemia (ISC) causes an interruption of neuronal and glial oxidative metabolism and can lead to irreversible loss of brain function. Neuronal damage caused by global ISC is associated with an imbalance in ionic homeostasis. $\text{Na}^+\text{K}^+\text{-ATPase}$ is the enzyme in cytoplasmic membrane responsible for the active transport of sodium and potassium ions in the nervous system, maintaining the ionic gradient necessary for neuronal excitability and regulation of neuronal cell volume. The aim of this study was to investigate the effect of resveratrol (RSV) treatment after global cerebral ischemia on the $\text{Na}^+\text{K}^+\text{-ATPase}$ activity in hippocampus and cortex at different time points of reperfusion.

Methods: Adults Wistar rats were submitted to 10 min of ischemia by the four-vessel occlusion method. RSV (30mg/Kg) was injected i.p. for 7 consecutive days before ischemia. The effects of RSV on $\text{Na}^+\text{K}^+\text{-ATPase}$ was determined at 1h, 24h, and 7 days after global ISC.

Results: Global ISC induced a statistically significant decrease in the $\text{Na}^+\text{K}^+\text{-ATPase}$ activity at 1h and 24h of reperfusion in the hippocampus (Control: 119 ± 13 , Control + RSV: 125 ± 17 , $n=5$; Ischemic 1h: 95 ± 12 , $p<0,05$, Ischemic 24h: 88 ± 17 , $p<0,001$, $n=6$) and cortex (Control: $126,6 \pm 18$, Control + RSV: $129,7 \pm 21$, $n=5$; Ischemic 1h: 90 ± 8 , $p<0,05$, Ischemic 24h: 66 ± 4 , $p<0,001$, $n=6$). The decline in the $\text{Na}^+\text{K}^+\text{-ATPase}$ activity was prevented in the animals treated with RSV within the first 24h of reperfusion (Hippocampus= Ischemic + RSV 1h: 120 ± 19 , Ischemic + RSV 24h: 116 ± 20 ; $n=6$ and Cortex= Ischemic + RSV 1h: 119 ± 6 , Ischemic + RSV 24h: 135 ± 21 ; $n=6$). The effect of RSV on $\text{Na}^+\text{K}^+\text{-ATPase}$ 7 days after global ischemia was not significant ($n=6$; $p>0,05$). Specific enzyme activity was expressed as nmol of inorganic phosphate released per minute per mg of protein.

Conclusions: Our results indicate that global ISC induced a significant decrease in the $\text{Na}^+\text{K}^+\text{-ATPase}$ activity in the hippocampus and cortex during different periods of reperfusion. RSV treatment prevented ischemia-induced changes in the $\text{Na}^+\text{K}^+\text{-ATPase}$ activity. We suggest that the maintenance of $\text{Na}^+\text{K}^+\text{-ATPase}$ activity afforded by RSV could be related to cellular neuroprotection.

Palavras-Chave: global cerebral ischemia; $\text{Na}^+\text{K}^+\text{-ATPase}$; resveratrol

Apoio Financeiro: CNPq, CAPES and Propesq/UFRGS

C.05.067

Liberação de S100B *in vitro* modulada por adrenalina em adipócitos de rato

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Introdução: A proteína S100B tem sido comumente utilizada como marcador de dano e/ou disfunção do sistema nervoso central devido a sua alta expressão e secreção por astrócitos. Entretanto, as fontes extracerebrais de S100B têm sido mais recentemente valorizadas na interpretação dos valores séricos dessa proteína.

Objetivos: O objetivo deste trabalho foi verificar a liberação de S100B em diferentes preparações de tecido adiposo *in vitro* e sua possível modulação por adrenalina.

Metodologia: Foram utilizados adipócitos isolados, fatias de tecido adiposo e cultura de lipócitos obtidas da linhagem celular GRx. Essas células foram tratadas com adrenalina na concentração de 10 µM por 1 ou 3 horas e a secreção de S100B foi avaliada pelo método de ELISA.

Resultados: Nos adipócitos isolados e no tecido adiposo, a adrenalina aumentou a secreção de S100B, somente após 1 hora, ($4 \pm 0,4$ para $10 \pm 0,7$ ng/ml, $P < 0,005$) e ($30 \pm 2,8$ para $40 \pm 3,7$ ng/ml, $P < 0,005$), respectivamente. Já nas culturas de lipócitos, houve aumento tempo-dependente na secreção basal de S100B no intervalo entre 1 ($0,05 \pm 0,0002$, $P < 0,005$) e 3 horas ($0,30 \pm 0,009$ ng/ml, $P < 0,005$) e não houve resposta pela adrenalina.

Conclusões: A secreção de S100B pelos adipócitos *in vitro* foi estimulada por adrenalina, sugerindo que este hormônio possa modular a secreção periférica de S100B *in vivo*. Estes dados confirmam a necessidade de se avaliar com cautela os níveis séricos de S100B, pois estes níveis podem ser provenientes tanto do tecido cerebral, quanto do tecido adiposo.

Palavras-Chave: S100B, adipócitos

Apoio Financeiro: CNPq, PROPESq-UFRGS, FINEP/ Rede IBN 01.06.0842-00, FAPERGS.

C.05.068

Dependência de estoques intracelulares de íons cálcio na toxicidade induzida por rotenona, um inibidor natural do complexo I mitocondrial.

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Objetivo: Investigar o envolvimento de vias dependentes de íons cálcio na neurodegeneração induzida por rotenona em culturas primárias derivadas de mesencéfalo ventral de ratos neonatos.

Métodos: Culturas primárias semipuras de mesencéfalo ventral de ratos Wistar neonatos (P2-P4) foram utilizadas para avaliação da toxicidade após exposição à rotenona (5, 10, 15, 20, 25 e 30 nM) por 7 dias. A viabilidade celular foi avaliada pelo método do MTT. Vias dependentes de cálcio foram investigadas com a utilização de nifedipina, vermelho de rutênio, benzamil, EDTA e tapsigargina.

Resultados: À exceção da concentração de 5 nM, a exposição ao composto rotenona resultou em perda celular dependente de concentração. Como ilustração, observamos valores de viabilidade celular de 79,92 % $\pm$ 4,76 e 50,91 % $\pm$ 5,60 para as concentrações de 10 e 30 nM de rotenona, respectivamente). Em culturas tratadas com 20 nM de rotenona, verificou-se que o benzamil (inibidor do trocador Na⁺/Ca²⁺) potencializou os efeitos da rotenona em concentração elevada (20 mM); adição do quelante EDTA promoveu aumento da toxicidade da rotenona nas concentrações de 0,5 e 1 mM; adição de nifedipina, bloqueador de canais de cálcio tipo-L, não alterou a viabilidade celular (0,1mM) ou mostrou-se tóxica (0,5 e 1 mM) sob as mesmas condições; a adição de tapsigargina (inibidor da bomba de Ca²⁺) potencializou o efeito neurotóxico da rotenona; A adição de vermelho de rutênio reduziu o efeito tóxico da rotenona quando utilizado em concentrações menores (0,5 e 1mM), porém mostrou-se tóxico na concentração de 5mM.

Conclusões: A exposição de culturas semipuras de mesencéfalo ventral à rotenona, um inibidor do complexo mitocondrial I, resultou em toxicidade dependente de concentração. Os experimentos realizados mostraram evidências da participação das reservas mitocondriais de íons cálcio concorrendo para estes efeitos celulares.

Palavras-Chave: Rotenona, Cálcio

Apoio Financeiro: FAPESPA/SEDECT

Indomethacin-loaded nanocapsules treatment reduces *in vivo* glioblastoma growth in a rat glioma model

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Objectives: Malignant gliomas have a dismal prognosis despite multi-modality treatments like neurosurgical resection, radiation and chemotherapy, mainly due to its high level of invasion. Nanoparticles of biodegradable polymers for anticancer drug delivery can provide a sustained, controlled, and targeted delivery as drug carriers. The aim of the present study was to investigate the effect of indomethacin-loaded nanocapsules treatment in a rat glioma experimental model.

Methods: Nanocapsule suspensions were prepared by interfacial deposition of preformed polymers. One million C6 cells were injected through esterotaxic surgical in the right striatum of male Wistar rats. After 10 days of glioma implantation, animals were treated with 1mg/Kg/day for 10 days with: drug-unloaded nanocapsules (NC), indomethacin (IndOH) or indomethacin-loaded nanocapsules (IndOH-NC). After treatment, the rats were decapitated and the brain sectioning and stained with H&E for pathological analysis. Mitosis was counted in ten high power fields of the periphery of the tumor, and the average of this counting was used as mitotic index. For tumor size quantification, images were captured using a digital camera connected to the microscope and tumor volume was determined.

Results: The rats treated IndOH-NC had a significant reduction in the tumor size (mm³) (Control: 152.72±39.26; NC: 148.73±22.38; IndOH: 105.99±51.90; IndOH-NC: 48.32±16.23; n=10, p<0.001). In addition, according to the pathological analysis, the gliomas of treated rats presented a significant reduction in the mitotic index (Control: 15.5±2.03; NC: 15.01±1.81; IndOH: 12.14±3.64; IndOH-NC: 5.6±1.72; n=10, p<0.001), which indicates a less invasive/proliferative tumor. Besides, our results showed a consistent improvement in the survival of the animals treated with IndOH-NC (Control: 20%; NC: 30%; IndOH: 40%; IndOH-NC: 80%; n=10, p<0.001).

Conclusion: Indomethacin-loaded nanocapsules are able to reduce *in vivo* glioblastoma growth. Although indomethacin is not an agent used in treatment of brain tumors, our results imply that this drug in nanocapsules formulation may be considered a potential candidate for glioma treatment.

Palavras-Chave: glioblastoma multiform, cell proliferation, indomethacin, polymeric nanocapsules, drug delivery

Apoio Financeiro: Supported by Capes, Propesq/UFRGS and Rede Brasil-França CNPq/MCT.

C.05.070

Brain mitochondrial dysfunction in a mouse model of sepsis

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Mitochondrial dysfunctions have been associated with the pathogenesis of sepsis. In the present work, we investigate brain mitochondrial function in a septic mouse model. Male Swiss mice, aged 6–8 wks, were subjected to cecal ligation and perforation (sepsis group) with saline resuscitation or to sham operation. Oxygen consumption was measured polarographically in an oximeter. Brain homogenates from septic animals presented higher oxygen consumption in the absence of adenosine 5'-diphosphate (state 4) compared with control animals (12.5 ± 1.1 vs. 8 ± 1.6 nmol oxygen·min⁻¹·mg protein, $p < 0.05$). The increase in state 4 respiration in animals in the cecal ligation and perforation group resulted in a drastic decrease in both respiratory control and adenosine 5'-diphosphate/oxygen ratios (1.7 ± 0.1 vs. 3.3 ± 0.6 , $p < 0.0001$), indicating a reduction in the oxidative phosphorylation efficiency. Septic animals presented a significant increase in the recovery time of mitochondrial membrane potential on adenosine 5'-diphosphate addition compared with control animals, suggesting a proton leak through the inner mitochondrial membrane. The septic group presented a general reduction in the content of cytochromes. Moreover, the activity of cytochrome c oxidase was specifically and significantly decreased in the brain during sepsis (31.8 ± 1.5 vs. 40.4 ± 3.3 nmol oxygen·min⁻¹·mg⁻¹ protein). Hydrogen peroxide generation by brain mitochondria from septic mice did not respond to substrates of electron transport chain or to adenosine 5'-diphosphate, showing that mitochondrial function may be compromised in a critical level in the brain during sepsis. The mitochondrial dysfunctions demonstrated here indicate that uncoupling of oxidative phosphorylation takes place in the brain of septic mice, compromising tissue bioenergetic efficiency.

Palavras-Chave: brain; mitochondrial dysfunction; sepsis; oxidative phosphorylation;

Apoio Financeiro: CNPq, FUIB, Faperj

Investigation of the effects from Cat's Claw extracts in C6 glioma cell line

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Objective: Glioblastoma is the most frequent and most malignant human brain tumor. The prognosis remains very poor, with most patients dying within 1 year after diagnosis. Cat's Claw, also known as "Uña de Gato" or "*Uncaria tomentosa*", is an effective treatment for several health disorders including chronic inflammation, gastrointestinal dysfunctions, tumors and infections. In this study we investigated a possible anti-tumoral effect of two purified fractions, alkaloid and phenolic, from Cat's Claw in C6 glioma cell line.

Methods: C6 glioma cells lineage were grown and maintained in DMEM medium supplemented with 5% fetal bovine serum and incubated at 37°C with 5% of CO₂. Cells were seeded in 24-well plates and treated with different Cat's Claw purified fractions (alkaloid and phenolic) at different concentrations (2.5, 5, 10, 25, 50, 100, 150mM) for 24 and 48 hours. Propidium iodide (PI) (5mM) uptake was used as a marker of cell death and sulphorodamine B assay was performed to investigate the percentage of cells at the end of the treatment. Organotypic hippocampal slice cultures were used to investigate the toxicity of Cat's Claw extracts in a nontumoral tissue.

Results: No PI uptake nor cell percentage alteration was observed after 24h of exposure to any fraction at any concentration (n=6; p>0,05). However, after 48 h of exposure to both, phenolic and alkaloid fractions, PI uptake increased in all tested concentrations higher than 50mM, suggesting cell death. At same time, cell percentage decreased in cultures treated with the phenolic fraction (mM, % of control±SD, n=6, p<0.05) at concentrations of 25 (78.2±4.2), 50 (67.8±14.2), 100 (54.4±7.2) and 150 (38.9±5.3). In cultures treated with the alkaloid fraction we observed the same effect only at concentration of 100 (43.3±22.6) and 150 (39.9±16.4). Using organotypic hippocampal cultures, PI uptake was not observed after the treatment with higher concentrations of both fractions (n=3, p>0,05) indicating no cellular death.

Conclusions: Cat's Claw extracts (phenolic and alkaloid) were able to induce cell death and a decrease in cell number of C6 glioma cell lineage after 48h of treatment. Also, they did not affect nontumoral cells to the extent that they affect tumor cells.

Palavras-Chave: glioma, proliferação, cat's claw, antitumor agent

Apoio Financeiro: CNPq and CAPES

HYPERMETHIONINEMIA INCREASES ACETYLCHOLINESTERASE ACTIVITY IN CEREBRAL CORTEX OF RATS.

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Objectives: Considering that very little is known about the mechanisms underlying the brain damage present in hypermethioninemia, in the present study we investigated the effect of chronic administration of methionine on acetylcholinesterase (AChE) activity in cerebral cortex of rats.

Methods: For chronic hypermethioninemia, rats received subcutaneous injections of methionine (1.34–2.68 µmol/g of body weight) from the 6th to the 28th days of age; control rats received the same volume of saline solution (J. Neurosci. Methods 160:1, 2007). Rats were sacrificed by decapitation 3 h, 12 h or 30 days. For acute hypermethioninemia, 29-day-old rats received one single injection of methionine (2.68 µmol/g of body weight) or saline (J. Neurosci. Methods 160:1, 2007) and were sacrificed 1 h, 3 h or 12 h later. Cerebral cortex was isolated and used for AChE activity determination (Biochem. Pharmacol. 7:88, 1961).

Results: Our results showed that chronic hypermethioninemia significantly increased AChE activity at 3 h ($p<0.05$), 12 h ($p<0.01$) or 30 days ($p<0.05$) after the last injection of methionine. However, acute administration of methionine did not alter cerebral cortex AChE activity at none time tested ($p>0.05$).

Conclusion: Our findings demonstrated that hypermethioninemia caused an increase of AChE activity that might be related, at least in part, to the neurological alterations observed in hypermethioninemic patients.

Palavras-Chave: hypermethioninemia, methionine, acetylcholinesterase

Apoio Financeiro: CNPq, PRONEX II, PROPESQ/UFRGS.

Epicatechin gallate increases glutamate uptake and S100b secretion in C6 cell lineage

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Introduction: There is a current interest in dietary compounds, such as green tea polyphenols, that can favor protection against a variety of brain disorders, including Alzheimer's disease, ischemia, and stroke.

Objective: The objective of the present study was to investigate the effects of (-)-epicatechin-3-gallate (ECG), one of three major green tea antioxidants, on C6 astroglial lineage cells.

Methods: Here, we evaluated cell morphology by phase contrast and integrity by propidium iodide (PI) assay. Specific astrocyte activities were performed by L-[³H]Glutamate uptake (nmol/mg prot/min) and S100B secretion (ng/mL) by ELISA, in the presence of 0.1, 1 and 10 µM ECG. Values of $P < 0.05$ were considered to be significant by one-way ANOVA followed by the Tukey's test.

Results: During 6 h of incubation, cell morphology was altered only at 10 µM ECG; however, after 24 h of treatment, cells become stellate in the presence of all concentrations of ECG. There was a small increase (6%) in PI incorporation after 24 h of incubation, but only at 10 µM ECG. After 6 h, 0.1-10 µM ECG induced a sustained increase in glutamate uptake, from 0.39 ± 0.04 (control) to 0.70 ± 0.06 (10 µM ECG), $P < 0.05$. In addition, a significant increase in S100B was observed by ECG treatment, from 3.4 ± 0.22 (control) to 4.6 ± 0.32 and 5.8 ± 0.44 , $P < 0.05$ (1 and 10 µM ECG, respectively) after 1 h. After 6 h of treatment, all doses of ECG induced a significant increase (about 60%) in S100B secretion.

Conclusions: These data demonstrate that ECG induces a significant improvement in glutamate uptake and S100B secretion in C6 cells, indicating that ECG could contribute to the neuroprotective role of astroglial cells. **Palavras-Chave:** ECG, catechin, astrocyte, C6, glutamate uptake, S100B

Apoio Financeiro: CNPq, PROPESq-UFRGS, FINEP/ Rede IBN 01.06.0842-00, FAPERGS.

Alpha-MSH is a neurotrophic factor for spinal motor neurons

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Objectives: Melanocortins (MC) are produced from cleavage of a longer peptide called pro-opiomelanocortin. Alpha-melanocyte-stimulating hormone (alpha-MSH) expression has been observed in spinal cord, and was upregulated in motor neurons and neuromuscular endplates after nerve injury. One of its receptors, the MC4R was induced in motor neurons and sensory dorsal root ganglion neurons after nerve injury. Moreover, alpha-MSH induced functional recovery after peripheral nerve injury as well as neurite outgrowth from several neuronal types in vitro. However, it is not known whether alpha-MSH might have direct effects on spinal motor neurons. **Methods:** In the present work we have tested the effects of alpha-MSH in purified rat spinal motor neuron cultures and astrocyte-motor neuron co-cultures. **Results:** A dose-dependent (≥ 1 mM) neurotrophic effect of alpha-MSH on trophic factor deprived motor neuron purified cultures was observed, reaching comparable levels of motor neuron survival as with GDNF addition. Moreover, protection from glutamate-induced excitotoxicity was also produced by alpha-MSH. Finally, in astrocyte-motor neuron co-cultures exposed to LPS to mimic an inflammatory environment, alpha-MSH protected motor neurons from cell death. **Conclusions:** We conclude that alpha-MSH is a neurotrophic factor with direct actions on motor neurons, able to protect them from excitotoxic and inflammatory insults.

Palavras-Chave: MSH, melanocortin, motor neuron, astrocyte, inflammation, neurotrophic factor

Protector effect of zinc on brain and cerebellum changes induced by mercury in young rats.

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Objectives: Animals in development are particularly sensitive to external insults, including heavy metals. Recently, we observed that several toxic effects of Hg on young rats were prevented when animals were pre-exposed to zinc (Zn) (Tox.Let., 146:17, 2003; Eco.Env.Saf., 66:441, 2007). Then, we investigated the effects of HgCl₂ on brain and cerebellum acetylcholinesterase (AChE) activities, one enzyme widely used as index of cholinergic function, and the possible preventive effects of ZnCl₂ on AChE and on other alterations induced by Hg in young rats.

Methods: Wistar rats were pre-treated by subcutaneous injections from 3rd to 7th postnatal day with one daily dose of saline (NaCl 90 mg/kg/day) or ZnCl₂ (27 mg/kg/day). On five subsequent days the animals received saline or HgCl₂ (5 mg/kg/day). Twenty-four hours after last dose the animals were anesthetized and killed by decapitation. The brain and cerebellum were removed for determination of AChE activity (Biochem.Pharmacol. 7:88, 1961) and metal content (*Fresenius J.Anal.Chem.* 367:479, 2000).

Results: HgCl₂ induced significant reduction of brain (15%; p<0.05) and cerebellum (30%; p<0.05) weights, inhibition of the cerebellum AChE activity (28%; p<0.05), and increase of the Hg levels in brain (about 24 fold; p<0.05) and cerebellum (about 26 fold; p<0.05). ZnCl₂ pre-treatment prevented totally the diminution of brain and cerebellum weights (p<0.05), and partially the inhibition of the cerebellum AChE activity (16%; p<0.05) and the increase of the Hg content in brain and cerebellum (about 9.5 fold, p<0.05) induced by HgCl₂.

Conclusion: The Hg exposure promotes accumulation of this metal in brain and cerebellum, decreasing the weights of these structures and inhibits the cerebellum AChE activity. The pre-treatment with Zn was effective in to prevent the alterations induced by Hg in young rats. **Palavras-Chave:** mercury, zinc, acetylcholinesterase, young rats.

Apoio Financeiro: CAPES, FIPE, CNPq

Efeito da Variação Glicêmica na Depressão Alastrante em Retina de Pinto

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Introdução: A depressão alastrante (DA) descrita por Leão (1944) é uma onda maciça generalizada de despolarização da substância cinzenta ou da retina que pode ser demonstrada em várias espécies, incluindo peixes, anfíbios, aves e mamíferos. A DA resulta num colapso temporário dos gradientes iônicos e num potencial transmembrana e é caracterizada por um potencial de campo negativo de 10-20 mV de amplitude e 1-2 min de duração, propagando em uma velocidade de alguns milímetros por minuto. Como a retina é um tecido que apresenta grande demanda de energia, ela requer um aporte constante de glicose e oxigênio e, a hipoglicemia, condição que facilita o início da DA cortical em ratos de idade avançada, parece levar à morte de células da retina e perda da acuidade visual em camundongos.

Objetivo: Este trabalho tem como objetivo avaliar o impacto das variações na concentração de glicose sobre o desencadeamento e propagação das ondas de DA em retinas *ex-vivo* de pinto.

Métodos: Retinas de pinto de 7-10 dias pós-eclosão (n=10) foram mantidas *ex-vivo* em condições de normoxia por até 4 horas, variando-se isosmoticamente a concentração de glicose do meio (repondo NaCl). Uma onda de DA foi evocada quimicamente, aplicando-se 20mM de KCl de maneira focal na retina e foi detectada como um círculo opalescente que se expande a partir do ponto do estímulo. As áreas onde foram constatadas lesões foram processadas com iodeto de propídeo para verificar se havia processo de morte celular.

Resultados: Em condições de hipoglicemia (4mM), ondas de DA espontâneas aparecem primariamente na região da mácula e depois de pelo menos duas DAs espontâneas, uma lesão puntiforme, positiva para iodeto de propídeo aparece próxima ao sítio de início da DA. A adição de concentrações progressivamente maiores de NaCl em soluções hipoglicêmicas é capaz de diminuir (113mM e 126mM) a velocidade de propagação, ou mesmo de reverter (139mM) o disparo da DA.

Conclusão: Concluímos que a variação da osmolaridade secundária a diminuição da glicose é responsável pelo disparo de ondas de DA e conseqüente lesão tecidual da retina. **Palavras-**

Chave: Depressão Alastrante, Glicemia, Retina

Apoio Financeiro: NYS SCIRB, CNPq, FAPERJ

Searching for neuroprotective properties in plants of genus *Pterocaulon*

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Plants from the genus *Pterocaulon* (Asteraceae) possess microbicide and antiviral properties. Since these species contain high concentrations of polyphenolic compounds, which are known to have antioxidant properties, the present study has been designed to evaluate the *in vitro* effect of the methanolic fractions of two species of genus *Pterocaulon*, *P. balancae* and *P. alopecuroides*, on the iron-induced lipid peroxidation in brain of young male Wistar rats. Brain lipid peroxidation was induced by incubating cortical homogenates with iron (25 μ M) and ascorbate (250 μ M). Then, the neuroprotective action was evaluated by co-exposing the homogenates with iron combined with ascorbate plus crescent concentrations of the extracts (25, 50, 75, 100 mg/mL). The antioxidant properties were analyzed by assessing the levels of lipid peroxidation measured through the thiobarbituric acid reactive substances (TBA-RS), and the concentration of the non-proteinic sulfhydryl groups (NPSH). It was observed that *P. balancae* and *P. alopecuroides* reduced TBA-RS levels (up to 146 %) and the effect was dependent on the concentration (*P. balancae*: [$F_{(6,21)}=14.63$, $P < 0.001$; $\beta=0.89$, $P < 0.001$] and *P. alopecuroides* [$F_{(6,21)}=22.58$; $P < 0.001$, $\beta=0.89$, $P < 0.001$]). In addition, the oxidation of NPSH induced by ascorbate plus iron was significantly prevented (up to 62 %) by the co-incubation with the fractions (*P. balancae*: [$F_{(6,21)}=4.59$, $P < 0.01$] and *P. alopecuroides*: [$F_{(6,21)}=7.28$, $P < 0.001$]). These results strongly indicate that the methanolic fractions of extracts from plants of genus *Pterocaulon*, apart from microbicide and antiviral properties, also have neuroprotective activities.

Palavras-Chave: Antioxidant, *Pterocaulon*, Neuroprotection

Apoio Financeiro: CNPq, FAPESC and FINEP research grant "Rede Instituto Brasileiro de Neurociência (IBN-Net)" #01.06.0842-00.

LACTATIONAL EXPOSURE TO INORGANIC MERCURIC DID NOT INHIBIT ACETYLCHOLINESTERASE ACTIVITY IN CEREBRUM AND CEREBELLUM OF LACTATING RATS AND SUCKLING PUPS

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Objectives: Considering that inorganic mercury is efficiently excreted into maternal milk (Biol. Trace Elem. Res. 28:27, 1991), the aim of this study was to investigate the effects of maternal exposure to mercuric chloride (HgCl₂) during mid-lactation period on acetylcholinesterase (AChE) activity in cerebrum and cerebellum of lactating rats and suckling pups.

Methods: Lactating *Wistar* rats (dams) were subcutaneously exposed to 5 mg/kg/day of HgCl₂ from 8th to 12th day of lactation (Tox. Lett. 146:17, 2003; Life Sci. 81:1264, 2007). Suckling pups were exclusively exposed to mercury via maternal milk. One day after the last dose of exposure, dams and pups were weighed, anesthetized and sacrificed by decapitation. Cerebrum and cerebellum were removed, weighed and used for AChE activity determination (Biochem. Pharmacol. 7:88, 1961).

Results: A significant loss of body weight was observed in dams exposed to HgCl₂ during lactation ($p < 0.05$). Besides, it was observed a significantly lower body weight gain in pups indirectly exposed to HgCl₂ during lactation ($p < 0.05$). Lactational exposure to HgCl₂ did not alter absolute cerebrum and cerebellum weights of dams and pups ($p > 0.05$). Cerebrum-to-body weight ratio was significantly increased in dams (about 23%; $p < 0.05$) and pups (about 22%; $p < 0.05$) exposed to HgCl₂. Cerebellum-to-body weight ratio was significantly increased only in dams (about 22%; $p < 0.05$) exposed to HgCl₂. Moreover, our results showed that AChE activity of cerebrum and cerebellum from dams and suckling pups were not affected by HgCl₂ exposure during lactation ($p > 0.05$).

Conclusion: Our findings demonstrated that maternal exposure to inorganic mercury during lactation period did not alter AChE activity in cerebrum and cerebellum of dams, as well as, of indirectly exposed pups.

Palavras-Chave: inorganic mercury, lactation, acetylcholinesterase.

Apoio Financeiro: CNPq, CAPES and FIPE/UFSM.

Phospholipases A2 (PLA2s) toxins isolated from *Micrurus lemniscatus* coral venom induce neuronal injury in embryonic hippocampal cell culture

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Previously we determined the outcomes of hippocampal injections of four PLA2s isolated from *M. lemniscatus* venom. Convulsions and severe hippocampal loss were induced by these PLA2s. **Objectives:** To study the effects of PLA2s isolated from *M. lemniscatus* in hippocampal cell culture. **Method:** Neurons were dissociated from hippocampi of E18-E19 Wistar rat embryos, after treatment with trypsin 0,25% and deoxyribonuclease (0,15mg/ml) neurons were cultured in B27-supplemented Neurobasal Medium. Cultures were kept at 37°C in a humidified incubator in 5% CO₂. Cells were plated at a density of 200 neurons/mm². In order to evaluation the neuronal viability and quantify cell death MTT assay and release of lactate dehydrogenase (LDH) from cells into the cell culture media (at day 6) were measured 24 or 48 h after treatment with MI-8 or MI-9 (1.0 – 1000 ng/ml). Morphological features of neurons were quantified by ethidium bromide. An ultrastructural analysis was also performed. Nitric Oxide (NO) determination was made with the apparatus NOA™ 280. **Results:** MI-8 (100 and 1000 ng/ml), MI-9 (10, 100 and 1000 ng/ml) induced neuronal injury by apoptosis and necrosis. Cultured neurons survival was significantly decreased when compared with the control group (ANOVA and Dunnett test): control (25±5,9); MI-8 10,0 (4,8±3,3), 100,0 (1,1±1,8) or 1000,0 (1,8±3,1) ng/ml; MI-9 1,0 (17,2±13,8); 10,0 (17,3±6,6); 100,0 (7,8±2,2); 1000,0 (2,4±2,1) ng/ml. Loss of cell viability was demonstrated by the release of intracellular LDH to the incubation medium. MI-8 (1000ng/ml) increased the NO production in the culture media after 48h of incubation **Discussion:** MI-8 and MI-9 caused neuronal death by apoptosis in neuronal culture. It has been postulated that snake presynaptic PLA2s block nerve terminals by binding to neuronal membranes and by catalyzing phospholipid hydrolysis, producing lysophospholipids and fatty acids. These enhance fusion of synaptic vesicle with release of mediators and concurrent inhibition of vesicle fission and recycling. The toxicity induced by PLA2s studied herein could also be attributed, at least in part, to those mechanisms. These PLA2 can be a useful tool for exploring the death-signaling pathways of neurotoxicity.

Palavras-Chave: hippocampal cell culture; neuronal injury; PLA2 neurotoxin; *Micrurus lemniscatus* venom

Apoio Financeiro: FAPESP; CNPq; FUNDAP

C.05.080

Effects of hypoxia on neuronal phenotype and viability in organotypic cultures.

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Perinatal asphyxia occurs in approximately 0.3% full-term newborn babies and this percentage has not decreased despite medical advances. There are evidences indicating that neurosteroids are important in neurodevelopment showing neuroprotective effects. We studied the potential protective effect of allopregnanolone (AP) in vitro using organotypic cultures from neocortex, striatum and hippocampus. Organotypic cultures were prepared from P3–P5 rats following Stoppini protocol with modifications (Cereb cortex 13:11, 2003). After 6 days in vitro cultures were exposed to 5µM AP or vehicle for 24h then subjected to hypoxia for 1h in an anaerobic chamber (Billups-Rothenberg, Inc.) and incubated at 5% CO₂ and 95% air at 37°C for 24h.

Results: First, the changes previously observed in our animal model were evaluated. Immunocytochemistry and confocal microscopy showed an increase of GFAP signal in the studied brain areas after hypoxia. Western blot studies supported these results (striatum 116%; hippocampus 101%; cortex 82%, n=3 p<0.05). The presence of the NOS enzyme was studied by NADPH diaphorase technique. After hypoxia we observed an increase in NADPH positive neurons in the neocortex (66%) and the striatum (40%) but not in the hippocampus (n=3 p<0.05). Cell survival was analyzed by measuring LDH. After 1h of hypoxia, all cultures showed a non-significant increase of LDH which was greater after 24h of hypoxia (striatum-cortex co-cultures 140%, hippocampus 111% n=3 p<0.05). LDH levels have no changes after AP pretreatment before hypoxia. However tissues AP treated showed a non significant slight decrease of the GFAP signal compared to control cultures.

Conclusion: Organotypic culture is a suitable method to study the effects of hypoxia in vitro. 24h pre-treatment with 5µM of AP does not change neuronal viability and it does not prevent astroglyosis induced by hypoxia.

Palavras-Chave: hypoxia, organotypic cultures, allopregnanolone, GFAP

Apoio Financeiro: PIP-5349 UBACYT M020 and PICT-727

CARACTERIZAÇÃO DA PERCEPÇÃO VISUAL EM MULHERES SUBMETIDAS ÀS DROGAS ANTINEOPLÁSICAS

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Objetivo: As drogas antineoplásicas são utilizadas no tratamento sistêmico do câncer por interferir nos mecanismos de sobrevivência, proliferação e migração celular, impedindo a duplicação ou causando a morte celular. O mecanismo de ação destas drogas pode alterar os sistemas sensoriais e perceptivos do indivíduo quando submetidos ao seu uso. Este estudo teve como objetivo medir a sensibilidade ao contraste visual de mulheres que foram submetidas ao uso de drogas antineoplásicas. **Método:** Respeitando os aspectos éticos necessários para a realização da pesquisa, participaram dos testes 10 voluntárias, com acuidade visual corrigida ou não, sendo cinco voluntárias que usaram drogas antineoplásicas administradas em seis ciclos de mesmo componente químico, com término do último ciclo, em média, 4,8 meses antes da sessão experimental (Grupo Experimental, GE) e cinco voluntárias sem patologia identificável e sem uso das drogas antineoplásicas (Grupo Controle, GC). As medidas de limiar sensorial foram realizadas no Laboratório de Percepção, Neurociências e Comportamento, utilizando estímulos visuais de frequências espaciais de 0,25; 1; 4 e 8 cpg (ciclo por grau de ângulo visual). Os estímulos eram gerados em tons de cinza e apresentados num monitor de vídeo a 150 cm da tela. Usou-se um delineamento experimental com medidas repetidas e o método psicofísico da escolha forçada. O procedimento para medir a sensibilidade consistiu na apresentação sucessiva simples de pares de estímulos e as voluntárias foram orientadas a escolher sempre dentre eles aquele que continha a frequência espacial. O outro estímulo neutro foi sempre um padrão homogêneo com a luminância média de 42,4 cd/m². **Resultados:** A análise de variância para medidas repetidas não mostrou diferença significativa entre o limiar de contraste do GE e do GC ($F(1,118)=1,3758$; $P<0,243$). Entretanto, o teste post-hoc Tukey HSD revelou diferença significativa na frequência de 8 cpg ($p<0,001$), onde o GC foi da ordem de 1,4 vezes mais sensível do que o GE. **Conclusões:** Estes resultados sugerem que a sensibilidade ao contraste nas frequências espaciais mais altas é alterada pela ação de drogas antineoplásicas.

Palavras-Chave: Percepção Visual da Forma; Drogas Antineoplásicas; Quimioterapia; Limiar sensorial.

Apoio Financeiro: CNPq

Influence of trace metals on adenosine deaminase activity in zebrafish (*Danio rerio*) brain

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Introduction: Trace metals are ubiquitous pollutants that are introduced in the aquatic environment by anthropogenic sources. These substances induce a wide range of deleterious effects mainly at the central nervous system (CNS). Zebrafish is widely used in toxicological and biochemical studies. Nucleotides and nucleosides have important roles in energetic and signaling systems in the CNS. Adenosine concentrations are regulated by Adenosine deaminase (ADA) activity. **Objectives:** Considering the neurotoxic effect of trace metals, the aim of this work is verify the *in vitro* effect of these pollutants on ADA activity in soluble and membrane fractions of zebrafish brain. **Methods:** The preparation of brain membranes was performed J. Neurochem. 61:1685, 1993. Adenosine deaminase activity was determined J. Biol. Chem. 263: 5266, 1988. The specific activity was expressed as nmol of NH₃. min⁻¹. mg⁻¹ of protein. The data were presented as mean ± S.D. and analyzed by ANOVA, followed by Tukey test. *P* values = 0.05 were significant. **Results:** We tested the *in vitro* effect of mercury (Hg²⁺), cadmium, chrome, lead, zinc, copper, cobalt and manganese on soluble and membrane ADA activity in zebrafish brain. The control activity was 8,7±1,1 and 16,6±2 for soluble and membrane fractions, respectively. Among the metals tested only Hg²⁺ was able to influence the ADA activity. Inhibition was observed in soluble fraction varying from 85% to 93% (5 to 250 µM of mercury) and in membrane fractions varied from 21% to 26 % (50 to 250 µM). Dithiothreitol (DTT) was added to the reaction medium in order to verify if it could prevent or reverse the inhibition on ADA activity. The results demonstrated that the inhibition promoted by 5 µM of Hg²⁺ on soluble fraction were partially and totally recovery by DTT when added after and before the soluble fraction, respectively (59% and 101% of control activity). On membrane fractions DTT revert partially the inhibition promoted by 250 µ M of mercury when added before the enzyme (67% of control activity). **Conclusions:** This study suggests that the inhibition of ADA activity promoted by mercury can be one of the neurotoxic targets of this trace metal.

Palavras-Chave: Trace metals, Adenosine Deaminase, Zebrafish, Brain, Adenosine.

Apoio Financeiro: CAPES, (IBN-Net) # 01.06.0842-00, CNPq, FAPERGS, Propesq-UFRGS.

Desenvolvimento de Tumores Intracranianos: Tumores Metastáticos X Tumores Gliais

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Os tumores intracranianos são formados pelos tumores primários cerebrais ou por metástases. A grande maioria destas últimas é originária do pulmão (até 64%). Dos tumores gliais primários, o glioblastoma multiforme (Gbm) é a forma mais maligna.

No intuito de analisar as interações tumorais com o parênquima cerebral, desenvolvemos um modelo de xenotransplante de células tumorais inoculadas no caudado putâmen de ratos adultos a partir de orientações estereotáticas. Nosso objetivo é caracterizar o padrão histopatológico das lesões, a expressão das células tumorais para a proteína de filamento intermediário Vimentina e a expressão da Proteína Glial Fibrilar Acidica (GFAP) dos astrócitos cerebrais para a caracterização da formação da cicatriz glial (gliose reativa). Para mimetizar uma metástase, utilizamos as células GLC4, uma linhagem de tumor de pequenas células de pulmão. Para estudar um tumor primário, utilizamos linhagens de Gbm estabelecidas em nosso laboratório. Duas semanas após a inoculação destas células os animais foram sacrificados.

Na análise histopatológica, a lesão por GLC4 mostrou uma evidente massa tumoral com gliose reativa, edema, necrose tumoral, figuras mitóticas e aberrações celulares, características típicas deste tipo de lesão. Já a lesão por Gbm apresentou uma massa única com figuras gemistocíticas, alta taxa de mitoses, células fibrosas e necrose tumoral. Na análise por imunohistoquímica encontramos em ambas linhagens expressão diferenciada de Vimentina entre as células tumorais, como observado em cultura celular, conservando seu fenótipo indiferenciado. Encontramos aumento na expressão de GFAP nos astrócitos circundantes às lesões tumorais, característico da gliose reativa. Além disso, resultados preliminares sugerem que o fenômeno de astrocitose se dá de forma específica, ou seja, as células GFAP positivas apresentam maior expressão desta proteína quando há a presença de um tumor externo.

Desta forma, nosso modelo possibilita uma melhor compreensão sobre as interações dos tumores com o parênquima cerebral *in vivo*, mostrando que os tumores inoculados apresentam padrões muito semelhantes àqueles presentes nas lesões de pacientes humanos. **Palavras-Chave:** tumores intracranianos, metástase, glioblastoma, gliose reativa

Apoio Financeiro: CAPES, CNPq, FAPERJ

Manganese oxidation state mediates toxicity in Neurons: role of protector agents such as Ascorbic Acid and d,l-Lactate.

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Manganese (Mn) is essential, but can induce neurodegeneration at high level. Evidence suggests that this metal may be to inhibit cellular energy production, ultimately leading to increased radical formation (1). On the other hand, the role of the Mn oxidation state on toxicity is not well understood (2). For that reason, our study was to investigate the toxic effects of Mn(II)-Chloride and Mn(III)-Citrate in undifferentiated cultured cortical (CTX) and cerebellar granular (CGC) neurons, which were exposed to 0–1 mM, for 120 h, with or without ascorbic acid (AA) and d,l-lactate (L) at 120h. Mn(II) induced higher toxicity than Mn (III) in CTX, according to the MTT assays, EC₅₀ of 143,1 ± 61,9 mM and 597,6 ± 98,9 mM respectively; but in CGC toxicity was significantly superior than CTX (P<0.0001), to Mn(II) 25,3 ± 1,2 mM and Mn(III) 25,6 ± 10,4 mM. In CGC Mn species treatments produced a declining of the GSht level, 25 - 35%, at concentrations producing < 20 % of cell death; conversely, in CTX, both metal species increased GSht levels by 35 – 45 % at lower concentration, and producing a declining at higher concentrations. The co-treatment with AA and L offered protection to both metal species in the two cell models. These results suggest that CGC are more sensibly than CTX; that Mn species induced injured cell by multiple pathways (inhibition of cellular energy production, oxidative stress and other not evaluated in this work); which is more acute to Mn (II) in CTX; and finally chronic exposition the oxidation state of manganese plays an important role in mediating its cytotoxicity.

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Palavras-Chave: Manganese toxicity, CGC, CTX, Neuroprotection

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Anti- and pro-oxidant effects of resveratrol in astroglial cells

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Objectives: The redox active compound, resveratrol (*trans* 3,5,4',- trihydroxy stilbene) is a polyphenolic present in grapes and red wine and has been proposed to have beneficial effects in brain pathologies that may involve oxidative stress. The major objective of the present study was to examine the protective effects of resveratrol (100 mM), under two models of oxidative stress induced by hydrogen peroxide (H₂O₂), in C6 astroglial cells.

Methods: C6 glial cells were seeded (5 x 10³ cells/well) and cultured in DMEM (pH 7.4) supplemented with 5% fetal bovine serum (FBS), at 37°C/ 5 % CO₂/95 % air. Models of H₂O₂ insult: (I) 1 mM H₂O₂ for 0.5 h and (II) 0.1 mM H₂O₂ for 6 h. It was analyzed the index of DNA damage, assessed by the comet assay as well as DCF-DA oxidation, GSH content and lipid peroxidation measured by fluorimetric assay.

Results: In both models, resveratrol prevented oxidative DNA damage (50%, and 30%, in models I and II, respectively). However, in model I, resveratrol decreased DCF-DA oxidation (100±6.1 to 62±3.2 UF/mg protein, P<0.001) and lipid peroxidation (6.3±0.4 to 3.8±0.3 nmol/mg protein, P<0.001) and increased GSH content (about 61%) compared of control. In model II, resveratrol increased DCF-DA (100±6.1 to 138±7.9 UF/mg protein, P<0.001) and lipid peroxidation (7.1±0.3 to 12.1±0.6 nmol/mg protein, P<0.001) and decreased GSH (22 to 54%), suggesting a pro-oxidant effect.

Conclusions: Our results provide evidence that resveratrol may contributes to brain health in disorders involving oxidative stress. However, the dual effect of resveratrol (presenting anti- and pro-oxidant activity) suggests more caution to evaluate the therapeutic effects of this compound in brain disorders.

Palavras-Chave: Resveratrol, astroglial cells, hydrogen peroxide, comet assay

Apoio Financeiro: CNPq, PROPESq-UFRGS, FINEP/ Rede IBN 01.06.0842-00, FAPERGS.

Orally administered epigallocatechin gallate attenuates light-induced photoreceptor damage

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Objectives: EGCG, a major component of green tea, has a number of properties which includes an antioxidant effect and the modulation of cell survival/death genes. The aim of this study was to deduce whether inclusion of EGCG in the drinking water of albino rats attenuates the effect of a light insult (2200 lx, for 24 h) to the retina. **Methods:** Adult Wistar rats (n=24) were used and subdivided into three groups: a control group maintained under dim cyclic light and two groups submitted to a light-insult where EGCG was absent (light) or present (400 mg/kg /day) in their drinking water (light + EGCG). Functional and biochemical analysis of the retina was assessed by using electroretinography, histochemical, western blot and RT-PCR assays. **Results:** TUNEL-positive cells were seen in the outer nuclear layer of the retina, indicating the efficacy of the light insult in inducing photoreceptor degeneration. Moreover, Ret-P1 and the mRNA for rhodopsin were also significantly reduced (about 50%) as well as the amplitude of both the a- and b-waves of the electroretinogram (about 60%), showing that photoreceptors in particular are affected by light. Müller cells reactivity detected by an increase in protein/mRNA of GFAP was induced by the light insult. However, antigens associated with bipolar cells (PKC α), ganglion cells (Thy-1) and amacrine cells (GABA), appeared unaffected. The light insult also caused a change in the content of various proteins (caspase-3, caspase-8, PARP, Bad, and Bcl-2) involved in apoptosis. A number of the changes to the retina caused by a light insult were significantly attenuated when EGCG was in the drinking water. The reductions of the a- and b-waves and photoreceptor specific mRNAs/proteins caused by light were significantly less (about 10-20%; $p < 0.05$). In addition, EGCG attenuated about 20% ($p < 0.01$) the changes caused by light to caspase-3, BAD and PARP after 2 days but did not appear to significantly influence the light-induced up-regulation of GFAP protein/mRNA. **Conclusions:** It is concluded that orally administered EGCG attenuates the detrimental effect of light to the retina of albino rats where the photoreceptors are primarily affected.

Palavras-Chave: EGCG; Photoreceptor degeneration; Bad; Bcl-2; Caspase-3; Neuroprotection; ERG

Apoio Financeiro: CNPq, China Scholarship Council, University of Oxford

antioxidant EFFECTs OF Ascorbic Acid and pro-oxidant effects of quercetin on CATECHOL – INDUCED cytotoxicity to GL-15 GLIOBLASTOMA CELLS.

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ABSTRACT

Flavonoids are secondary plant phenolic metabolites with significant antioxidant and chelating properties. Catechol (CAT) is a metabolite of benzene that generates reactive oxygen species and quinones. The oxidative stress is involved in several diseases of the central nervous system. Ascorbic acid has shown scavenging properties and also inhibited quinones formation. **AIM:** In this study, we investigated the effects of quercetin and ascorbic acid on CAT-induced cytotoxicity to GL-15 glioblastoma cells.

METHODS: GL-15 cells were maintained in DMEM supplemented with 10% fetal calf serum, 2 mM L-glutamine, penicillin (100 IU/ml), and streptomycin (100 µg/ml). Cells were treated with CAT (3-1000 µM) for 72 h to determine the EC₅₀. Cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were also treated with CAT in the presence of quercetin (0.6-100µM) or ascorbic acid (20 – 3000µM) for 72 h. **RESULTS:** CAT was cytotoxic to GL-15 cells after 72 h (EC₅₀ = 252µM). Ascorbic acid (20-3000µM) and quercetin (0.6 -100µM) were not cytotoxic to GL-15 cells after 72h. The cytotoxicity induced by 300µM CAT was reverted by ascorbic acid (20-3000mM). However, the flavonoid quercetin (0.6-100µM) increased the cytotoxic effect of CAT.

CONCLUSIONS: The flavonoid quercetin increased CAT-induced cytotoxicity and ascorbic acid protected GL-15 glioblastoma cells *in vitro*.

Supported by Fapesb, Capes, CNPq

Palavras-Chave: Catechol, Flavonoids, Glia, Oxidative Stress

Apoio Financeiro: Fapesb, Capes e CNPq

Expression and Activation of the Nuclear Factor κ B in a Murine Model of Neurotuberculosis

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Introduction: Neurotuberculosis is the most severe form of tuberculosis (TB), particularly in immunocompromised patients. Experimental models of neuro-TB can help the comprehension of its physiopathology. Several mediators are involved in the molecular mechanisms of the infection and inflammation by mycobacteria, such as nuclear factor κ B (NF κ B). NF κ B activation, a cytoplasmic transcriptional factor that migrates to the cellular nucleus under stimuli, is involved with the modulation of the expression of various genes that are fundamental to immune and inflammatory responses.

Objectives: The objective here is to characterize the NF κ B production and activation in our murine model of neuro-TB.

Materials and Methods: Induction of meningitis and tuberculomas were possible with stereotaxic inoculation of Bacilo Calmette Guérin (BCG) in mice cerebellum. Diagnostic was confirmed by detection of alcohol-acid resistant bacilli (BAAR), in tuberculous lesions. One to six days; one, two, four and eight weeks after injection, mice were perfused and brains were paraffin-embedded. Sections (8 μ m) were incubated with anti-NF κ B primary antibody, biotinylated second antibody and an ABC kit which were detected using diaminobenzidine as chromogen.

Results: The subcellular location of NF κ B indicates its state: activated (nuclear) or latent (cytoplasmic). There was increased production and activation of NF κ B in the tuberculous lesions, it was characterized by its nuclear translocation from cytoplasm. We identified nuclear and cytoplasmic NF κ B expression in inflammatory cells within the subarachnoid space (meningitis) and in the cerebellar parenchyma (tuberculomas). We also observed NF- κ B expression in cells in the adjacent parenchyma and surrounding blood vessels.

Conclusions: Soluble mediators production (as NF κ B) by the host, producing in response to invasion of the pathogen in the CNS, has been involved in the pathophysiology of the neuro-TB, such as demonstrated in this experimental model. The final aim is to elucidate the physiopathology of this serious illness and understand the molecular events involved in the production of the lesions.

Palavras-Chave: tuberculosis, central nervous system, experimental model, nuclear factor κ B

Apoio Financeiro: CAPES

Neurotoxic effects of toxin IV-IV isolated from *Tityus serrulatus* scorpion venom

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The Brazilian scorpion *Tityus serrulatus* is the main cause of the scorpion accidents in this country. Its venom is composed by toxins with neurotoxic activity. The IV-IV is a toxin with a chromatographic profile similar to the TsTX toxin, one of the best studied toxins from the *T. serrulatus* venom, and its effect isn't known mainly in CNS. Our aim was to study the behavioral, electrographic and histopatologic alterations evoked by IV-IV toxin and to compare its effects with the ones caused by TsTX and to determine IV-IV toxin structure. Cannulas and electrodes were implanted in hippocampus of male Wistar rats (250g). One day after surgery, the animals were injected intra-hippocampally with 1µl of the IV-IV toxin (1.0mg/ml, n=11). After injection, behavioral and electrographic analyses were made during 4 hours. Seven days after, the animals had their brain removed and processed for the histological analyses. In order to elucidate the homology between IV-IV and TsTX toxins, the N-terminal sequencing and mass spectroscopy of the IV-IV toxin were made and compared with the TsTX toxin (dates obtained from the literature). The control group injected with 1µl of the ringers doesn't show behavioral and electrographic alterations and the number of cells in hippocampal region was: CA1 ipsilateral (i): 53.63±1.29, CA1 contralateral (c): 55.23±0.91, CA3i: 33.6±0.90, CA3c: 32.63, CA4i: 28.06±1.51 and CA4c: 29.43±1.27. The IV-IV toxin evoked postural loss in 9.0% of animals, paralysis of the members and yawnings in 18.1%, mastigatory moviment, lung edema and clonic convulsion in 45.4%, respiratory difficulty in 54.4%, myoclonus in 72.7%, wet dog shakes in 100% and tonic convulsion with death in 36.3% of the animals. In electrographic record were observed intense and moderate discharges and spikes. Neuronal loss in CA1i (43.66±2.65), CA1c (55.23±0.91) and CA3i (33.6±0.90) were observed. Four different amino acids were observed in the N-terminal region of the toxin. A molecular mass of 6985 Da was determined, i.e 92 Da less that TsTX toxin. The IV-IV toxin has homology with TsTX toxin and it may be more potent. The IV-IV toxin has neurotoxic effect in hippocampus.

Palavras-Chave: scorpion venom, toxin, *Tityus serrulatus*, hippocampus, seizure

Apoio Financeiro: CAPES, Fundação Butantan

Partial blockage of the effects of toxin IV-IV from *Tityus serrulatus* venom by glutamatergic antagonists.

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Scorpions from the genus *Tityus* are the more dangerous in Brazil. Scorpion toxins act on ionic channels releasing neurotransmitters among them glutamate. There are no studies about IV-IV toxin from *T. serrulatus* venom. Our aim was to analyze if antagonists of glutamate receptors can block the effects of IV-IV toxin. Cannulas and electrodes were implanted in male Wistar rats (250g). They were injected with toxin (0.75µg/µl) and antagonists (n=6-8): MK-801 1mg/Kg, CNQX 2µg/µl, AP3 100µg/2µl, MCPG 0.2mg/3µl or AP-5 1µg/µl, and observed for 4 hs. After 7 days, the rats had the brains removed and processed for histology. The control group injected with ringer (1µl) showed no epileptiform changes. Its cell number in hippocampus was: CA1 ipsilateral (i): 53.6±1.3; CA1 contralateral (c): 55.2±0.9; CA3i: 33.6±0.9; CA3c: 32.6±1.3; CA4i: 28.0±1.5 and CA4c: 29.4±1.3. MK-801+toxin caused postural loss (PL) in 75%; myoclonus (M) in 37,5%; paralysis of the members (PM), mastigatory moviment (MM), respiratory difficult (RD), wet dog shakes (WDS) and death in 12,5% of the animals and evoked electrographic (EEG) changes like intense (ID) and moderate discharges (MD) and spikes (S). Neuronal loss occurred in CA1c (51.7±0.9 cells). CNQX+toxin caused MM in 33.3%, M in 50%, WDS in 100% and yawnings (Y), RD, lung edema, tonic convulsion and death in 16,6% of the rats and EEG changes like MD, and S. Neuronal loss occurred in CA3i (26.9±0.9), CA3c (29.4±1.0) and CA4i (24.5±0.6). AP-3+toxin caused Y and M in 33% and WDS and MM in 83.3% of the rats. No EEG changes were observed. Neuronal loss was observed in CA1i (49.3±2.3) and CA1c (51.8±0.8). MCPG+toxin caused MM in 33.3% and WDS in 66.6% of the rats. ID, MD and S were observed in EEG. Neuronal loss occurred in CA3i (26.9±1.7), CA3c (29.5±2.3), CA4i (26.0±0.9); and CA4c (26.3±2.7). AP-5+toxin caused Y in 33.3%, MM in 66%, WDS in 100% and tonic-clonic convulsion with death in 16.6% of the rats. ID was observed in EEG. Neuronal loss occurred in CA1i (49.5±1.4), CA1c (51.6±1.2), CA3i (29.3±1.7), CA3c (31.3±0.8). The antagonists block partially the effects evoked by toxin and the metabotropics seem to be more efficient than the ionotropics.

Palavras-Chave: *Tityus serrulatus*, antagonists, scorpion toxins, glutamate

Apoio Financeiro: CAPES, Fundação Butantan

DESEMPENHO MOTOR DE RATOS ISQUÊMICOS APÓS TRATAMENTO COM CÉLULAS-TRONCO DA MEDULA ÓSSEA E BLOQUEIO MICROGLIAL COM MINOCICLINA.

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Objetivos: Avaliar se o tratamento com células-tronco da medula óssea associado ao bloqueio da ativação microglial utilizando minociclina melhora o desempenho motor de ratos adultos submetidos à isquemia focal do córtex motor. **Métodos:** Os animais foram submetidos à isquemia focal do córtex motor através de microinjeção do peptídeo vasoconstritor endotelina-1. Os seguintes grupos experimentais foram delineados: ratos tratados com injeção endovenosa de solução salina estéril 24 horas após a lesão (N= 6, grupo 1); ratos tratados com 5×10^6 de células-tronco (N= 5, grupo 2); ratos tratados com 5×10^6 de células-tronco concomitante com tratamento com minociclina (25-50 mg/Kg) para o bloqueio microglial (N=6, grupo 3). O desempenho motor dos animais foi avaliado através dos testes comportamentais de remoção do adesivo modificado e do campo aberto, 24h, 72h e 7 dias após a isquemia. Os animais foram anestesiados e perfundidos com solução salina 0,9% e paraformaldeído a 4% no tempo de sobrevivência de 7 dias. **Resultados:** As médias e os desvios padrão para o teste do campo aberto dos grupos 1, 2 e 3, 24h após o início do tratamento, foram, respectivamente, 235 ($\pm 134,72$); 333 ($\pm 120,65$) e 515 ($\pm 189,66$) cm ($p > 0,05$). A análise estatística (ANOVA de um critério) seguida de teste de hipótese (Bonferroni) realizada não demonstrou diferença significativa entre os grupos neste teste. Para os grupos 1, 2 e 3 os resultados do teste de remoção do adesivo foram, respectivamente, 0,56 ($\pm 0,27$), 0,77 ($\pm 0,51$) e 0,92 ($\pm 0,39$) para o tempo de sobrevivência de 24h após isquemia; 0,64 ($\pm 0,29$), 0,72 ($\pm 0,07$) e 0,88 ($\pm 0,55$) para o tempo de sobrevivência de 72h e, 0,83 ($\pm 0,20$), 0,85 ($\pm 0,36$) e 1,70 ($\pm 1,05$) sete dias após a indução isquêmica. Esta última medida demonstrou ser significativa ($p < 0,05$, ANOVA-Bonferroni). **Conclusão:** Os resultados demonstram melhora no desempenho motor dos animais tratados com células-tronco e minociclina em comparação ao grupo tratado apenas com células-tronco. Estes resultados sugerem que a ativação microglial dificulta os efeitos terapêuticos de células-tronco da medula óssea transplantadas na fase aguda da resposta inflamatória durante o processo isquêmico.

Palavras-Chave: AVC isquêmico, Células-tronco, Medula óssea, Minociclina, Comportamento

Apoio Financeiro: Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq - Brasil

Efeito da dieta hiperlipídica sobre a susceptibilidade de células hipocâmpais ao dano oxidativo induzido pelo peróxido de hidrogênio

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O efeito da composição da dieta sobre a vulnerabilidade cerebral ao dano oxidativo tem sido pouco investigado. O nosso objetivo foi analisar os efeitos de uma dieta hiperlipídica (42% de lipídeos) sobre a suscetibilidade de células hipocâmpais ao dano oxidativo induzido pelo peróxido de hidrogênio (H₂O₂) avaliado através da viabilidade celular. Ratos Wistar receberam durante o período pré-natal e até os 6 meses de idade uma dieta hipoprotéica (7%). Durante os 6 meses seguintes, os animais foram divididos, um grupo permaneceu hipoprotéico (desnutrição protéica) e outro grupo recebeu uma dieta hiperlipídica (42% de lipídeos). O grupo controle recebeu dieta normoprotéica (25%) durante todo o período. Os ratos foram decapitados, os cérebros foram dissecados e os hipocâmpos, imediatamente, fatiados em “chopper”. As fatias hipocâmpais do mesmo animal foram divididas em duas placas: sem H₂O₂ e expostas ao H₂O₂. Foi realizado o ensaio de avaliação da viabilidade celular (determinada pela redução do brometo de 3-[4,5-dimetiltiazol-2-il]-2,5-difeniltetrazólio, MTT). As fatias hipocâmpais dos animais submetidas à dieta hipoprotéica e à dieta hiperlipídica expostas ao H₂O₂ apresentaram uma redução na atividade mitocondrial comparadas ao controle (cerca de 20%, Mann Whitney, ambos p < 0,01). Não houve diferença significativa na redução do MTT entre fatias obtidas de animais normonutridos submetidas ou não ao H₂O₂. Fatias de animais submetidas à dieta hiperlipídica apresentaram um aumento no delta da redução do MTT em fatias expostas ao H₂O₂ e não expostas de aproximadamente 60% (normo = 0,0333 ± 0,01; hiper = 0,0536 ± 0,01; teste T, p=0,0123), enquanto que a desnutrição protéica não alterou este parâmetro. A exposição à dieta hiperlipídica aumenta a susceptibilidade hipocâmpal à injúria oxidativa induzida pelo peróxido de hidrogênio. **Palavras-Chave:** Susceptibilidade ao dano oxidativo; dieta hipoprotéica; dieta hiperlipídica; fatias hipocâmpais; ratos

Apoio Financeiro: PIBIC CNPq/UFRGS; BIC/FAPERGS; BIC/UFRGS.

TRATAMENTO COM INIBIDOR DE CALPAÍNA PROMOVE NEUROPROTEÇÃO DE CÉLULAS GANGLIONARES DA RETINA E DE FIBRAS DO NERVO ÓPTICO DE RATOS APÓS ESMAGAMENTO.

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O nervo óptico é um modelo experimental ideal para estudo dos tractos do sistema nervoso central (SNC). A sua localização anatômica facilita a realização de lesões experimentais (esmagamento ou transecção) “in vivo”, e o estudo das mesmas permite entender e manipular as lesões degenerativas do sistema nervoso central. A degeneração Walleriana, que ocorre em consequência dessas lesões, é provocada pelo aumento de cálcio intracelular e isto leva a ativação de proteases neutras, chamadas calpaínas. Em trabalho anterior de nosso grupo, com gambá *Didelphis aurita*, foi observado que o inibidor exógeno da calpaína foi capaz de oferecer neuroproteção às fibras do SNC após lesão por esmagamento.

OBJETIVOS: avaliar se após o esmagamento do nervo óptico de ratos Wistar, o inibidor exógeno de calpaína é capaz de realizar uma proteção similar àquela observada em nosso trabalho anterior, e prevenir a morte celular por apoptose das células ganglionares da retina. **MÉTODOS:** ratos tiveram os nervos ópticos submetidos ao esmagamento e num deles foi aplicado o inibidor da calpaína localmente. Após 4 dias, as fibras nervosas foram analisadas através de microscopia óptica e eletrônica de transmissão; e a morte celular na camada de células ganglionares foi avaliada através da técnica de TUNEL, após 9 dias da cirurgia. **RESULTADOS:** a análise qualitativa por Microscopia Óptica e Eletrônica mostrou que o grupo tratado com o inibidor da calpaína apresentou preservação da citoarquitetura do nervo óptico, quando comparados com o grupo que recebeu apenas o veículo. Na análise da morte celular por apoptose observou-se 50% de proteção no grupo tratado com o inibidor de calpaína quando comparado com o grupo não tratado. No grupo tratado houve 0,32% de células TUNEL positivas sobre o total de células ganglionares da retina, enquanto no grupo não tratado houve 0,64% de células TUNEL positivas.

CONCLUSÃO: Estes resultados sugerem que o inibidor de calpaína é capaz de exercer um papel neuroprotetor sobre as células ganglionares da retina e nervo óptico de ratos após uma lesão por esmagamento, abrindo a possibilidade de novas estratégias terapêuticas em lesões de SNC.

Palavras-Chave: nervo óptico, neuroproteção e inibidor de calpaína

METHYLMERCURY INTOXICATION INDUCES CHANGES IN MOSAIC AND DENSITY OF NADPH DIAPHORASE POSITIVE CELLS IN THE GANGLIONAR LAYER IN CHICK RETINA

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Objective

Retinal tissue represents an important target of methylmercury (MeHg) toxicity, however, few studies have described its effect in the retinal ontogenesis (Braz.J.Med.Biol.Res.39:415, 2006). The nitreergic system have important role on the retinal development and previous studies showed that MeHg alters NADPH-diaphorase pattern in retinal cultures. Thus, the aim of this study was to characterize the effect of MeHg on the spatial distributions and density of NADPH diaphorase positive cells (NADPHd⁺) in chick retinas in different periods of development.

Methods and results

Retinas of White Leghorn chick were obtained from embryonic day 15 (E15) and posthatching day 1 (P1). Control and intoxicated tissues (MeHg 5 μ M for 3 hours) were fixed for 30 min in 4% paraformaldehyde. Whole-mount retinas were incubated in NADPH-diaphorase histochemistry buffer for 4 hours at 37 °C. Only right eyes were used to analyze the neurons of ganglion cell layer (GCL). The raw images of the NADPHd⁺ neurons were digitalized. The characterization of cellular mosaic was done by Density Recovery Profile (DRP) (Vis.Neurosci.6:95, 1991). Our results demonstrated in E15 a decrease in the density of NADPHd⁺ in GCL when compared with control. However, no significant changes were detected in P1. Topographic maps showed high density only in the dorsal region as demonstrated by DRP analysis. Mosaic results demonstrated significant variation in packing factor just in E15, with 0.21 ± 0.04 for MeHg 5 μ M and 0.27 ± 0.01 for control (t test, $p < 0.05$). DRP results for cellular mosaic in P1 retina did not showed any difference (t test, $p > 0.05$).

Conclusion

MeHg changed the density and distribution of the mosaic of NADPHd⁺ neurons in GCL of chick embryos retina, suggesting that embryonic retina was more affected by mercury intoxication than the hatched retina.

Palavras-Chave: Methylmercury, retina, NADPH diaphorase, mosaic

Apoio Financeiro: PARD-ufpa/CNPq; CNPq; CAPES; FINEP; iBNET

AVALIAÇÃO A LONGO PRAZO DO EFEITO NEUROPROTETOR DO FATOR NEUROTROFICO CILIAR (CNTF) CONJUGADO A DOMÍNIO DE TRANSLOCAÇÃO DE PROTEÍNA SOBRE MOTONEURÔNIOS

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Objetivos: O CNTF tem ação neuroprotetora sobre motoneurônios (MN). Contudo, efeitos colaterais impedem seu uso clínico. Previamente mostramos que CNTF ligado a domínio de translocação de proteína (TAT-CNTF) tem ação neuroprotetora sobre MN após axotomia em ratos neonatos (P2) e não produz efeitos colaterais no tratamento por 5 dias (d) (P2-P7). Presentemente investigamos se a ação neuroprotetora da TAT-CNTF administrada de P2-P7 é suficiente para garantir a sobrevivência dos MN até a idade de 60d e se o tratamento por 15d (P2-P17) não reduz o ganho de peso corporal (RPC) observada nos ratos tratados por 5d com CNTF.

Métodos e Resultados: Ratos Wistar (P2; n=5/grupo) tiveram o nervo ciático esquerdo (NCE) esmagado e foram tratados com CNTF (1,2mg/g; 5d), TAT-CNTF (1,2mg/g; 5d e 15d) ou PBS (5d e 15d). Após 20, 30, 45 e 60d foi avaliada a recuperação funcional motora (RFM; Walking Track Test-WTT). Após o último WTT (60d) os ratos tiveram o NCE seccionado e o coto proximal exposto a uma solução de Amina Dextrano Biotinilado (BDA). Após 7d a intumescência lombar foi removida e processada para revelação histoquímica dos MN contendo BDA (MN+). O número total de MN+ foi obtido a partir da sua contagem corrigida (Abercrombie). Os ratos do grupo TAT-CNTF apresentaram RPC com relação ao grupo PBS a partir do 9º dia de tratamento, porém inferior ao produzido pelo CNTF a partir do 2º dia. Em ambas condições houve reversão desse efeito, porém esta ocorreu mais precocemente nos ratos do grupo TAT-CNTF (P25) quando comparados ao grupo CNTF (P32). O WTT revelou que a RFM foi semelhante entre os ratos dos grupos CNTF e TAT-CNTF e 50% superior à dos ratos controle após 60d da lesão. O número total de MN+ foi também semelhante entre os ratos dos grupos CNTF (399±11) e TAT-CNTF (400±19, 5d; 409±11, 15d) e maior que dos ratos controle (254±8, 5d; 286±9, 15d; p<0,01).

Conclusões: Nossos resultados confirmam a ação neuroprotetora da TAT-CNTF e evidenciam que mesmo quando administrada por um período prolongado não induz efeitos colaterais. Estes dados reforçam a hipótese de que a TAT-CNTF pode ser uma importante estratégia no tratamento de doenças neurodegenerativas e neurotraumas.

Palavras-Chave: CNTF, TAT-CNTF, motoneurônios, neuroproteção, neonato

Apoio Financeiro: FAPESP (04/14210-8; 05/54613-7) e CNPq (47.3909/2004-9)

Efeito do alumínio *in vitro* sobre a captação de glutamato em fatias de hipocampo e córtex de ratos adultos.

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Objetivo: Investigar os efeitos do alumínio *in vitro* sobre a captação de glutamato em fatias de hipocampo e córtex de ratos adultos. **Métodos:** Ratos Wistar machos adultos de 60 dias foram decapitados, o cérebro foi imediatamente removido e submergido em Solução Salina Balanceada de Hanks (HBSS) a um pH 7,2. Fatias coronais de córtex e hipocampo a 400µM de espessura foram obtidas e transferidas para placas de 24-well e pré-incubadas em meio HBSS a 35° C (em banho-maria) por 60 min para estabilização. Após as fatias foram divididas em quatro grupos: grupo controle e 3 grupos tratados com cloreto de alumínio (concentração de 1mM, 10µM e 100µM). As fatias foram pré-incubadas em meio HBSS a 35° C por 60 min. na presença e ausência de alumínio e após foram incubadas durante 5 e 7 min (hipocampo e córtex, respectivamente) com Glu-H3. A radioatividade foi quantificada por cintilação e a dosagem das proteínas pelo realizada pelo método de Peterson. **Resultados:** O alumínio, nas concentrações de 1mM e 100µM, não alterou significativamente a captação de glutamato nas duas estruturas estudadas. No entanto, na concentração de 10µM houve uma diminuição significativa de aproximadamente 50% na captação de glutamato em hipocampo (p= 0,015) e de 55% em córtex (p< 0,001) quando comparados ao grupo controle. **Conclusão:** Nossos resultados indicam que o alumínio, em baixas concentrações, é capaz de alterar *in vitro* a captação de glutamato em fatias de hipocampo e córtex de ratos adultos.

O FATOR NEUOTRÓFICO CILIAR LIGADO A DOMÍNIO DE TRANSLOCAÇÃO DE PROTEÍNA EXERCE AÇÃO NEUROPROTETORA SOBRE NEURÔNIOS SENSITIVOS DOS GÂNGLIOS DAS RAÍZES DORSAIS DE RATOS NEONATOS

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Objetivo: O Fator Neurotr fico Ciliar (CNTF) ligado a dom nio de transloca  o de prote na (TAT-CNTF) possui a  o neuroprotetora sobre motoneur nios e n o produz efeitos colaterais associados ao CNTF. Neste estudo investigamos o efeito da TAT-CNTF sobre neur nios sensitivos (NS) dos g nglios das ra zes dorsais (GRD) L4 e L5 ap s o esmagamento do nervo ci tico de ratos neonatos com 2 dias de vida (P2).

M todos e Resultados: Ratos Wistar (P2; n=5/grupo) tiveram o nervo ci tico esquerdo (NCE) esmagado e foram tratados por 5 ou 15 dias (d) com CNTF (1,2mg/g; 5d), TAT-CNTF (1,2mg/g; 5d e 15d) ou PBS (5d e 15d). Ap s 20, 30, 45 e 60d foi avaliada a recupera  o funcional sensitiva (RFS) atrav s da medida da intensidade do est mulo limiar para a resposta de retirada da pata (LRR) provocada pela estimula  o el trica na regi o plantar. Ap s a  ltima avalia  o (60d) o NCE foi seccionado e o coto proximal exposto a uma solu  o de Amina Dextrano Biotinilado (BDA). Ap s 7d os GRD L4 e L5 foram dissecados e processados para revela  o histoqu mica dos NS contendo BDA (NS+). O n mero total de NS+ foi obtido a partir da sua contagem corrigida pela f rmula de Abercrombie. A avalia  o do LRR revelou que a RFS foi semelhante entre os ratos dos grupos CNTF e TAT-CNTF e 50% inferior   dos ratos do grupo controle em todos os tempos analisados. O n mero total de NS+ foi semelhante entre os ratos dos grupos CNTF tratados por 5d (L4:1568 79; L5:2366 151) e TAT-CNTF tratados por 5d (L4:1476 124, L5:2340 158) ou 15d (L4:1626 118; L5:2045 221). Estes valores do n mero total de NS+ foram superiores aos computados nos correspondentes g nglios dos ratos controle tratados com PBS por 5d (L4:497 17; L5:813 22) ou 15d (L4:517 12; L5:830 38), respectivamente (p<0,01).

Conclus es: Nossos resultados mostraram que a CNTF   capaz de proteger neur nios sensitivos axotomizados em ratos neonatos e que a adi  o de um dom nio de transloca  o de prote na a essa mol cula (TAT-CNTF) n o alterou essa capacidade. Al m disso, ambas as mol culas parece ter estimulado a regenera  o axonal sensitiva contribuindo assim para uma melhor recupera  o funcional sensitiva. **Palavras-Chave:** CNTF, TAT-CNTF, g nglio da raiz dorsal, neuroprote  o, neonato

Apoio Financeiro: FAPESP (04/14210-8; 05/54613-7) e CNPq (47.3909/2004-9)

Indomethacin-loaded nanocapsules treatment protects against oxygen and glucose deprivation in rat hippocampal organotypic cultures

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Objectives: The blood-brain barrier (BBB) limits the penetration of drugs into the CNS. Biodegradable polymeric nanoparticles could be used as potential drug carriers across the BBB, an important requirement when one searches the development of novel drug for neurodegenerative disease. The brain is highly dependent on continuous blood flow for the supply of oxygen and glucose, for this reason ischemic insults result in severe cellular degeneration. Here we investigated the neuroprotective effect of indomethacin-loaded nanocapsules (IndOH-NC) in an *in vitro* model of ischemia.

Methods: Nanocapsule suspensions were prepared by interfacial deposition of preformed polymers. With the purpose to evaluate the cytotoxic effect as well as the efficiency of these formulations we used organotypic hippocampal slice cultures treated with drug-unloaded nanocapsules (NC) as vehicle control or indomethacin-loaded nanocapsules (IndOH-NC). In addition, we evaluated whether these formulations exert a protective effect when cultures are exposed to 60 minutes of oxygen-glucose deprivation (OGD). For this, formulations were added to the cultures for 2h before OGD and maintained for 24h after OGD. Cellular death was quantified by measuring of propidium iodide (PI) uptake, a marker of dead cells.

Results: Our results show that NC and IndOH-NC (50 or 100 μ M) did not cause a significant PI incorporation in hippocampal slices, indicating that these formulations are not toxic (Control: 3.32 ± 2.31 ; NC: 3.63 ± 1.49 ; IndOH-NC: 2.68 ± 2.18 ; n=9 for 50 μ M and Control: 1.49 ± 0.57 ; NC: 2.54 ± 2.80 ; IndOH-NC: 1.27 ± 1.46 ; n=7 for 100 μ M). When 100 μ M of IndOH-NC was added to the hippocampal cultures exposed to OGD we observed a decrease in cell death compared with cultures which did not receive IndOH-NC (Control: 1.49 ± 0.57 ; NC: 2.54 ± 2.80 ; IndOH-NC: 1.27 ± 1.46 ; OGD: 64.57 ± 5.91 ; OGD+NC: 57.5 ± 11.79 ; OGD+IndOH-NC: 30.81 ± 11.97 ; n=7, p<0.001).

Conclusion: Although further studies are necessary to evaluate the mechanisms involved in the protective effect observed, these data suggest that indomethacin-loaded nanocapsules could offer new potentially highly effective strategies for the treatment of brain ischemia.

Palavras-Chave: indomethacin-loaded nanocapsules, organotypic culture, ischemia, neuroprotection

Apoio Financeiro: Supported by Capes, CNPq and Rede Brasil-França CNPq/MCT.

Viral encephalitis, enriched environment and neuroprotection: behavioral and neuropathological changes after arbovirus piry infection in adult albino swiss mice.

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Objectives: Environmental effects on viral encephalitis evolution have not been described in detail. We investigate the effects of enriched environment on viral encephalitis progression induced by the arbovirus Piry (PIRYV) in adult albino Swiss female mice. Behavioral changes and neuropathological features were compared during disease progression with emphasis in the olfactory pathways and hippocampus.

Methods: Fifty-nine 2 months old mice, were kept in impoverished environment (IE, n=31) or in enriched environment (EE, n=28) for three months and then submitted to the following tests: open field (OF), burrowing (B), dark/light box (DLB) and elevated plus maze (EPM). After these behavioral tests, 5 µl of viral infected brain homogenate (10^{-5} v/v) or of normal brain homogenate were nasally instilled in the infected (PIRYV, n=34) and control (C, n=25) groups respectively. All subjects from different conditions were tested again after 20 (IEPIRYV, n=10, IEC, n=7, EEPIRYV, n=5, EEC, n=7) or 40 (IEPIRYV, n=8, IEC, n=6, EEPIRYV, n=11, EEC, n=5) days post-infection (PI) in OF, B, DLB and EPM. After behavioral tests, all subjects were sacrificed and fixed brain sections were immunolabeled for virus antigens or histochemically reacted to detect biotinylated *Lycopersicum sculentum* lectin, to detect activated microglia.

Results: Behavioral changes were observed in the 20th day P.I. in DLB, OF and B tests (ANOVA, $p < 0.05$). An increased time spent on the light side of the box and a reduction in the exploratory and B activity in the 20th but not in the 40th day PI were detected only in IEPIRYV. Microglial activation was mainly present along the olfactory pathways and in CA3 ventral and dorsal in the infected group with higher intensity in IE than in EE. Coincidentally with behavioral findings virus antigens were detected in the brain parenchyma early (20th day) in the disease but not late (40th day). At 40 days PI, microglial activation was still present in IEPIRYV but not EEPIRYV.

Conclusion: Thus, environmental enrichment seems to protect infected animals against viral induced behavioral changes via reduction of microglial activation. Molecular mechanisms associated to neuroprotection remain to be investigated.

Palavras-Chave: Viral encephalitis, enriched environment, neuroprotection, behavior, arbovirus Piry, albino Swiss mice.

ANTI OXIDANT ACTIVITY OF DICHALCOGENIDE IN RAT BRAIN

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ABSTRACT

Objective:

Organoselenide compounds have been documented as promising pharmacological agents against a number of diseases associated with oxidative stress. This has elicited an increased interest in the synthesis of potentially bioactive compounds, including novel organic chalcogens. In the present study we appraised the antioxidant potential of organic chalcogen, measuring the ability of the compounds to inhibit the formation of thiobarbituric acid reactive species (TBARS) induced by the neurotoxic agents Fe(II) and the thiol peroxidase mimetic activity of diphenyl diselenide (PhSe)₂, Diphenyl ditelluride (PhTe)₂, dinaftal diselenide (NafSe)₂, dinaftal ditelluride (NafTe)₂ and 1-(2-(2-(2-(1-aminoethyl)phenyl)diselanyl)phenyl)ethanamine.

Methods: The standard assay for thiobarbituric acid-reactive substances (TBARS) was performed at 37°C, using spectrophotometry to quantify the malondialdehyde (MDA) content (Anal Biochem.95:351, 1979). While the thiol peroxidase activity was determined by using thiophenol assay (*J. Am. Chem. Soc* 2557: 116, 1994).

IC₅₀ determination

The IC₅₀ was calculated by the method of Dixon and Webb (1964). Data are the means of four to six experiments.

Results

The present results demonstrate that the order of inhibition was in the order of (PhTe)₂ > (NafTe)₂ > (NafSe)₂ > 1-(2-(2-(2-(1-aminoethyl)phenyl)diselanyl)phenyl)ethanamine >> (PhSe)₂. While the order of Thiol Peroxidase were 1-(2-(2-(2-(1-aminoethyl)phenyl)diselanyl)phenyl)ethanamine >> (NafTe)₂ > (PhTe)₂ > (PhSe)₂ > (NafSe)₂.

Conclusion:

The present results demonstrated that organic chalcogen can be considered potential antioxidant compounds. Among them the most potent was 1-(2-(2-(2-(1-aminoethyl)phenyl)diselanyl)phenyl)ethanamine.

Palavras-Chave: Dichalcogenide, rat brain, , antioxidant, iron, TBARS,

Apoio Financeiro: Supported by CNPq/TWAS

Neuroprotección por quercetina: papel de la modulación del estado redox celular tiol-dependiente

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Estudios epidemiológicos indican que los flavonoides de la dieta pueden evitar y/o retrasar la aparición de diversas neuropatologías. Sus propiedades neuroprotectoras pueden deberse a: su capacidad atrapadora de radicales libres, quelación de metales, modulación de receptores y enzimas, e interacción con cascadas intracelulares. Recientemente, se ha postulado que algunos flavonoides también pueden inducir la expresión de enzimas detoxificantes y antioxidantes.

Los sistemas de glutatión y tiorredoxinas son los sistemas antioxidantes celulares tiol-dependientes mayoritarios, y ocupan un importante papel en los procesos de protección y detoxificación celular. Así, compuestos capaces de modular los niveles fisiológicos de estos tioles resultan de gran interés por su potencial como protectores celulares.

El objetivo de este trabajo es profundizar en los mecanismos de neuroprotección del flavonoide quercetina, analizando su capacidad moduladora del estado redox del glutatión celular (GSH/GSSG) y de la tiorredoxina mitocondrial ($\text{Trx2}^{\text{red}}/\text{Trx2}^{\text{ox}}$).

Se utilizaron cultivos primarios de neuronas cerebelares de rata tratados con quercetina 25mM, a los que luego de 24 hs, se les provocó un daño oxidativo con peróxido de hidrógeno (H_2O_2) 60mM. Se evaluó la viabilidad celular (por MTT), los niveles de glutatión (HPLC con detección por fluorescencia), y el estado redox de Trx2 (Redox Western blot) a diferentes tiempos durante 24hs post-daño oxidativo.

La adición de H_2O_2 a cultivos no tratados con quercetina disminuyó significativamente la viabilidad celular (24% respecto al control) y modificó transitoriamente el estado redox de GSH/GSSG (+33 mV a las 4 hs). El pretratamiento con quercetina aumentó los niveles de GSH total (152,6% respecto al control), y previno la muerte neuronal (83,6% respecto al control) y la alteración del estado redox de GSH/GSSG inducidas por H_2O_2 . Resultados preliminares indican que en los tiempos evaluados la quercetina no previene la oxidación de la Trx2 provocada por H_2O_2 .

Estos resultados aportan evidencias sobre la modulación del estado redox celular tiol-dependiente como mecanismo de neuroprotección por quercetina.

Palavras-Chave: flavonoides, neuroprotección, glutatión, tiorredoxina 2, estrés oxidativo

Apoio Financeiro: PEDECIBA

Novel modular recombinant gene therapy vectors for acute CNS injuries

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Objectives: Successful introduction of therapeutic genes into the central nervous system (CNS) requires the further development of efficient transfer vehicles, which avoid viral vector-dependent adverse reactions while maintaining high transfection efficiency. The rational combination of modular domains is an emerging strategy for the development of modular proteins for gene delivery. Here, we explore the capability of this type of vectors for the in vivo gene delivery to the postnatal rat CNS and their potential as vectors for neuroprotective gene therapy after acute excitotoxic injuries. **Methods:** The modular vector termed NLSCt composed of beta-galactosidase with the insertion of a nuclear localization motif, and integrin targeting RGD motif and a polylysine tail, was injected into the normal or excitotoxically lesioned postnatal cortex and transgene expression, lesion volume, inflammation and neurological evaluations were performed. **Results:** Transient transgene expression was observed both in the excitotoxically injured and non-injured brain. In the injured brain, an increased and widespread expression occurred in the entire lesioned area and retrograde transport of the vector towards distant thalamic nuclei and transgene expression were observed. Neurons, astrocytes, microglia and scattered endothelial cells expressed the transgene. No acute inflammatory reaction was triggered up to 6 days after vector injection into normal brain, as observed by absence of leukocyte recruitment, demyelination, interleukin-1beta expression or increase in astrocyte/microglial activation. When the NLSCt vector carrying the gene for the antioxidant enzyme Cu/Zn Superoxide dismutase was injected intracortically two hours after an excitotoxic lesion, a reduction in lesion volume and a improvement in neurological performance of the animals were observed. Interestingly, injection of the vector carrying the gene for the GFP displayed less pronounced but significant neuroprotection. **Conclusions:** the modular recombinant NLSCt vector shows promising properties for gene therapy intervention in the acutely lesioned postnatal and adult CNS. **Palavras-Chave:** Gene therapy, Neuroprotection, Inflammation

Análise da distribuição das enzimas NADPH-diaforase e citocromo oxidase na *substantia nigra* e *striatum* de camundongos infectados pelo *Toxoplasma gondii*.

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O **T. gondii** é um protozoário intracelular obrigatório que pode invadir qualquer célula nucleada do hospedeiro. A distribuição deste parasita é cosmopolita, com uma soroprevalência de até 80% no Brasil. Os pacientes com infecção aguda geralmente apresentam infecção assintomática, com posterior desenvolvimento de cistos em diferentes tecidos, mais comumente nos músculos estriados e no sistema nervoso central [1]. Estudos epidemiológicos atuais sugerem que a infecção pelo **T. gondii** pode contribuir para a ocorrência de alguns casos de esquizofrenia, uma doença neuropsiquiátrica pervasiva que tem sido associada com anormalidade funcional do sistema dopaminérgico[2,3]. Objetivo: avaliar a atividade das enzimas NADPH-diaforase e citocromo oxidase (CO) no *striatum* e *substantia nigra* de cérebros de camundongos infectados com **T.gondii**. Metodologia: camundongos albinos suíços foram infectados com um inóculo de 10⁴ taquizoítos da cepa RH por via intraperitoneal. Os cérebros foram removidos após oito dias de infecção, seccionados em criostato e processados por histoquímica para NADPH-diaforase e CO. Resultados: os resultados demonstraram a presença do **T. gondii** nas áreas estudadas. A análise densitométrica das secções reagidas para a NADPH-diaforase revelou uma menor reatividade enzimática quando comparadas ao animal não-infectado, tanto no *striatum* (controle: D.O.= 123.22±17.4; infectado: D.O.= 100.9±15.7; p≤0.05) quanto na *substantia nigra* (controle: D.O.= 153.13±14.9; infectado: D.O.= 108.4±12.5; p≤0.05). A mesma queda na reatividade enzimática no animal infectado também ocorreu para a enzima CO, tanto no *striatum* (controle: D.O.= 99.22±1.61; infectado: D.O.= 94.84±0.92; p≤0.05) quanto na *substantia nigra* (controle: D.O.= 134.01±2.82; infectado: D.O.= 105.5±7.7; p≤0.05). Conclusão: estes resultados indicam que a presença pelo **T.gondii** está associada a alterações tanto nitrérgicas quanto metabólicas na *substantia nigra* e *striatum*, o que possivelmente ocasiona alterações no sistema de transmissão dopaminérgico.

1-The Lancet. 363:1965-1976. 2004; 2-Parasitology. 113:49-54. 1996; Parasitology. 122:515-520. 2001;

Palavras-Chave: Toxoplasma gondii, NADPH-diaforase, citocromo oxidase, striatum, substantia nigra.

Apoio Financeiro: CNPq

EXPRESSION OF IMMUNOMOLECULES BY MICROGLIAL CELLS DURING THE RECOVERY OF ACUTE EAE

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CD4, a molecule commonly expressed on the surface of T-helper lymphocytes with a recognized critical role in the antigen presentation process, has been also reported in monocytes and macrophages, although its role in these cells remains unknown. It is well established, that microglia, the resident macrophage population in the central nervous system (CNS), becomes activated after a variety of injuries. This activation produced both morphological and functional changes that include the upregulation or the novo expression of a variety of molecules in their surface. Adult resting microglial cells were CD4-, however, it has been reported the expression of this molecule in activated microglia in certain types of injury such as aspiration lesion and spinal cord injury. The objective of our study was to perform a detailed analysis of CD4 expression in activated microglial cells along the course of experimental autoimmune encephalomyelitis (EAE). Among EAE paradigms, we used the acute monophasic disease induced in Lewis rat because the spontaneous recovery that takes place makes this model very useful for the study not only of the inductive but also the resolution of the inflammatory process. To accomplish that female Lewis rats, MBP-injected, were sacrificed at different phases along the course of EAE accordingly to their clinical score. Spinal cords were analyzed by flow cytometry analysis for CD11b, CD4 and CD45, histochemistry for NDPase and single and double immunohistochemistry for Iba1 and CD4. We found CD4 expression in both macrophages (CD11b+CD45_{high}) and activated microglial cells (CD11b+CD45_{intermediate}). Microglial CD4 expression was found mainly during recovery scores and was maintained until 40 days post-induction. In agreement with flow cytometry analysis, immunolabelled sections demonstrated colocalization of Iba1 and CD4 in cells with ramified morphology. In conclusion, our results indicate that in this EAE model, activated microglial cells expressed CD4 and interestingly, this expression took place mainly during the recovery, suggesting a role of microglial CD4 in the resolution of the immune response.

Palavras-Chave: microglia, EAE, CD4

Apoio Financeiro: Fundació Marató TV3 and Fundació Koplowitz.

PAPEL DE TGF-beta1 NA PROGRESSÃO DE GLIOBLASTOMAS

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Os gliomas são os tumores neuroepiteliais primários mais comuns do sistema nervoso central mostrando-se refratários aos tratamentos convencionais. A heterogeneidade celular dos gliomas causa controvérsia na sua classificação e equívoco no diagnóstico. O fator de crescimento transformante beta 1 (TGF-beta1) é uma citocina que controla eventos como proliferação, diferenciação e migração. A sinalização deste fator se dá por 2 receptores transmembrana do tipo serina/treonina cinases, TGFRI e TGFRII. Tem sido proposto que os níveis de ativação de TGF-beta1 estejam associados ao grau de malignidade tumoral. Este trabalho consiste em estudar o efeito de TGF-beta1 na proliferação e migração de células de gliomas humanos *in vitro*. Para isso foram utilizadas, 3 linhagens de glioblastoma humano (Gbm02, Gbm95 e U87). Ensaio de imunocitoquímica para TGF-beta1 e seu receptor, TGFRII, revelaram uma distribuição similar nas 3 linhagens de glioblastoma. A análise do efeito de TGF-beta1 na proliferação foi realizada por ensaio de incorporação de timidina-³H. Para isso, as células foram incubadas em MSS (meio sem soro) por 24h com TGF-beta1 nas concentrações de 1,0 e 10 ng/ml e nas últimas 6h, foi adicionado um pulso de timidina-³H. Observamos um aumento de 6X e 4X, respectivamente, na proliferação das linhagens de Gbm 02 e Gbm 95 em resposta ao TGF-beta1 (10 ng/ml). Entretanto, a linhagem U87 não respondeu ao tratamento. A quimiotaxia das células de glioblastomas por TGF-beta1 foi testada na câmara de boyden. Essas células foram mantidas em meio com soro (controle positivo), meio sem soro (controle negativo) e em presença de TGF-beta1 (5,0 e 10 ng/ml) por 4h. Observamos que o TGF-beta1 promove quimiotaxia nas linhagens de Gbm95 e Gbm02. Não observamos efeito na linhagem U87. Em conjunto, nossos dados demonstram que TGF-beta1 induz proliferação e quimiotaxia em células tumorais de Gbm02 e Gbm 95, e não exerce efeito na linhagem U87. Esses dados ressaltam a heterogeneidade desses tumores, demonstrando que o TGF-beta1 pode ser um fator importante para a tumorigênese celular. **Palavras-Chave:** Glioblastoma, TGF-beta1

Apoio Financeiro: FAPERJ, CNPq, CAPES.

SELENIUM COMPOUNDS AND GUANOSINE PREVENT THE PRODUCTION OF REACTIVE SPECIES INDUCED BY GLUTAMATE

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This study was performed to investigate the reactive species (RS) production induced by glutamate, as well as the protective effect of selenium organic compounds (ebselen and diphenyl diselenide) and guanosine, a modulator of glutamatergic system, in different brain structures of rats. RS production was determined spectrofluorimetrically using the fluorescent marker 2',7'-dichlorofluorescein diacetate (H₂DCF-DA). Brain slices (striatum, cortex, hippocampus) were incubated for 2 h at 37°C with glutamate (1 mM). When tested, the compounds ebselen (0.01-100 µM), diphenyl diselenide (0.05-100 µM) and guanosine (0.5-200 µM) were added 15 min before the glutamate. Fluorescent signals were recorded at an excitation wavelength of 488 nm and an emission wavelength of 525 nm. Glutamate caused an increase in H₂DCF oxidation of approximately 40% in striatum, cortex and hippocampus. Ebselen protected against RS production from 0.5 µM in cortex and striatum, and from 1 µM in hippocampus. Diphenyl diselenide was effective in protect against H₂DCF oxidation from 0.5 µM in cortex and striatum, and from 10 µM in hippocampus. When guanosine was tested to prevent RS production it was more effective in cortex (10 µM), followed by hippocampus (20 µM) and striatum (100 µM). Guanosine (10 µM and 100 µM) also was tested associated with ebselen (1 µM) or diphenyl diselenide (1 µM) producing better effects than the compounds alone. The results present in this study show the protective effects of guanosine, ebselen and diphenyl diselenide against glutamate induced – RS production, especially when these compounds were associated. Guanosine could be acting by increasing glutamate uptake by astrocytes, while ebselen and diphenyl diselenide probably are acting neutralizing the RS. In this way the association of glutamatergic system modulators and antioxidants seems to be efficient in protect against glutamatergic excitotoxicity damage.

Palavras-Chave: Glutamate; guanosine; selenium organic compounds; 2',7'-dichlorofluorescein; oxidative stress.

Apoio Financeiro: FINEP, CAPES, CNPq, FAPERGS

Evaluation of the protective role for pyruvate, NAC and EGTA against acute Zn^{+2} neurotoxicity in a model of immature hippocampal slices.

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Zinc (Zn^{2+}) is an essential structural element of proteins and a signaling messenger that is released by neuronal activity at central excitatory synapses in cerebral cortex and hippocampus. It has been also shown that this metal can be toxic in several conditions such as global ischemia or epilepsy in which Zn^{2+} may activate neuronal cell death pathways. The aim of this work was to determine the neurotoxicity of Zn^{+2} in hippocampal slices of young rats (post natal day 14; PN14) exposed *in vitro* to the metal, as well as, a possible neuroprotective effect of pyruvate and the antioxidant N-acetyl-cysteine (NAC) against Zn^{2+} neurotoxicity. Additionally, it was analysed the effect of metal chelator EGTA (ethylene glycol tetra acetic acid). The cellular viability was evaluated by MTT assay. The hippocampal slices were exposed in the absence (control) or presence of $ZnCl_2$ (Zn^{2+} ; 10-100 μ M) for 2 h and in the presence or not of pyruvate (1-4mM); NAC (0.5-1mM) or EGTA (0.02-1mM). The results are expressed as mean $\pm$ S.E.M. and shown that the Zn^{2+} (100 μ M) reduced the slice viability in approximately 20% $\pm$ 2.2 ($p < 0.05$). The treatment with pyruvate did not protect the slices from Zn^{2+} neurotoxicity. Similarly, it was not observed a neuroprotective effect of NAC against Zn^{2+} injury. However, in the same experimental conditions, the reduction of slice viability caused by Cd^{2+} (40% $\pm$ 0.5; $p < 0.001$) was counteract by NAC. EGTA partially reversed the Zn^{2+} neurotoxicity (increasing for 88% $\pm$ 4 the slice viability). Pyruvate has been claimed as a very effective protector against Zn^{2+} neurotoxicity in models of cultured neurons and astrocytes. However, our results do not show such neuroprotective action of pyruvate on immature hippocampal slices acutely exposed to Zn^{2+} . Moreover, NAC was also hopeless to protect the cell viability impairment caused by Zn^{2+} . It suggests an inefficiency of NAC to chelate Zn^{2+} or the metal action may be independent of oxidative stress. Therefore, additional studies analyzing the action of Zn^{2+} on specific mitochondrial enzymes and the use of other viability assays will be important to comprehend the Zn^{2+} neurotoxicity on immature hippocampal slices. **Palavras-Chave:** Zinc; hippocampus; neuroprotection; pyruvate; NAC; EGTA

Apoio Financeiro: Supported by CNPq, FAPESC and FINEP (IBN-Net #01.06.0842-00)

Neurological and neuropsychological deterioration in miners with occupational elemental mercury exposure

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The occupational exposure to mercury in the small gold mining constitutes a serious problem in Andacollo town located in Coquimbo, Chile.

This investigation represents the first epidemiological intervention to determine neurological and neuropsychological impairment in a population exposed to elemental mercury in Chile.

The main pathways for the exposure to elemental mercury are the direct contact through the skin and inhalation of vapours. The most evident clinical signs of neurological damage are: sensorial deficiency and paresthesias in the limbs, to lately present motor and cognitive impairment, including paralysis. We choose three clearly defined populations: workers occupationally exposed to mercury of Andacollo town; a group corresponding to inhabitants of Andacollo town not occupationally exposed to mercury ("internal control") and a third control group called "external control" which corresponds to inhabitants of Coquimbo city. A sociodemographic survey was applied to the individuals to determine if they met with the inclusion criteria in each one of the groups. Lately, the included ones were invited to participate in the study and were requested to sign the informed consent. A complete neurological examination was performed to all individuals and also a five neuropsychological test battery plus a depression-detecting test was applied. Moreover, blood samples were collected to test total mercury and the status of oxidative stress biomarkers activity in them, which constitutes an early indicative of plausible neurotoxic after-effects.

Taken together, the data obtained in this study concludes that the population exposed to mercury presents neurological and neuropsychological impairment associated to exposure to this heavy metal and this is an occupational but not environmental health problem.

Palavras-Chave: mercury, neurotoxicity, neuropsychology

Apoio Financeiro: Conicyt by FONIS SA05I20032

ANXIOGENIC EFFECTS OF METHOTREXATE IN FEMALE AND MALE RATS TREATEDS AFTER SINGLE DOSE IN THE FIRSTS HOURS OF LIFE AND EVALUATED DURING ADULTHOOD

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Objectives: Methotrexate is a chemoterapic drug widely used for acute linfoblastic leukemia (ALL) treatment, which is the most common type of neoplasia among children. In this study was evaluated the long-term Methotrexate effects in rats submitted to elevated plus maze test.

Methods: Male and female newborn Wistar rats (n=48), with about 12 hours of life were divided in control (CTL), sham operated (SHO) and treated (MTX) groups. MTX group received a 0.5µl of intrathecal single dose of Methotrexate (20 µg/kg) and SHO received saline. Aged 45 days, animals were submitted to elevated plus maze. The apparatus consisted of a 4 wood-made arms with 2 of them closed and two open elevated 50 cm of the floor. Animals were exposed during 5 minutes to the apparatus. The frequency of entries and time spent in the closed or open arms were registered. The statistical analysis was performed using ANOVA and Tukey's HSD *post hoc* test, having treatment groups (CTL vs SHO vs MTX) and gender (female vs male) as independent variables and entries frequency or time spent as dependent variables. Differences were considered significant when the *p*-values were smaller than 0.05.

Results: MTX male group showed a increased number of entries in the closed arms than SHO and CTL ($F_{2,42}=19,08$; $p<,0001$), suggesting motor hyperactivity. Besides, MTX male group exhibited a decreased number of entries in the open arms ($F_{2,42}=19,08$; $p<,0001$), showing elevated anxiety levels. The percentage into open arms decreased on the MTX male group ($F_{2,42}=20,52$; $p<,0001$) and its percentage into closed arms increased ($F_{2,42}=8,20$; $p<,0010$) in comparison to than SHO and CTL groups confirming the drug anxiogenic effect. MTX female group showed decreased number of entries in the closed arms ($F_{1,42}=4,65$; $p<,0369$, Tukey $p<,0255$) and increased percentage into the open arms ($F_{1,42}=4,65$; $p<,0369$, Tukey $p<,0441$) in comparison to MTX male group, suggesting that female has less sensibility for Methotrexate effects than male rats.

Conclusions: Our study showed that Methotrexate induced long term anxiety state and motor hyperactivity. Further, females seem to be less susceptible for Methotrexate long-term behavioral effects. **Palavras-Chave:** Methotrexate; Anxiety; Motor hyperactivity; Elevated Plus Maze.

Apoio Financeiro: CNPq

Effects of the flavonoid agathisflavone, isolated from *Caesalpinia pyramidalis*, on growth, viability, and morphology of human glioblastoma cells

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OBJECTIVES: Glioblastomas are the most aggressive primary brain tumor. Potential differentiation or apoptosis inducing factors can offer a therapeutic alternative to chemotherapy. In this study, we investigated if the flavonoid agathisflavone, isolated from *Caesalpinia pyramidalis*, could interfere on cell proliferation and viability, and whether it induces differentiation in human GL-15 glioblastoma cells.

METHODS: GL-15 cells were grown in supplemented DMEM. Cells were treated with agathisflavone (0.1–100µM) for 24 or 72 h. Cell proliferation was measured by ³H-thymidine incorporation; cell viability was determined by trypan blue exclusion and measuring lactate dehydrogenase (LDH) activity on the medium; cell death was evaluated by flow cytometry after Annexin-V/propidium iodide (PI) staining; changes on cell morphology were evaluated after Rosenfeld's staining and by immunocytochemistry for GFAP and βIII-Tubulin.

RESULTS: Significant reductions on cell viability were evident only after exposure to 100µM agathisflavone for 24–72h (P<0.05). ³H-Thymidine incorporation showed that 100µM agathisflavone also induced an efficient growth inhibition, which reached 78–83% after 72 h exposure. Moreover, flow cytometry analysis revealed that 50µM agathisflavone induced apoptosis after 24 h. GL-15 cells in control conditions have a bipolar morphology and express low levels of GFAP and βIII-tubulin. It was observed that the majority of cells treated with 50–100µM agathisflavone retracted the cell body and emitted thin GFAP and βIII-tubulin positive processes. A subpopulation of cells showed a star-like astrocyte phenotype with increased GFAP immunostaining (76 %). On the other hand, another subpopulation of cells was marked with βIII-tubulin (63 %).

CONCLUSIONS: These findings indicate that 100µM agathisflavone was cytotoxic to GL-15 cells, and reduced cell proliferation. Furthermore, 50µM agathisflavone induced apoptosis. The ability to modify GFAP and βIII-tubulin expression in specific subpopulations of cells indicates that this flavonoid also has a morphogenic potential to glioblastoma cells.

Palavras-Chave: glioblastoma, flavonoids, agathisflavone

Apoio Financeiro: FAPESB and CNPq

Excitotoxicidade induz alteração na expressão das GTPases Rho na retina de pintos

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Objetivos: O glutamato é um importante neurotransmissor, mas em concentrações elevadas torna-se uma potente neurotoxina. Sua toxicidade está diretamente relacionada com a morte neuronal em diversas patologias, tais como isquemia e glaucoma e uma das possibilidades seria via GTPases Rho, que recentemente foram correlacionadas à morte celular por excitotoxicidade em neurônios cerebelares (1). Assim, o objetivo deste trabalho foi investigar se o tratamento excitotóxico promove alterações na distribuição das GTPases Rho (RhoA, RhoB, Cdc42 e Rac1) na retina de pintos.

Métodos: Agonistas de glutamato NMDA(2mmol), AMPA(20nmol) ou KA(200nmol) e salina tamponada (PBS, controle) foram injetados intraocularmente em pintos de 2 dias. A retina foi coletada após 1, 3 e 7 dias de sobrevida, fixada em PFA 2%-15', seccionada transversalmente e os cortes incubados para TUNEL e imunofluorescência e analisados em microscópio confocal.

Resultados: Muitas células apoptóticas (TUNEL-positivas) foram observadas principalmente na camada nuclear interna no 1º dia, diminuindo nos outros dias de sobrevida, sendo praticamente inexistente no 7º dia. Após os tratamentos, a distribuição de RhoA e RhoB não apresentou alterações significativas no padrão de marcação. A expressão de Cdc42 no segmento de fotorreceptores aumentou, assim como em possíveis processos de células gliais de Müller. Dentre as GTPases Rho, as alterações em Rac1 foram as mais expressivas, sendo possível observar uma marcação esférica e intensa na camada nuclear interna em todos os tratamentos, semelhante à marcação de células apoptóticas detectadas pelo método de TUNEL.

Conclusão: Esses resultados corroboram dados do laboratório em outros modelos de degeneração retiniana, que mostraram uma alteração no padrão de expressão e de atividade de Rac1. Assim, sugere-se que Rac1 esteja envolvida no processo de morte celular, embora a participação de outras GTPases não possa ser descartada.

Referência bibliográfica: 1- Nat Neurosci. 10:436-43, 2007.

Palavras-Chave: Excitotoxicidade, GTPases Rho, Retina

Apoio Financeiro: FAPESP e CNPq

Piry Virus (Rhabdoviridae: *Vesiculovirus*) Acute Encephalitis in C57BL/6J: Microglial Inflammatory Response Assessed by Stereological Investigation

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Objective: To determine target areas of Piry Virus (Rhabdoviridae: *Vesiculovirus*) in the central nervous system and to evaluate qualitatively and quantitatively the microglial inflammatory response in the pyramidal layer of mouse hippocampus after acute infection. **Methods:** 8 C57Bl/6J mice, female, 32 weeks old, were grouped as follow: control (n=4) or infected (n=4) groups. They were inoculated intranasally with saline solution or infected cerebral homogenate with the Piry virus (dilution = 10⁻⁵). In the 7th day, when infected animals presented signs of acute encephalitis they and control group were perfused with aldehyde fixatives. Random and systematic sample of 70µm sections were immunolabeled for Piry virus antigen using a specific antibody to it, stained for tomato lectin, *Lycopersicon esculentum*, and counterstained by Nissl. Morphological analyses were done using an optical microscope, Nikon, with low and high power objectives. The number of microglial cells in the pyramidal layer of hippocampus was assessed using the optical fractionator technique with StereoInvestigator software (MicroBrightField, USA). **Results:** The virus immunolabeling showed tropism for neurons in the olfactory tract and a few proximal microglial cells. Labeled neurons were found in the brainstem, diencephalon and in the hippocampus, where CA3 was the main target. The number of microglial cells in the hippocampal pyramidal layer was higher in the infected (23.064±5.494) than in the control group (5.174±3.141) (p<0.05, t-test). Qualitative microglial changes were seen between groups and among different regions. Microglia were characterized by cells with long and ramified branches in the control group, whereas in the infected group microglia branches were shorter, especially in CA3 pyramidal layer where cells were overtly activated and had the appearance of typical phagocytes. **Conclusion:** CA3 pyramidal layer seems to be the main hippocampal target of Piry virus and microglial activation is proportional to the immunolabeling for Piry antigens.

Palavras-Chave: Piry virus, hippocampus, microglia.

Apoio Financeiro: CNPq, FINEP, CAPES

Early axonal injury after methylmercury (MeHg) intoxication in adult cats: a 3-D morphometrical study of the axon terminals of the primary visual cortex.

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Although many experimental studies investigated methyl mercury neurotoxicity in mammalian visual system early axonal changes induced by mercury contamination remain to be analyzed in details. The aim of the present study is to investigate possible axonal morphological changes in the cat primary visual cortex early after methylmercury intoxication by using microscopic three-dimensional (3-D) reconstruction and cluster analysis. Six adult male cats (**Felix catus**), with 12h day-light cycle and *ad libitum* water and food regime, received a single dose of MeHgCl (6,4 mg/kg) dissolved in milk. Six other control cats received only milk. After 30 days, biocytin was injected by iontophoresis into the area 17 near the border with area 18. Twenty four hours after iontophoresis, all cats were perfused with saline followed by 4% paraformaldehyde. Brain sections (200µm thick), were processed to reveal biocytin labeled terminals. Axonal microscopic 3D reconstructions using Neurolucida software (Microbright Systems Inc.) allowed to estimate the densities of boutons, branching points and segments for each terminal. Cluster analysis based on boutons, branching points and segment densities of the control group axon terminals revealed, as expected, type I and type II families. Axon terminals from intoxicated cats showed statistical significant decrease of all morphometric measurements when compared to controls (two tailed t-test, $p < 0.05$) and it was not possible to identify typical type I and II axons between them. Boutons, branching points and segment densities of intoxicated group, decreased 73, 77 and 37% respectively, relative to the control values. The concentrations of MeHg and inorganic Hg (Hgi) was measured in the brain parenchyma of intoxicated cats and corresponded on average to $1,39 \pm 0,3$ and $6,79 \pm 0,6$ ppm (mean $\pm$ s.e.m.) respectively. The results showed that morphological axonal alterations, associated with MeHg, appear early in the disease (30 days after contamination) in the absence of evident clinical signs, revealing new aspects of the neuronal pathology of the methylmercury intoxication in the visual cortex.

Palavras-Chave: methylmercury, visual cortex, axon terminals, cat, biocytin

Apoio Financeiro: CNPq

IMPACTO LONGITUDINAL DA HIPÓXIA CRÔNICA CONSTANTE INTRA-UTERINA NO CÉREBRO EM DESENVOLVIMENTO

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Objetivo: O nosso trabalho visa analisar como a hipóxia crônica fetal durante toda a gestação interfere no desenvolvimento do cérebro bem como desenvolver intervenções terapêuticas que permitam reverter ou atenuar as alterações decorrentes.

Métodos: Camundongos fêmeas grávidas foram mantidas em câmara hipóxica (Coy Laboratory Products Inc., MI, EUA) com atmosfera contendo 11% de oxigênio, temperatura e umidade mantidas constantes, durante todo o período gestacional. Após o nascimento foram avaliados os parâmetros biométricos, histopatológicos do encéfalo e funcionais (reflexo de endireitamento, abertura de olhos, locomoção, equilíbrio, memória e aprendizagem) da prole desde o nascimento até a idade adulta.

Resultados: Nossos resultados preliminares demonstram que 100% dos animais submetidos à hipóxia crônica constante pré-natal apresentam redução de aproximadamente 20% do tamanho e peso corporais, bem como atraso na abertura dos olhos e redução do reflexo de endireitamento. Até P180 não foi observado recuperação do equilíbrio (teste da barra).

Conclusão: Nossos estudos vão possibilitar a análise do impacto longitudinal da hipóxia intra-uterina crônica constante no desenvolvimento do cérebro ao determinarem a compensação realizada pelo sistema nervoso central ao longo da vida do animal.

Palavras-Chave: hipóxia ; intra-uterina ; desenvolvimento cerebral

Apoio Financeiro: FAPERJ ; CAPES ; CNPq ; NIH (EUA) ; UFRJ (PIBIC)

Expression of Inhibitor of Apoptosis Protein Survivin in Childhood Medulloblastoma

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Objectives: Medulloblastoma is the most common Central Nervous System neoplasia in childhood, accounting for about 20% of the cases. Due to the high rate of mortality and treatment-related morbidity, the identification of biological factors can help predict response to treatment could lead to a more precise selection of patients who can benefit from a more aggressive therapy as well as improve risk stratification (1). Recently, Inhibitor Apoptosis Protein Survivin has emerged as a promising prognostic factor in several tumor types and also as an interesting therapeutic target due to its rare expression in adult differentiated tissues (2). Therefore, we analyzed Survivin expression in a series of 43 pediatric medulloblastoma tumor samples.

Methods: Immunohistochemical detection of Survivin was performed with an anti-Survivin antibody (Sigma) at a dilution of 1:800. The detection system used was the labeled streptavidin bition with a coupled HRP-peroxidase method (LSAB2 – Dako). Results were evaluated for both intensity (none to 4+) and percentage of nuclear and/or cytoplasmic staining. Statistical analysis was performed in SPSS software.

Results: Median age was 7 years. Survivin expression did not correlate with histological subtype and occurrence of metastasis ($p>0,05$). Survivin expression ranged from completely absent to fully present in a notably higher pattern of nuclear localization than cytoplasmic (86% x 14%, respectively). However, the most important finding so far was that patients with nuclear Survivin expression had a worse 5-year survival than those with no expression and patients with cytoplasmic Survivin expression had a better one. Although it was not statistically significant, because of this trend we are currently analyzing a greater number of cases in order to improve the series standard error.

Conclusions: Survivin expression does not appear to correlate with 5-year survival rate. Nevertheless, its cellular localization may contribute to this prognostic feature.

1- Clinical Cancer Research 14:971, 2008.

2- Carcinogenesis 28:1133, 2007.

Palavras-Chave: Medulloblastoma; Survivin; Imunohistochemistry.

Apoio Financeiro: Swissbridge Foundaion and Programa de Oncobiologia

EFEITOS DA INJEÇÃO CENTRAL DA TOXINA TX3-4 DA ARANHA *PHONEUTRIA NIGRIVENTER* EM RATOS WISTAR

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Objetivos: O objetivo desse trabalho foi avaliar os efeitos da injeção intracerebroventricular (i.c.v.) da toxina Tx3-4, que bloqueia os canais de cálcio dependentes de voltagem, sobre parâmetros do eletroencefalograma (EEG), do eletrocardiograma (ECG) e sobre os valores de pressão arterial (PA) .

Métodos e Resultados: Foram utilizados ratos Wistar machos, com peso entre 250 e 300 gramas, divididos em 5 grupos (n=7 cada), de acordo com a dose da toxina microinjetada (1,5µg; 0,750µg; 0,325µg; 0,160µg) e grupo controle (PBS). Os animais foram submetidos à cirurgia para implantação da cânula guia sobre o ventrículo lateral esquerdo e de eletrodos para registro de EEG e de ECG. Após seis dias, foi realizada a canulação da artéria femoral esquerda. No dia seguinte, EEG, ECG e PA foram registrados por 15 minutos antes da aplicação da toxina. Em seguida, foi realizada a microinjeção i.c.v. de 3µL de toxina em 6 minutos e os mesmos parâmetros foram registrados por 60 minutos. Para análise dos registros foram utilizados 3 minutos anteriores e 60 minutos posteriores à injeção da toxina. A toxina Tx3-4 causou diminuição da frequência cardíaca nas doses de 1,5mg (379,9±7) e 0,75mg (353,9±4) em relação ao PBS (412,2±5) (p<0,01) e aumento da pressão artéria média em todas as quatro doses utilizadas, PBS (111,2±0,5), 1,5mg (119,9±1,1), 0,75mg (115±0,8), 0,325mg (115,6±0,6) e 0,160mg (116,0±0,6) (p<0,01). A energia total do EEG do hemisfério direito foi diminuída para as doses 1,5µg (175mV) e 0,750µg (174mV), com relação ao PBS (212mV) (p<0,01). Essa diminuição não se repetiu no hemisfério esquerdo, onde as bandas de frequência alfa e beta do EEG diminuíram, enquanto delta e gama aumentaram na dose de 1,5µg (valores normalizados). Os animais foram sacrificados dois dias após o experimento, durante esse período não foi registrada nenhuma morte.

Conclusão: A toxina Tx3-4 mostrou baixa toxicidade quando injetada centralmente para as doses estudadas. Esses resultados, aliados à capacidade da Tx3-4 bloquear canais de cálcio dependentes de voltagem, encorajam o estudo dessa toxina como recurso terapêutico em casos de isquemia cerebral.

Palavras-Chave: Tx3-4, neurotoxicidade

Apoio Financeiro: CNPq, Fapemig e Capes

Buspirone partially protects an astrocytic cell line from the ethanol effects

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The human astrocytoma cell line 1321N1 expresses 5HT1A receptors. Our objectives were to study i) the response of this cell line to ethanol (E) ;ii) if buspirone (B, a 5HT1A partial agonist) has a protective effect. The 1321N1 cells were cultured in slides with the following culture medium: DMEM, high glucose, 1% w/v glutamine, 10% v/v fetal bovine serum and 1% penicillin-streptomycin at 37° C, 5% CO2. When confluence reached 80% the culture medium was replaced by OPTIMEM for 3 hours before the experimental treatment for three days with: a) OPTIMEM only (control, C); b) OPTIMEM + 400nM B; c) OPTIMEM + 25mM E; d) OPTIMEM + 25mM E and 400nM B; e) OPTIMEM + 75mM E; f) OPTIMEM + 75mM E and 400nM B. Cells were fixed with 4% p/v paraformaldehyde in phosphate buffer 0.1M pH 7.4 for 15 min at room temperature. We assessed nuclear morphology in Hoechst 33342 stained cells. The expression of 5HT1A receptors, GFAP and S100B protein were assessed by immunofluorescence. The results were: 1) a tendency to an increase in E25 in the number of abnormal nuclei/field with an increase in E75 (C: 9.06 ± 4.15 ; E25: 20.18 ± 7.98 ; E75: 39.53 ± 22.74 , $P < 0.01$) reversed when B was added (E25+B: 14.27 ± 6.97 ; E75+B: 19.85 ± 10.51); 2) a maximal decrease of total cell number/field in the 75mM E treatment (C: 78.6 ± 56.49 ; E25: 40.08 ± 29.54 ; E75: 15.6 ± 7.8) partially reversed when B was added (E75+B: 39.92 ± 19.45); 3) more than 80% of the cells coexpressed S100B, GFAP and 5HT1A receptors. E altered nuclear morphology and the number of processes in each cell. These results show that buspirone partially protects the astrogloma cell line from the detrimental effects of the ethanol. Since this cell line has 5HT1A receptors, they are likely to be involved in the protective effects.

Palavras-Chave: Astrocytoma, ethanol, buspirona, 5HT1A

Apoio Financeiro: PICT 31964; PIP 5034; UBACYT M072.

Interaction between metastatic lung tumor and brain parenchyma

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Objectives: Lung tumors present the largest incidence of central nervous system metastasis worldwide (50-60%)¹. Such pattern demonstrates that these tumors have affinity to the brain parenchyma. Tumoral cells often overexpress epidermal growth factor receptor (EGFR)². On the other hand, brain parenchyma cells overexpress EGFR only in case of lesion³. Our work demonstrated the interaction between the cells of a lung tumor and the brain parenchyma using a brain metastasis *in vitro* model. More specifically, we analyzed the role of soluble factors secreted by tumoral cells and brain parenchyma cells in relation to: (1) brain extracellular matrix (ECM) elements expression and (2) tumoral multidrug resistance (MDR) proteins expression.

Methods: Cortical astrocytes of newborn rats (P0) were treated for 3 days *in vitro* with conditioned medium from cells of lung tumor non-oat cells lineage (A549) on absence or presence of EGFR inhibitor. After treatment we used immunofluorescence technique to observe possible alterations on ECM astrocytes proteins laminin (LN) and fibronectin (FN) expression. A549 cells were treated for 3 days *in vitro* with conditioned medium of cortical astrocytes P0. To analyze alterations on MDR proteins expression, more specifically the efflux pumps MRP1 (MDR related protein) and BCRP (breast cancer resistance protein) we used flow cytometry assay. The expression levels were represented by intensity fluorescence mean (IFM).

Results: Factors secreted *in vitro* by A549 tumoral cells increased LN and FN expression levels in cortical region astrocytes from rats. The increase of cerebral ECM elements was dependent of EGFR activation. Furthermore, MRP1 expression in A549 cells increased when they were treated with conditioned medium from astrocytes (IFM = $4,7 \pm 0,7$ $p < 0,05$) then with control medium (IFM = $2,8 \pm 0,3$). No alteration was observed regarding BCRP expression on tumoral cells.

Conclusions: Our results suggest that metastatic lung tumors secrete factors capable to alter the cerebral ECM, possibly favoring its installation. In parallel, brain parenchyma cells secrete factors capable to influence tumoral cells to acquire MDR phenotype.

References: (1) Folia Neuropathol 44:42, 2006; (2) Clin Cancer Res 9:1070, 2003; (3) J Neurosci 26:7532, 2006.

Palavras-Chave: metastatic lung tumor; brain parenchyma; extracellular matrix; multidrug resistance

Apoio Financeiro: CAPES, FAPERJ, CNPq, PRONEX-MCT, FUJB-UFRJ, FAF

Molecular mechanisms in gliomagenesis

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The need to develop personalized treatments, in the case of tumor processes, drives us not only to a better knowledge of the molecular events involved in cancer pathogenesis, but also the design of experimental models that allow us the study of the repercussions of the activation of certain oncogenes and/or the inactivation of certain tumor suppressor genes on the response to antitumoral therapy. Therefore, we combined both aspects, developing a genetically modified mouse models of astrocytomas, a tumor type with a fatal prognosis, that allow us to study the influence of certain mutations on the pathogenesis, and the response to certain treatments. Consequently, it is crucial to generate animal models of astrocytoma that allows not only a better understanding, at the molecular level, of the etiopathology behind this neoplasia, but also facilitate the development of new therapeutic approaches. The proposed models are based on the combination of inducible systems of the initial oncogenic events, and a non-invasive systems for in vivo monitoring that allow us to follow-up the entire tumor process.

Palavras-Chave: glioma; oncogene; tumor suppressor; astrocyte; cancer; genetically modified mouse models

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Role of PARP-1 in the regulation of astrocyte proliferation

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Poly(ADP-ribose) polymerase-1 (PARP-1) is an ubiquitous enzyme that catalyzes the synthesis of poly(ADP-ribose) on the glutamate residues of target proteins and, owing to this mechanism, participates in several DNA cleavage and rejoining-dependent reactions, such as DNA replication, recombination and repair. In addition, PARP-1 plays also a pivotal role in transcriptional regulation, although the determinants for its binding to undamaged DNA have not been defined.

Objectives: In this study, we investigated the role of PARP-1 in the genesis and development of the oncogenic process in glial cells. With this purpose we characterized how PARP-1 deficiency would influence the rate of astrocyte proliferation both under basal conditions and in the presence of an oncogenic event.

Results: Primary astrocytes obtained from Adprt^{-/-} mouse cortices showed a lower rate of proliferation than wild-type cells. However, this effect was more evident when primary astrocytes were infected with a viral vector encoding the oncogenic HRasV12 protein, thus suggesting the PARP-1 may affect the cellular response to oncogenic events. Interestingly, despite the activation of cellular checkpoints was similar in all cases, no signs of cellular senescence were observed after the oncogenic stress. Furthermore, we were also unable to find any features of apoptosis, thus suggesting that PARP-1 deficiency results in a proliferative disadvantage. In all cases restoration of PARP-1 levels rescued the phenotypic changes.

Conclusions: Our findings demonstrate that PARP-1 is involved in the regulation of the progression through the cell cycle. Since lack of PARP-1 inhibits astrocyte proliferation, especially in the presence of an oncogenic event, the possibility also exists PARP-1 deficiency may be a proliferative disadvantage for glial-derived tumors.

Palavras-Chave: astrocytes, PARP-1, Ras

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Neuroprotective action of erythropoietin against cell death induced by hypoxia on SHSY5Y cells

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OBJETIVES: On exposure to hypoxia, brain tissue becomes sensitive to cell injury and death, which could cause neurodegenerative outcomes. Erythropoietin (Epo) has emerged as a multifunctional factor that could play a significant role in tissues outside the hematopoietic system. We decided to investigate whether Epo might be able to prevent deleterious effects of hypoxia.

METHODS: Human neuroblastoma SH-SY5Y cells exposed to hypoxia were used. Treatments with Epo were made before and after hypoxia injury. Morphological alterations were observed by scanning electron microscopy (SEM). Cell viability was determined by MTT assay. Apoptosis was detected by nuclear fluorescence microscopy (Hoechst). Expression of Bcl-2 members (Bcl-2, Bcl-x_L and Bax) was analyzed by RT-PCR.

RESULTS: Hypoxia exposure for 16 or 24 h caused morphological changes like lost of neuritogenesis and cell detachment. Under this condition, a significantly reduced cell viability dependent on the exposure-length (H16 71±9%, H24 28±15%; P<0.05 with respect to controls; n=4) and a significant increase in cell apoptosis were detected (H16 35±2% vs. controls P<0.05; n=5). These results are in accordance with cell morphology alterations and membrane blebbing observed by SEM. The effects triggered by hypoxia in this undifferentiated neuroblastoma cell line were totally prevented by a previous treatment with Epo (25 U/ml, 9 h) as shown by data of cell viability: H16 71±7%, E9/H16 100±4%; P<0.05; n=5, and percentage of apoptotic cells: H16 28±1%, E9/H16 7±2%; P<0.001; n=5. A post treatment with Epo up to 48 h after hypoxia did not overcome cell damage. Cell exposure to hypoxia showed downregulation of RNAm levels of the antiapoptotic member Bcl-x_L (RT-PCR) to near 45±12% of the control levels (P<0.05; n=3), while the previous treatment with Epo has the opposite effect (Bcl-x_L RNAm: 147±12%; P<0.05).

CONCLUSIONS: these results demonstrate that short treatments with Epo could not reverse the damages caused by SH-SY5Y cell cultures under low oxygen condition. However, Epo prevented the apoptosis induced by hypoxia, suggesting a neuroprotective action partially dependent on the modulation of Bcl-x_L.

Palavras-Chave: Hypoxia, Erythropoietin, Neuroprotection, SH-SY5Y cells

Apoio Financeiro: The national Council of Scientific and Technical Research (CONICET, Argentina) and the National Agency for Scientific and Technologic Promotion (ANPCYT, Argentina)

Effect of chronic caffeine administration on glutamate uptake in hippocampal slices from male and female aged-rats.

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Objective: Glutamate uptake (Glu) is essential for the maintenance of physiological glutamate levels in the synaptic cleft. Caffeine is a psychostimulant with distinct neuroprotective effects in male and female subjects. Previous studies have been shown that caffeine can affects Glu release, but its effects on Glu uptake were not investigated yet. In this study, we evaluated the effects of caffeine administration for 30 days on Glu uptake in hippocampal slices from aged-rats. We aimed to compare glutamate uptake between male and female aged-rats after caffeine treatment. **Methods:** Wistar males and females rats (>1 year old) were used. Animals were treated with caffeine (10mg/kg, i.p) or saline 0.9 % during 30 consecutive days. After treatment slices (400µm) from the hippocampus of all groups were obtained for Glu uptake assays. Statistical analysis was performed by two-way ANOVA followed by Newman-Keuls post hoc test. **Results:** Hippocampal slices from male-aged rats presented 25% of increase in the Glu uptake than their female counterparts (from 0.3117 ± 0.02845 to 0.2358 ± 0.01379 males and females, respectively; $P < 0.05$). Hippocampal slices from caffeine-treated male rats presented a decrease of 33% in the Glu uptake (from 0.3117 ± 0.02845 to 0.2157 ± 0.02869 control and caffeine males, respectively to $P < 0.05$). Caffeine did not modify Glu uptake in slices from female aged-rats. **Conclusion:** Our study may explain the distinct effects of caffeine depending on gender with participation of glutamatergic system. In fact, Glu uptake in hippocampal slices presented differences according to gender, since male rats presented an increase in the Glu uptake. Besides, the inhibitory effect of caffeine on Glu uptake was evidenced only in hippocampal slices from male rats. Thus, data presented here point to a role of glutamate uptake in the distinct effects of caffeine in the central nervous system. **Palavras-Chave:** Caffeine, age, gender, glutamate uptake.

Apoio Financeiro: IBNnet, CNPq, FAPERGS

Prostaglandin E₂ decreases Na⁺,K⁺-ATPase activity in rat hippocampus ex vivo

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Objectives: There is evidence that prostaglandin E₂ (PGE₂) facilitates seizures, but little is known about the molecular mechanisms involved. Na⁺,K⁺-ATPase is a membrane protein which plays a key role in the maintenance of neuronal excitability, and a decrease in Na⁺,K⁺-ATPase activity facilitates the appearance of seizures. In this study we tested the hypothesis that PGE₂ decreases Na⁺,K⁺-ATPase activity, in order to shed some light on the mechanisms underlying the excitatory action of PGE₂.

Methods: Male adult Wistar rats (250 g) were anesthetized, placed in a rodent stereotaxic apparatus, and then two cannula were inserted bilaterally into the lateral ventricles (coordinates relative to bregma: AP 0 mm, ML 1.5 mm, V 2.5 mm). Seven days later the animals were injected with PGE₂ (1 - 100 ng/site, 2 µl) or its vehicle (phosphate-buffered saline, pH 7.4) and sacrificed thirty minutes thereafter. The hippocampi and cerebral cortices were dissected and homogenized in Tris-HCl buffer (30 mM, pH 7.4). Na⁺,K⁺-ATPase activity was determined in homogenates by a colorimetric method assessing ouabain-sensitive ATP hydrolysis. In another set of experiments, PGE₂ (0 - 10 µM) was added directly to the reaction medium used for determination of enzymatic activity, in order to investigate whether PGE₂ would decrease Na⁺,K⁺-ATPase activity in vitro. Data were analyzed by one or two-way ANOVA followed by the Student-Newman-Keuls test, when appropriate.

Results: Statistical analysis revealed that the intracerebroventricular injection of PGE₂ (100 ng/site) decreased Na⁺,K⁺-ATPase activity in the hippocampus (113.75 ± 7.12 versus 90.94 ± 4.83 nmol Pi/mg protein/min) [F(3,18)=4.221; p < 0.02], but not in the cerebral cortex [F(3,18)=0.834; p > 0.05] ex vivo. Conversely, PGE₂ did not decrease Na⁺,K⁺-ATPase activity when added directly to the reaction medium [F(3,12)=0.022; p > 0.05].

Conclusions: We conclude that the intracerebroventricular injection of PGE₂ (100 ng/site) decreases Na⁺,K⁺-ATPase activity in rat hippocampus ex vivo, an effect that requires an intact cellular system.

Palavras-Chave: PGE₂, Na,K-pump, epilepsy, neurotoxicity

Apoio Financeiro: CAPES, CNPq (grant 301552/2007-0), CNPq/Pibic

Avaliação dos mecanismos de Neuroproteção promovida pela Guanosina em fatias de hipocampo de ratos submetidas à Privação de Glicose e Oxigênio

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Guanosina (GUO) apresenta papel neuroprotetor frente à privação de glicose e oxigênio (PGO) em fatias de hipocampo, de maneira dependente dos níveis de cálcio e ativando canais de potássio. Objetivamos estudar os mecanismos de neuroproteção da GUO, avaliando a captação de glutamato e o subtipo de canal de potássio envolvido neste efeito. Fatias de hipocampo de ratos adultos foram incubadas com tampão isquêmico (2-deoxi-glicose e nitrogênio) por 15 min. Na reperfusão de 2 h, o tampão isquêmico foi substituído por tampão fisiológico. Nestes períodos, as fatias foram pré-incubadas por 15 min com inibidores de canal de potássio: 4-AP 50 μ M, apamina 300nM, glibenclamida 20 μ M e TEA (tetraetilamônio) 25mM seguidas da adição de GUO 100 μ M. A viabilidade celular foi avaliada pelo método de redução do MTT. A avaliação da captação de L-[³H]Glutamato (0,33 μ Ci/ml) pelas fatias hipocamapais foi quantificada por cintilação líquida. Fatias submetidas a 15 minutos de PGO apresentam diminuição significativa da viabilidade celular de $30 \pm 7,7\%$ em relação ao grupo controle (100%). A GUO adicionada ao meio durante o período de 2 h reperfusão promove um significativo aumento na viabilidade celular ($99,5 \pm 3,3\%$). A pré-incubação com apamina e glibenclamida, não alterou a neuroproteção promovida pela GUO. Entretanto, na presença dos inibidores 4-AP e TEA, houve uma reversão do efeito neuroprotetor da GUO ($81 \pm 13,9$; $82 \pm 9,1$ %, respectivamente). Ocorreu uma diminuição significativa na captação de Glutamato nas fatias submetidas a 15 min de PGO ($0,044 \pm 0,006$ nmol/min/mg de proteína), 15 min de PGO na presença de GUO ($0,028 \pm 0,008$) e 15 min de PGO seguido de 2 h de reperfusão ($0,0153 \pm 0,004$) em relação ao grupo controle ($0,085 \pm 0,0044$). Entretanto quando a GUO ($0,0603 \pm 0,0018$) foi adicionada ao período de 2 h de reperfusão foi observada uma recuperação na captação de Glutamato. A neuroproteção promovida pela GUO envolve a participação de canais de potássio dependentes de cálcio. Além disso, o efeito neuroprotetor da GUO está relacionado a um aumento na captação de glutamato após a PGO e reperfusão.

Palavras-Chave: Guanosina, Privação de glicose e oxigênio, Glutamato, Canais de Potássio, Neuroproteção, Fatias hipocamapais.

Apoio Financeiro: CAPES, FAPESC, IBN/Net, CNPq

Selenium compounds counteract the stimulation of ecto-nucleotidase activities in rat cultured cerebellar granule cells: putative correlation with neuroprotective effects.

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Glutamate, the mainly excitatory neurotransmitter in brain, showed to stimulate ecto-nucleotidase activities in cerebellar granule cells increasing extracellular adenosine levels. The compounds ebselen and diphenyl diselenide [(PhSe)₂], display neuroprotective activities mediated in part by their antioxidant and anti-inflammatory properties and were described to prevent glutamate toxicity and by modify glutamatergic synapse parameters. In this study, we investigate the effects of ebselen or (PhSe)₂ on glutamate-induced stimulation of ecto-nucleotidase activities in rat cultured cerebellar granule cells. Glutamate increased ATP hydrolysis at 10 mM ($36 \pm 7\%$; 29.5 ± 2 nmol Pi/ mg protein/ min) and 100 mM ($43 \pm 9\%$; 31 ± 2.5 nmol Pi/ mg protein/ min) and AMP hydrolysis at 10 mM ($44 \pm 8\%$; 28.5 ± 3 nmol Pi/ mg protein/ min) and 100 mM ($47 \pm 9\%$; 31 ± 5 nmol Pi/ mg protein/ min) when compared to control group (22 ± 2 and 20 ± 2 nmol Pi/ mg protein/ min, for ATP and AMP, respectively). Both selenium compounds prevented the stimulatory effect caused by 100 mM glutamate at control levels. Further, we investigated the association of organoselenium effects with their antioxidants properties searching for redox site modulation by using the alkylant agent N-ethylmaleimide (NEM). Pre-treatment with NEM alone increased ATP ($55.5 \pm 8\%$; 32 ± 3 nmol Pi/ mg protein/ min) and AMP ($61 \pm 20\%$; 22 ± 2.6 nmol Pi/ mg protein/ min) hydrolysis when compared to control group (21 ± 2.4 and 15 ± 1.4 for ATP and AMP, respectively) and this stimulatory effect was abolished by ebselen (3 mM) or (PhSe)₂ (1 mM). Moreover, ATP and AMP hydrolysis remained increased $46 \pm 12\%$ and $79 \pm 16\%$, (29 ± 3 and 24 ± 2 nmol Pi/ mg protein/ min) respectively when 100 mM glutamate and 10 mM NEM were co-incubated and although this effect was not overlaid, ebselen and (PhSe)₂ were less effective in preventing the AMP hydrolysis stimulation by association of glutamate and NEM. Our results suggest that selenium compounds can avoid the increase on glutamate-induced stimulation of ecto-nucleotidase activities probably due their antioxidant properties. **Palavras-Chave:** Selenium compounds; ecto-nucleotidases; glutamate; cerebellar granule cells

Apoio Financeiro: Capes, CNPq and PRONEX

Avaliação do possível efeito protetor do extrato de *Panax ginseng* sobre a apoptose induzida em astrócitos

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Objetivo: O presente estudo propôs verificar se o extrato hidroalcoólico de *Panax ginseng*, planta amplamente utilizada para melhora de memória e cognição, poderia ter algum efeito protetor na morte celular por apoptose.

Métodos: A cultura de astrócitos foi obtida a partir do córtex de ratos com 3 dias de idade. Os núcleos dessas células foram corados com Hoechst 33342 e a porcentagem de células apoptóticas foi calculada por análise da morfologia nuclear. Inicialmente, fez-se uma curva concentração-tempo-efeito para verificar a possível toxicidade do extrato e permitir a escolha de uma concentração ideal para incubação das células. A morte dos astrócitos foi induzida por staurosporina, cafeína ou etanol por 6 horas e as células foram previamente incubadas com uma concentração de 100 microgramas/ml do extrato para verificar o seu efeito protetor.

Resultados: O extrato de *Panax ginseng* foi atóxico em todos os tempos e concentrações testados na cultura de células. A partir disso, a dose de 100 microgramas/ml do extrato foi escolhida para verificar a possível proteção do extrato frente a agentes apoptóticos. A staurosporina induziu apoptose nas células, pois houve um aumento de morte significativo de células incubadas com a mesma quando comparadas com as células do grupo controle e as células incubadas com o extrato [$F_{(3,56)} = 5,81$; $P < 0,05$]. Por sua vez, a incubação prévia do extrato hidroalcoólico de *Panax ginseng* não foi capaz de prevenir essa apoptose induzida pela staurosporina. A incubação das células com cafeína e etanol não causou morte estatisticamente diferente do grupo controle, devido à concentração ou ao tempo de incubação escolhidos, dificultando a análise de um possível efeito protetor do extrato.

Conclusão: Os dados sugerem que em astrócitos de rato, o extrato hidroalcoólico das raízes de *Panax ginseng* não foi tóxico nas concentrações e tempos testados e que o extrato hidroalcoólico das raízes de *Panax ginseng* não apresentou efeito antiapoptótico no modelo de indução de apoptose por staurosporina.

Palavras-Chave: Apoptose, astrócitos, *Panax ginseng*.

Apoio Financeiro: CAPES, AFIP, CEBRID

Chronic intranasal instillation of *residual oil fly ash* (ROFA) induces lipid peroxidation (LPO) in hippocampus, striatum and cerebellum of rats.

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Objectives: Epidemiological studies had linked the transition metals with neurodegenerative diseases. The particulate matter, a component of air pollution, present these metals adsorbed in the surface of the particles. In our study, the objective was to analyse the LPO in hippocampus, striatum and cerebellum of rats exposed to ROFA by intranasal instillation and treated or not with n-acetylcysteine (NAC) for 30 days.

Methods: Male Wistar rats, 45 days of life, were divided in four groups (n=10): G1: ROFA (20µg/10µl saline) by intranasal instillation (i.i)+saline (10µl) intraperitoneally (i.p); G2: saline (10µl) i.i+saline (1 ml/Kg) i.p; G3: ROFA (20µg/10µl saline) i.i+NAC (150 mg/Kg) i.p; and G4: saline (10µl) i.i+NAC(150 mg/Kg) i.p. Animals were euthanased. The hippocampus, striatum and cerebellum were dissected. The structures were homogenized and tested for LPO.

Results: ROFA induced an increase in malondialdehyde concentration in hippocampus (P=0.001), striatum (P=0.033) and cerebellum (P=0.030) in G1 when compared to other groups.

Conclusions: NAC protected these structures from an increase of LPO. Therefore, the present study suggests that ROFA administrated by intranasal instillation may pass through olfactory bulb and it may induce LPO in hippocampus, striatum and cerebellum.

Financial support: CAPES, CNPq, FFFCMPA

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Palavras-Chave: Poluição Atmosférica, neurotoxicidade, ROFA, metais de transição neurodegeneração

Apoio Financeiro: CAPES, CNPq, UFCSPA

ANÁLISE DA EXPRESSÃO E FOSFORILAÇÃO DA PROTEÍNA RB EM LINHAGENS DE GLIOBLASTOMAS

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Objetivos: Cerca de 90% dos glioblastomas apresentam mutações em proteínas da via de RB (retinoblastoma), levando à inativação de RB por hiperfosforilação, com o consequente aumento na proliferação celular. Entretanto, além de bloquear a progressão do ciclo celular, RB pode inibir a apoptose. Perguntamos se a inibição da apoptose em glioblastomas ocorre com RB mesmo no seu estado hiperfosforilado. Esta hipótese, se comprovada, explicaria porque em glioblastomas a hiperfosforilação da proteína RB é mais freqüente do que a inativação gênica de *Rb-1*. Comparamos a expressão e a fosforilação da proteína RB em linhagens de glioblastoma humano. As linhagens com altos níveis de expressão e fosforilação de RB serão analisadas quanto à resistência a apoptose e o índice proliferativo após silenciamento de RB por RNAi.

Métodos: As linhagens de glioblastomas humanos U87 (ATCC), GBM95 e GBM02 (produzidas no laboratório), foram cultivadas em meio DMEM-F12 suplementado com 10% de soro fetal bovino e antibióticos. As células foram submetidas à imunocitoquímica com anticorpos anti-RB e anti-RB fosforilado nos resíduos de serina (Ser) 807/811 e treonina (Thr) 821/826, e analisadas através de microscopia de fluorescência.

Resultados: Todas as linhagens testadas apresentaram uma expressão similar de RB: pontilhada, nuclear e citoplasmática. O percentual de marcação de RB nas linhagens U87, GBM95 e GBM02 foi de $87.9 \pm 0.6\%$; $96.5 \pm 1.5\%$ e $86.01 \pm 5.9\%$, respectivamente. Os níveis de fosforilação de RB nos resíduos de Ser 807/811 e Thr 821/826 foram semelhantes entre as linhagens utilizadas: $66.5 \pm 9\%$ (Ser) e $87.5 \pm 6.4\%$ (Thr) para U87; $68.5 \pm 12.8\%$ (Ser) e $95 \pm 5\%$ (Thr) para GBM95 e $71.7 \pm 5.1\%$ (Ser) e $83.4 \pm 2\%$ (Thr) para GBM02.

Conclusões: As linhagens testadas apresentam alta expressão e fosforilação de RB, corroborando os dados da literatura. Estas linhagens serão transfectadas com RNAi de silenciamento para RB e a resposta das células frente à indução de apoptose será avaliada.

Palavras-Chave: Glioblastoma, RB, ciclo celular, fosforilação

Apoio Financeiro: Apoio financeiro: FAPERJ, IBRO, TWAS, FUJB, CNPq.

Guanosine modulates BDNF immuncontent and protects organotypic hippocampal slice culture against glucose and oxygen deprivation

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Objective: Our group has already demonstrated some neuroprotective actions of guanine based purines (GBPs) in both, in vivo (anticonvulsant) or in vitro assay (against oxygen and glucose deprivation in hippocampal slices), however the exactly mechanism need to be elucidate. Organotypic hippocampal slice cultures are a valuable alternative to animal experiments, whereas they maintain the cell architecture and interneuronal connections, allowing a long in vitro period of survival. They have been used to investigate molecular mechanisms involved in cytotoxicity, such as during oxygen and glucose deprivation. In order to investigate the mechanism which GBPs exert their neuroprotective actions we investigated the effect on viability of guanosine treatment against oxygen and glucose deprivation in organotypic hippocampal slice cultures and trying to clarify the mechanism of the neuroprotective effect observed, we presents preliminary results demonstrating a possible involvement of the brain derived neurotrophic factor (BDNF) with the neuroprotective effect observed.

Methods: Organotypic hippocampal slice cultures were prepared from 6 to 8-days-old male *Wistar* rats and were maintained in control conditions for 14 days. At day 14, the slices cultures were exposed to a model of oxygen and glucose deprivation. During the ischemic conditions, some of the slice cultures received guanosine 100uM. After ischemia, the slice culture medium was changed with control medium and was also added guanosine 100uM for the next 24 hours. After the recovery period the cellular damage was assessed by fluorescent image analysis of propidium iodide (PI) uptake. After the cellular damage assay, the slice cultures were homogenized and the immuncontent of BDNF was verified using Western blotting.

Results: Guanosine treatment (100uM, during the ischemia period and the 24 hours of recovery) protected slice cultures against oxygen and glucose deprivation (30 ± 7.2 % of control, n=10, p<0.05). Our preliminary results demonstrated that in both control and ischemic conditions guanosine decrease (decreases 23 ± 8.2 [n=6] and 37 ± 7.0 % [n=3] in relation of control conditions without GUO) the immuncontent of BDNF in the slice culture homogenate whereas there was no decrease after ischemia and 24 hours of recovery in control conditions.

Conclusion: Guanosine protects the organotypic hippocampal slices against hypoxic ischemia insult and regulates the immuncontent of BDNF in control and injured slices

Palavras-Chave: Guanosine, Ischemia, BDNF, Neuroprotection

Apoio Financeiro: CNPq

Atorvastatina reduz a incidência de convulsões induzidas pelo Ácido Quinolinico em camundongos e previne a morte celular hipocampal

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As estatinas (inibidores da enzima 3-hidróxi-3-metilglutaril coenzima A (HMG-CoA) redutase) tem sido demonstradas como possíveis fármacos neuroprotetores. Avaliamos o efeito neuroprotetor da atorvastatina na excitotoxicidade glutamatérgica induzida pelo Ácido Quinolinico (AQ). Camundongos machos adultos foram pré-tratados com atorvastatina via oral (1 ou 10 mg/kg) ou salina (0,9%), diariamente, durante sete dias. Os animais foram submetidos à cirurgia estereotáxica para implantação da cânula guia no ventrículo lateral. O AQ foi infundido i.c.v (36,8 nmol) e os animais foram observados para ocorrência de convulsões. Após 24 horas, os animais foram sacrificados e fatias hipocampus foram avaliadas quanto à viabilidade celular pelos métodos de redução do MTT e captação de iodo de propídio (IP). A administração de 1 mg/kg de atorvastatina diminuiu em 13% ($P>0,05$) a incidência convulsões induzida pelo AQ. Entretanto, o pré-tratamento de atorvastatina 10 mg/kg foi capaz de prevenir 50% ($P<0,05$) das convulsões induzidas por AQ. Houve uma diminuição da viabilidade celular verificada pelo método do MTT (em D.O., $1,194\pm0,280$) 24hs após infusão i.c.v de AQ em relação ao grupo controle ($1,554\pm0,220$). Este efeito foi prevenido pelo pré-tratamento com atorvastatina 10mg/kg, tanto nos animais que não convulsionaram ($1,572\pm0,281$) quanto naqueles que convulsionaram ($1,529\pm0,269$; $P<0,05$) após a infusão do AQ. A atorvastatina per se não apresentou mudanças na redução do MTT ($1,534\pm0,254$). A quantificação de morte celular necrótica mostrou aumento da captação do IP nos animais infundido com AQ ($0,402\pm0,100$) em relação ao controle ($0,167\pm0,037$). Os animais tratados com atorvastatina 10mg/kg e AQ, apresentaram diminuição da captação de IP ($0,239\pm0,054$; $0,225\pm0,09$; não convulsionados e convulsionados; $P<0,05$). A atorvastatina per se não apresentou mudanças na captação de IP ($0,158\pm0,040$). A atorvastatina previne em 50 % dos animais as convulsões induzidas pela infusão i.c.v. de AQ e reduz a morte celular hipocampal. Desta forma, as estatinas apontam como uma possível estratégia na prevenção do dano cerebral causado pela exacerbação da toxicidade glutamatérgica em enfermidades neurodegenerativas **Palavras-Chave:** Atorvastatina, Acido Quinolinico, neuroproteção, convulsão

Apoio Financeiro: CAPES, CNPq, IBN(Net) # 01.06.0842-00.

Effect of long-term cyclosporine treatment on brain glutamatergic system

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Objective: Cyclosporine (CsA) dependent neurotoxicity occurs in up to 60% of transplant patients and the clinical predisposition and mechanisms of CsA-induced neurotoxicity remain controversial and poorly understood. Glutamate is the major excitatory neurotransmitter in CNS and increased levels of glutamate in synaptic cleft could render neurons susceptible to death. In the extracellular space, glutamate is taken up by astrocytes via glutamate transporters and in the astrocyte, glutamate is converted to glutamine via the astrocyte-specific enzyme, glutamine synthetase (GS). In this order, we decided to investigate the glutamate uptake and GS activity in hippocampus, striatum and cortex slices of rats submitted to chronic CsA treatment.

Material and Methods: Male Wistar rats were divided in control group (received vehicle - corn oil), and treated group received 15 mg/kg, by daily gastric gavage during 8 weeks. Glutamate uptake in slices of hippocampus, striatum and cortex was performed according to Thomazi et al. (2004). Incorporated radioactivity was measured using a liquid scintillation counter (Wallac 1409). GS enzymatic assay was performed as previously described by Petito et al. (1992) with some modification. CsA treatment did not affect glutamate uptake in hippocampus when compared to control group (0.73 ± 0.20 and 0.73 ± 0.19 , respectively) and even in striatum (0.84 ± 0.28 and 0.81 ± 0.22 , respectively) and cortex slices (0.95 ± 0.33 and 1.02 ± 0.31). GS activity was also not affected by CsA treatment when compared to controls, neither in hippocampus (1.52 ± 1.07 and 1.31 ± 0.42 , respectively), striatum (1.20 ± 0.43 and 1.44 ± 0.59) and cortex (1.99 ± 0.43 and 2.10 ± 0.35).

Conclusion: In the present study chronic CsA treatment did not alter glutamate uptake and GS activity, suggesting that CsA-induced neurotoxicity might not be related with these parameters of the glutamatergic system. Despite that, further studies are necessary to better elucidate the involvement of others parameters of glutamatergic system on CsA neurotoxicity.

Palavras-Chave: Cyclosporine, neurotoxicity, glutamate uptake, glutamine synthetase

Apoio Financeiro: IBNnet, CNPq

Transfer of zinc and cadmium into the nervous tissue

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Objectives: In view of the extensive metabolic role of zinc in all animal tissues (with an essential participation in the glutamate synaptic transfer) and the well known antagonistic and synergic action of cadmium with this metal, we propose to examine, preliminarily the transfer of these metals into brain tissues. Some authors [1] suggest that cadmium would be hardly taken up by the brain parenchyma because of the protection by the barrier system. This uptake by brain and cerebellum will be compared to the liver and examined with the administration of Zn and Cd alone, and both associated in various concentrations. As the uptake could be enhanced by inducing metallothionein synthesis, copper will also be analyzed. **Methods:** Daily intraperitoneal administration (Wistar rats) during 10 days of Zn and varying concentrations of Cd, alone, as well as associated with Zn. Control with administration of isotonic NaCl solution. Determination of the total content of Zn, Cd and Cu by atomic absorption spectrophotometry (brain, cerebellum and liver). **Results:** *Zinc* – In the liver there was significant increase of its concentration, even by Cd treatment alone. No increase was observed in the brain and the cerebellum. In the liver this uptake was enhanced by the addition of Cd. *Cadmium* – Not detected in the controls, showed a proportional increase in the brain, cerebellum and liver. Zn had no influence on the uptake by brain and cerebellum. *Copper* – No difference was detected in all tissues examined. **Conclusions:** There is a significant transfer of Cd into the brain and cerebellum in the experimental conditions, but not of Zn. The enhancement of the uptake of Zn by Cd in the liver may be due to the increase of the metallothionein synthesis. This was not observed in the brain or cerebellum. These eventual increases (Zn or Cd), however, did not reflect upon the concentrations of Cu, which can also be sequestered by metallothionein. **References:** [1] Biometals 15:371, 2002.

Palavras-Chave: zinc, cadmium, neurotoxicity, metallothionein

Apoio Financeiro: CAPES; Fundação Luiz Englert

Neurotoxicidad y actividad anticonvulsivante de los extractos etanólicos de hojas secas de *Rollinia fendleri* (ANNONACEAE) y corteza de *Cusparia trifoliata* (RUTACEAE).

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OBJETIVOS: La *Rollinia fendleri* y la *Cusparia trifoliata* son utilizadas en la medicina tradicional venezolana como antiparasitarios e hipoglicemiantes (1). Sin embargo, existe escasa información sobre su toxicidad (2), y actividades biológicas a nivel del sistema nervioso central (SNC). En vista del auge de su uso en la población Venezolana y el reporte de efecto sedante por otras especies relacionadas, evaluamos su potencial neurotóxico y posible efecto anticonvulsivante.

MÉTODOS: El extracto etanólico de las hojas de *R. fendleri* (ER) y de la corteza de *C. trifoliata* (EC), fue administrado por vía intraperitoneal (ip), en ratones NMRI. Se determinó DT₅₀ y DL₅₀, neurotoxicidad mediante el Rotarod (3) y la DE₅₀ de su potencial anticonvulsivante en el modelo de electroshock máximo (MES).

RESULTADOS:

Ambos extractos producen los siguientes efectos: pérdida del tren posterior (ER= 350 mg/Kg ; EC= 650mg/kg), con similares tiempo de inicio y duración de acción (min) 17±0,9; 35±3, respectivamente. ER produce sedación (1000 mg/Kg) y ptosis palpebral (2000mg/Kg), mientras que EC produce convulsiones en dosis de 500 mg/Kg (tiempo de inicio= 8±3 min, duración de acción= 20±8 min). La DT₅₀ de EC es 325 mg/Kg y de ER 625 mg/Kg. La DL₅₀ es: ER (>2000mg/Kg) y EC (450 mg/kg). ER no presenta efectos neurotóxicos significativos a 500mg/kg mientras que EC los presenta a 350 mg /Kg). ER (500 mg/kg) protege del evento convulsivo inducido por MES (tiempo efecto pico 30 min) mientras EC protege a 250 mg/kg (tiempo efecto pico 4h).

CONCLUSIONES

ER mostró menor toxicidad que EC y produce un efecto anticonvulsivante a dosis menores de la DT₅₀. EC tuvo una DE₅₀ menor, aunque cercana a la DT₅₀, lo que indica que su margen terapéutico es mas estrecho que ER.

REFERENCIAS: 1.-Planta Med. 65:250-254, 1999. 2.-Nat Prod Res. 21(3): 254-9.,2007. 3.- Cleve Clin Q 51: 293-305, 1984.

Palavras-Chave: Rollinia fendleri, Cusparia trifoliata, neurotoxicidad, anticonvulsivante.

Apoio Financeiro: CDCH 06.00.6438.2006

THERMOGRAPHIC EVALUATION AFTER SCIATIC NERVE CRUSH IN RATS

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OBJECTIVE: The objective of this study was analyze the hindpaw skin temperatures after a right sciatic nerve crush in rats using a thermographic method.

METHODS: The experiment was carried out on 35 adult male Wistar rats. Animals were anesthetized and the right sciatic nerve was exposed. 19 rats received a crush lesion and 16 rats were sham-operated. Thermographic measurements were obtained prior to surgical procedure and 1, 3 and 7days after nerve injury. Images the surface temperature (plantar hindpaw skin) were digitally recorded at an interval de 2 min using an infrared digital thermographic camera (ELETROPHYSICS, model PV320T, sensitivity at de 0.08°). The thermographic procedures were conducted in a constant- room temperature maintained at 23°C ($\pm 1^{\circ}\text{C}$) and humidity of 30-50%. The rats were lightly anesthetized with sodium pentobarbital (30 – 40 mg/kg, i.p) and placed in a prono position in a body-shaped depression; and the dorsal surface of hindpaw was fixed with an adhesive tape. The left and right plantar hindpaw skin temperatures were measured and the difference between the right and left paws temperature (TD) was calculated (symmetry of the temperature). The data were analyzed using a T test and showed as mean $\pm$ s.e.m.

RESULTS: Before the surgical procedure no differences were found in the TD between the Sham (-0.16 ± 0.24) and Crush (0.21 ± 0.16) groups. Furthermore, in the 1st and 3rd postoperative days, the Crush group showed asymmetric temperatures between right and left paws (0.93 ± 0.18 and 0.46 ± 0.15 , respectively) and these values were different to the Sham group (0.12 ± 0.15 and -0.02 ± 0.08 ; $p < 0.05$). However, in the 7th day the temperature of both hindpaws showed a symmetrical value again. The qualitative observation of the thermographic images point out that the right hindpaws in the crush rats were abnormally hotter than the left side.

CONCLUSION: The thermography contributes to detect in the experimental crush model the existence an early modification in the hindpaw temperature in injured side in relation to the control side. Thermography has proved to be useful as research tool in diagnostics and evaluations of injuries in human.

Palavras-Chave: Thermography, sciatic nerve crush, temperature, hindpaw

Apoio Financeiro: CAPES

Administration of zinc and cadmium (*in vivo*) and biosynthesis of metallothionein in the nervous tissueBraga, M.M.¹; Leite, M. C.¹; Guerra, A. S.¹; Souza, D. O.¹; Dick, T.¹ - ¹UFRGS - Bioquímica

Objectives: The administration of Zn causes a higher concentration of this metal in the liver enhanced by cadmium with and without Zn, but not in the brain or cerebellum. This may be due to the induced biosynthesis of metallothionein (MT-I/II). As the largest proportion of these metals may be sequestered inespecifically by many metabolites and proteins, and specifically by MT-I/II, only a smaller part may be available for physiological and toxic actions. This can be estimated by the differential determination of d-aminolevulinate dehydratase (d-ALA-D), without and with dithiotreitol (DTT). The MT-I/II and the d-ALA-D, with and without DTT [1], will be determined in brain, cerebellum and liver. **Methods:** Daily intraperitoneal administration (Wistar rats) during 10 days of Zn and varying concentrations of Cd, alone, as well as associated with Zn. Control with administration of isotonic NaCl solution. Determination of MT-I/II by immunoenzyme assay (ELISA) in the three tissues. Determination of d-ALA-D (with and without DTT) in the three tissues. **Results:** *Liver* - Compared to the controls, increased concentration of MT-I/II by administration of Zn (alone) and Cd (alone) as well as simultaneous administration. No significant change of the total or differential activity of d-ALA-D. *Brain and Cerebellum* - Slight increase of the concentration of MT-I/II observed by the Cd treatment. The total or differential activity of d-ALA-D was not altered, but the difference (%) was much higher in these tissues as in the liver. **Conclusions:** Cd induces slight increase of MT-I/II in brain and cerebellum and pronounced increase in liver. Zn has shown only inducing effect in liver. The differential activity of d-ALA-D (with and without DTT) in all these tissues did not show variation of the toxic availability of the metal ions, indicating an efficient sequestration in the case of the cerebellum and brain. The differential activity in these tissues, higher as in the liver, indicated a larger toxic availability. **Reference:** [1] Bellinaso, M.L. - Dissertação de Mestrado – Departamento de Bioquímica - UFRGS, Porto Alegre, 1985.

Palavras-Chave: zinc, cadmium, neurotoxicity, aminolevulinate dehydratase, metallothionein

Apoio Financeiro: CAPES, Fundação Luiz Englert

Beta- NADH diminui a sensibilidade da succinato desidrogenase à inibição pelo metilmalonato

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A acidemia metilmalônica é um erro inato do metabolismo autossômico recessivo caracterizado por acúmulo tecidual de ácido metilmalônico (MMA) e outros metabólitos. Foi demonstrado que o MMA é capaz de inibir a succinato desidrogenase (SDH) cerebral *in vitro*. Vários são os fatores que interferem na atividade e estabilidade da SDH, como a presença de ativadores (CoQH₂, ATP e ITP) e de inibidores (malato, malonato e oxalacetato) e o tecido usado. Além disso, as condições do meio reacional alteram a sensibilidade da enzima a inibidores, como o oxalacetato e malonato. Contudo, até a presente data, não se investigou se variações no meio reacional, particularmente aquelas que modificam o estado redox da enzima, alteram a sensibilidade da SDH ao MMA. Neste estudo avaliamos se a pré-incubação com b-NADH modifica a atividade da SDH e sua inibição por MMA em frações enriquecidas de mitocôndrias do córtex cerebral de ratos.

Métodos

Foram usadas frações enriquecidas em mitocôndrias obtidas de cortex cerebral de ratos Wistar machos adultos por centrifugação diferencial. A preparação enzimática foi pré-incubada com b-NADH (0-480 mM) por 20 min e a inibição da SDH pelo MMA (2,5; 5 e 10 mM) foi avaliada, determinando os parâmetros cinéticos $K_{m_{ap}}$, $V_{máx}$ e K_i espectrofotometricamente pela oxidação do INT na presença de concentrações variáveis de succinato.

Resultados

Valores de significativamente diferentes de $K_{m_{ap}} = 0,216$ e $K_{m_{ap}} = 0,272$ mM ($p < 0.05$) foram obtidos para a enzima pré incubada com b-NADH e não incubada, respectivamente, sem que houvesse alteração no V_{max} . O K_i mostrou-se diferente ($p < 0.05$) para a enzima incubada com b-NADH ($K_i = 21$ mM), e incubada sem b-NADH ($K_i = 11$ mM), mostrando que a enzima pré-incubada com NADH é menos suscetível à inibição por MMA.

Conclusão

Os resultados indicam que a pré-incubação com um meio redutor (presença de NADH), protege a enzima contra a inibição por MMA. Estes dados indicam que condições que favorecem a oxidação dos grupos prostéticos da SDH (estresse oxidativo) podem facilitar a inibição da SDH, e consequentes crises metabólicas por acúmulo de MMA.

Palavras-Chave: succinato desidrogenase, metilmalonato, estresse oxidativo, regulação redox

Apoio Financeiro: Capes, CNPq (500120/2003-0)

METHYLMERCURY CHRONIC ADMINISTRATION EFFECT ON ELECTRON TRANSPORT CHAIN IN MICE BRAIN

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Objective: Methylmercury (MeHg), an environmental contaminant, has been shown to cause neurological deficits in both animals and humans. The main postulated mechanisms of neurotoxicity are likely associated with intracellular calcium homeostasis impairment, glutamate homeostasis alteration and oxidative stress induction. However, the participation of the mitochondrial function in these toxic events is virtually unknown. Therefore, the objective of the present work was to investigate the effect of MeHg chronic administration on the activities of the complexes I, II and II-III, of the respiratory chain in mice cerebral cortex homogenates.

Methods: Sixty-day-old male mice were treated with different MeHg doses (20 and 40 mg . L⁻¹) diluted in drinking water freely available, during eleven days, as follows: 1) Control group: drinking water *ad libitum*; 2) Low MeHg dose; 3) High MeHg dose. Enzyme activities were measured spectrophotometrically.

Results: Results demonstrated that MeHg chronic administration provoked a significant complex I inhibition (up to 26%) [$F_{(2,17)} = 10.27$; $P < 0.001$] in a dose-dependent manner ($b = -0.67$; $P < 0.001$). It was also observed a significant reduction in complexes II (up to 37 %; [$F_{(2,15)} = 10.46$; $P < 0.001$]) and II-III (up to 34 %; [$F_{(2,14)} = 13.62$; $P < 0.001$]) in MeHg treated mice.

Conclusions: These data showed a MeHg-mitochondrial impairment in mice brain suggesting that energy depletion could be involved in the neurotoxic mechanisms induced by MeHg.

Palavras-Chave: methylmercury, neurotoxicity, respiratory chain complex activities

Apoio Financeiro: FINEP-research-grant “Rede Instituto Brasileiro de Neurociência (IBN-Net)”#01.06.0842-00, CNPq and FAPESC.

RESVERATROL AND QUERCETIN MODULATE PI3-K/AKT PATHWAY AND DECREASE THE CELL NUMBER OF GLIOMA LINEAGE C6 AND U87MG

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Objectives: In this work, we analyzed the capacity of resveratrol and quercetin to induce cell death in the glioma lineage C6 (from rat) and U87MG (from human) and the potential pathways involved in the cell response to these compounds.

Methods: The gliomas were treated for 48h with the drugs in the following doses (uM): resveratrol -10 and 50; quercetin – 25 and 100. The number of cells was determined by counting in a hemocytometer. Data are presented as a percentage of control in three different experiments and * indicates $p < 0,05$ in ANOVA followed by Tukey's test. We investigated the phosphorylation level of Akt, the mRNA expression and the immunocontent of PTEN, the mRNA expression of p53, the immunocontent of Bax through Western Blot and qRT-PCR, after 4h of treatment.

Results: After 48h, the number of C6 cells were reduced from 100(control cells) to $*17,4 \pm 3,4$ (R10), $*3,4 \pm 0,5$ (R50), $*40,9 \pm 3,6$ (Q25), $*19,9 \pm 1,7$ (Q100) and $*7,1 \pm 2,2$ (R50+Q100). In U87, the number of cells were reduced from 100 (control cells) to $*68,1 \pm 10,4$ (R10), $*42,4 \pm 7,8$ (R50), $*71,3 \pm 6,1$ (Q25), $*53,2 \pm 7,6$ (Q100) and $*52,9 \pm 11$ (R10Q25). These compounds decreased the Akt phosphorylation to $*71,54 \pm 13$ (R10) and $*47 \pm 10$ (Q25) (C6); $*73 \pm 6$ (R25) and $*36 \pm 7$ (Q25) (U87MG). There were no differences in Bax and PTEN immunocontent but resveratrol increased the PTEN mRNA by $*2,5$ times. The same increase was observed to p53 in all treatments.

Conclusion: Resveratrol was more effective than quercetin in decreasing cell number and no significant additive effect was observed. The C6 lineage is more sensible than U87. The drugs decreased the phosphorylation of Akt, a protein involved in proliferation and survival, and increased the mRNA of p53, that is a DNA damage sensor and a tumor suppressor. We have previously demonstrated that both drugs could protect hippocampus slices against cell death induced by oxygen and glucose deprivation (1,2). Due to their effect in protect healthy brain tissue and destroy tumor cells, resveratrol and quercetin may be used against tumor development and progression as well as in the prevention of neurodegenerative maladies.

Reference: 1-Neurobiol Dis 24:170, 2006; 2-AntiCancer Drugs 17:663, 2006.

Palavras-Chave: Resveratrol, Quercetin, Glioma

Apoio Financeiro: CNPq, FAPERGS

Effects of adenosine A_{2A} receptor on LPS-induced aspartate uptake in primary astrocyte culture

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Objectives: Under physiological conditions, the extracellular concentration of glutamate (Glu) is controlled by a family of excitatory amino acid transporters (EAATs). The ability of astrocytes to uptake glutamate via EAATs during excitotoxicity conditions may protect neurons from damage. Previous results showed that astrocytes exposed to lipopolysaccharide (LPS) presented an increase in the Glu uptake. Adenosine is a neuromodulator in the central nervous system (CNS) controlling neurotransmitters release including Glu. Adenosine via activation of A_{2A} receptor subtype is strictly involved in neuroinflammatory process, and its blockade preventing neuroinflammation. This study examined the role of adenosine A_{2A} receptors on LPS induced Glu uptake increase in cultured astrocytes. **Methods:** Primary cortical astrocyte cultures were prepared from 1 day-old rats and were used at confluency. EAATs activity was determined using the [³H]d-aspartate uptake. The cells were pretreated with CGS 21680 (30nM), an A_{2A} receptor agonist or SCH 58261 (50nM), an A_{2A} antagonist for 15 minutes then the astrocytes were treated with LPS (10 µg/ml, 30min). **Results:** The LPS treatment increased [³H]d-aspartate uptake (7,16±0,14, vs 5,94±0,22nmol[³H]d-aspartate/mg protein/minute) but the pretreatment with CGS 21680 or SCH 58261 did not prevent this effect (7,59±0,45; 7,61±0,26 nmol[³H]d-aspartate/mg protein/minute). **Conclusions:** Our findings suggest that A_{2A} receptors do not affect the LPS action on glutamate uptake. However, the effects on A_{2A} receptors remain to be more clarified because this receptor is a promising target to neuroprotective strategies.

Palavras-Chave: astrocyte, LPS, A_{2A} agonist, A_{2A} antagonist, aspartate uptake

Apoio Financeiro: IBN.net/Finep/Fapesp, CNPq, Fapergs

SWIMMING TRAINING MODIFIED OXIDATIVE STRESS PARAMETERS AND MITOCHONDRIAL FUNCTION IN BRAIN MICE.

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Objectives: In the last decade it became clear that regular exercise beneficially affects brain function. Exercise-induced modulation of the redox state is an important means, by which exercise benefits brain function, increasing the resistance against free radical induced damage and facilitating the recovery from oxidative stress. The aim of the study was evaluated oxidative stress parameters and the activities of the respiratory chain complexes (RCC) in brain mice exposed to a gradate swimming training. Swiss male mice were divided in two groups of ten animals: Exercises - E (submitted to a gradual swimming training during seven days) and Sedentary - S (allowed swimming for just thirty seconds each day).

Methods: Oxidative stress parameters including lipoperoxidation, measured by thiobarbituric acid reactive substances (TBARS), glutathione (GSH) levels, activity of the antioxidant enzymes glutathione peroxidase (GPx) and glutathione reductase (GR) and mitochondrial activities of the respiratory chain complexes (RCC; I and II) were determined in two different brain parts (cerebrum and forebrain).

Results: The results presented here demonstrated that the exercise training significantly decreased lipoperoxidation in cerebrum (47.7 ± 1.6 nmol/g; $P < 0.05$) when compared to sedentary group (65.2 ± 6.4 nmol/g), while the glutathione levels significantly decreased in exercise group ($E = 1.8 \pm 0.4$ / $S = 4.0 \pm 0.6$ μmol/g; $P < 0.01$). All parameters related to oxidative stress in forebrain was not modified with the exercise training, including the antioxidants enzymes. In addition, we observed that the exercise cause a significant decrease in the activity of complex I in cerebrum ($E = 39.2 \pm 1.5$ / $S = 49.2 \pm 3.4$ mmol/min/mg; $P < 0.05$), however in forebrain the complex I was significantly increased with the exercise ($E = 57.7 \pm 3.7$ / $S = 48.5 \pm 2.3$ mmol/min/mg $P < 0.05$).

Conclusion: In conclusion, the gradual swimming training intervened in the parameters of oxidative stress and mitochondrial function and these changes were distinct agreement of brain parts.

Palavras-Chave: SWIMMING TRAINING, OXIDATIVE STRESS, MITOCHONDRIAL FUNCTION

Apoio Financeiro: CNPq FAPESC e IBN-NET, Redoxoma

Efeitos comportamentais da administração periférica de citrato de alumínio em ratos submetidos ao modelo de memória do labirinto em T elevado

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Objetivos: Investigar se a administração periférica de citrato de alumínio (CA) interfere na aprendizagem de ratos submetidos a um modelo de memória e comparar o desempenho destes ratos intoxicados e dos ratos senis submetidos ao mesmo modelo.

Métodos: Foram utilizados ratos Wistar machos divididos em 3 grupos: experimental (10 sujeitos de 6 meses, submetidos à intoxicação aguda de 50 mg/kg de CA por administração intraperitoneal), controle (10 sujeitos de 6 meses) e senil (10 sujeitos com 14 meses). 24 horas após a intoxicação, todos os sujeitos foram submetidos ao teste do labirinto em T elevado (1), o qual se constituiu de uma linha de base, duas esquivas inibitórias e uma fuga, mensurando-se a latência das respostas. 72 horas depois, foi realizado o teste de memória (uma esquiva inibitória e uma fuga).

Resultados: Na linha de base, na esquiva inibitória 2 e no teste de fuga não houve diferenças entre os grupos. Já na esquiva inibitória 1, o grupo experimental permaneceu no braço fechado por mais tempo que o grupo senil. No teste de memória houve o aumento da latência (tempo de permanência no braço fechado) do grupo experimental em relação aos demais. Em relação aos testes de fuga, o grupo senil apresentou latência de fuga menor que a do grupo controle.

Conclusão: Os resultados sugerem que o grupo experimental não teve aprendizado prejudicado para os testes de esquiva inibitória; os animais do grupo controle e os do grupo experimental permaneceram por mais tempo no braço fechado (esquiva inibitória), caracterizando-se uma tendência natural desses animais. Quanto ao teste de fuga, os grupos experimental e controle demoraram mais para sair do braço aberto e entrar, com as quatro patas, no braço fechado (fuga), o que sugere efeito amnésico ou ansiolítico da intoxicação, visto que os animais expostos nos braços abertos de labirintos elevados fogem rapidamente para os braços fechados. Estes resultados preliminares não nos permitem conclusões mais aprofundadas, pois ainda estamos testando doses maiores e menores com outros grupos e com a mesma metodologia, para podermos avançar em interpretações mais consistentes.

Referências: 1- Biochemistry and Behaviour 49:3, 1994.

Palavras-Chave: Intoxicação, memória, modelo animal, alumínio

Apoio Financeiro: Universidade Federal do Pará e Fapespa

IMPAIRED BRAIN MITOCHONDRIAL FUNCTIONING IN LDL RECEPTOR KNOCKOUT FEMALE MICE

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Objectives: It is believed that mitochondrial dysfunction could play a role in atherosclerotic disease, a leading cause of death in westernized societies. Therefore, the objective of the present work was to investigate the activities of the respiratory chain complexes (RCC; I, II and IV) in the LDL receptor knockout female mice (LDLr -/- KO mice) brain mitochondria. **Methods:** LDL receptor null mice were randomly divided into two groups and were fed with either standard or cholesterol (1.25%) supplemented diets. Wild-type mice (WT) were used as controls. Mitochondria from cerebral cortex, cerebellum and hippocampus were isolated by conventional differential centrifugation. RCC activities were measured spectrophotometrically. **Results:** Cholesterol blood concentrations were 97.0 ± 9.3 ; 270.2 ± 13.9 ; 984.1 ± 54.2 mg/dl for WT, knockout animals and null animals fed with cholesterol. It was observed a significant increase in complexes II and IV activities in cerebral cortex (complex II: $F_{(2,12)} = 10.18$; $P < 0.01$; complex IV: $F_{(2,12)} = 7.36$; $P < 0.01$) from both groups of null animals, and the effect was always higher in the cholesterol fed group. In addition, complexes I and IV were significantly reduced in the cerebellum (complex I: $F_{(2,12)} = 10.18$; $P < 0.01$; complex IV: $F_{(2,12)} = 8.28$; $P < 0.01$), while complex II activity remained unchanged. Similar results were observed for complexes I, II and IV in the hippocampus (complex I: $F_{(2,12)} = 17.18$; $P < 0.001$; complex IV: $F_{(2,12)} = 5.96$; $P < 0.05$). **Conclusion:** These data strongly indicate that RCC activity is compromised in LDL receptor knockout mice brain. In addition, the altered mitochondrial function appears to be related to the cholesterol blood concentrations. Finally, it could be proposed that impairs brain electron transfer chain activity could compromise energy metabolism in brain from atherosclerotic patients. **Palavras-Chave:** Atherosclerosis, oxidative stress, respiratory chain

Apoio Financeiro: CNPq FAPESC e IBN-NET, Redoxoma

ATP-induced cell death in GL261 mouse glioma line

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Objectives: Extracellular nucleotides participate in diverse processes in the Central Nervous System (CNS). Specially, ATP is implicated in proliferation of different neural cells, neurotransmission and synaptic modulation **(1)**. In contrast, this molecule can induce death in some cells, mainly via P2X₇ receptor **(2)**. Gliomas are the most common CNS tumor and exhibit high resistance to conventional treatments and poor prognosis. Our study suggests the involvement of purinergic system in glioma growth and invasion since extracellular ATP induces neuronal death without affecting glioma survival **(3)**. The objective of our work was to investigate the cytotoxicity of ATP in mouse GL261, rat C6 and human U87 glioma cells.

Methods: Glioma cells lines were treated with ATP 5mM for 2h, and cellular viability protocols such as lactate dehydrogenase (LDH) release, propidium iodide (PI) incorporation and mitochondrial viability (MTT) were performed. Pharmacological experiments using purinergic agonists such as adenosine, ADP, UTP and BzATP and antagonists such as TNP-ATP and oATP were used. In RT-PCR experiments, mRNAs for the purinergic receptors were analyzed.

Results: The experiments indicated that only GL261 was sensitive to ATP (9.3 ± 3.1 vs 64.6 ± 12.1 % LDH release, $p < 0.05$). This cell death was observed only at mM concentrations of ATP and the death could be detected as soon as 1h after ATP treatment. BzATP, at 300 μ M, stimulated cell death (100 vs 53.4 ± 5.7 % cell viability, $p < 0.05$) and oATP 1mM blocked the effect of ATP 5mM (78.5 ± 7.7 %, $p < 0.01$), on cellular viability. In contrast, other gliomas showed a high resistance to ATP cytotoxicity. The P2X₇ mRNA was found in gliomas GL261 and U87.

Conclusions: The pharmacological and expression data suggest that P2X₇ plays an important role in ATP-induced death in GL261, but these data do not exclude other receptor(s) to participate in this effect. Furthermore, the presence of P2X₇ mRNA was not sufficient to cause cellular death, since the ATP-resistant cell line U87, also presents the transcript.

References: 1- Pharmacol Rev 58(1):58, 2006; 2- J Physiol 519: 335, 1999; 3- J Neuro Oncol 71(2):135, 2005.

Palavras-Chave: glioma; ATP; cell death; P2X₇

Apoio Financeiro: CNPq; FAPERGS

PROTECTIVE EFFECTS OF PROLACTIN IN PRIMARY ASTROCYTES CELL CULTURE EXPOSE TO METHYLMERCURY

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Objectives: This study aimed to investigate protective effects of prolactin (PRL) on disturbances caused by MeHg (methylmercury) in cellular viability, GFAP expression and mitogenesis in astrocyte cell cultures of newborn mice.

Methods: MeHg (5µM) exposure for 18 h was performed in serum free medium. We also evaluated the PRL treatment and co-treatment of MeHg (5 µM) + PRL (0.1, 1 and 10 nM) in order to analyze cellular proliferation by mitotic index (IM). The expression of GFAP was assessed by immunofluorescence GFAP stain and cellular viability by MTT method (1).

Results: Our results showed that MeHg treatment decreased cellular viability (about 85% ± 6.0 SD), decreased rate of cellular proliferation (Control: 0,90 and MeHg: 0,05, number of cell in metephasis was scored from cellular pool of the sample of each group) and changing GFAP standard stain. We also demonstrated that PRL treatment protected against MeHg toxicity, recovered the rate of cellular proliferation (PRL 1nM: 0,20 and PRL 10nM: 0,45) and integrity of the GFAP filaments.

Conclusion: These findings confirm the hypothesis that PRL can act as a cytoprotective agent in primary astrocytes culture expose to MeHg.

References: 1- J of Immunol Methods 65:55, 1983.

Palavras-Chave: methylmercury, prolactin, astrocytes and cytoprotection

Apoio Financeiro: Capes, Instituto Evandro Chagas and Fadesp

PROTECTIVE EFFECT OF SELENO COMPOUNDS ON THE METHYLMERCURY INHIBITORY EFFECT ON CREATINE KINASE ACTIVITY IN ADULT MICE BRAIN

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Methylmercury (MeHg), an organic form of mercury, has been shown to cause severe and irreversible neurobehavioral and neuropsychological disorders in both humans and animals. The objective of the present work was to investigate the potential protective effect of chronic subcutaneous administration of sodium selenite - Na₂SeO₃ - and diphenyl diselenide - (PhSe)₂ - on various kinases activities in adult Swiss albino male mice exposed to MeHg poisoning. Adult male mice were treated with MeHg dose of 40 mg . L⁻¹ diluted in drinking water during 21 days and the seleno compounds were daily subcutaneously injected (5 µmol . kg⁻¹). Control animals received drinking water *ad libitum*. The activities of pyruvate kinase (PK), adenylate kinase (AK) and mitochondrial creatine kinase (mitCK) were assessed in homogenates or mitochondrial preparations from mice cerebral cortex 24h after the last subcutaneous administration. Enzyme activities were measured spectrophotometrically. It was initially demonstrated that MeHg administration provoked a marked inhibition of mitCK in mice brain (up to 97 % of inhibition). However, PK and AK activities were not modified by the treatment. Next, we observed that the inhibitory effect of MeHg on this mitochondrial enzyme activity was partially prevented by the administration of Na₂SeO₃ and (PhSe)₂. In addition, Na₂SeO₃ alone, elicited a mild but significant inhibition of mitCK. Altogether, these data provide evidence for the first time, that MeHg poisoning provokes a severe inhibition mitCK activity in mice cerebral cortex. Moreover, this effect could be prevented by using seleno compounds. This strongly suggests that impairment of brain energy metabolism might be involved in the MeHg deleterious mechanisms leading to neurotoxicity and that, particularly, (PhSe)₂ could be consider as a neuroprotective agents in MeHg-induced brain poisoning.

Palavras-Chave: methylmercury, creatine kinase, neurotoxicity, seleno compounds

Apoio Financeiro: CNPq, FAPESC and FINEP research grant “Rede Instituto Brasileiro de Neurociência (IBN-Net)” #01.06.0842-00

Tratamento pré e pós-cirúrgico com o gangliosídeo GM1 diminuem a morte neuronal no sistema nervoso central

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O objetivo deste trabalho foi testar os efeitos do tratamento pré e pós-cirúrgico com monosialogangliosídeo (GM1), em diferentes concentrações no bloqueio da apoptose induzida por privação de suporte trófico e sobrevivência pós- cirúrgica de neurônios do sistema nervoso central, *in vivo*. **METODOLOGIA:** Os efeitos do tratamento serão analisados em células ganglionares da retina (RGCs) axotomizadas por ablação do colículo superior seu principal alvo de projeção. Foram usados ratos Lister hooded, recém-nascidos, os quais foram anestesiados por hipotermia e sofreram ablação de colículo superior. Os animais pré-tratados receberam injeção intraperitoneal 2 horas antes da cirurgia de GM1 (40ng/mL e 4µg/mL) ou de salina (0.9% NaCl em água destilada). Os animais pós-tratados receberam injeção intraperitoneal logo após o término da cirurgia (zero), 8h e 16h após a cirurgia de GM1(100ng/mL) ou de salina. Todos os animais tiveram sobrevida de 24hs. A quantificação dos resultados foi realizada em cortes transversais de retina de 10µm observadas em M.O. sob aumento de 100X. Os resultados mostram a porcentagem de células apoptóticas em relação ao total de células na camada de RGCs. **RESULTADOS:** Nos animais pré-tratados o número de células apoptóticas foi reduzido de 7,36±1,68% no controle, para 2,85±1,01% com aplicação de GM1 40ng/mL e para 2,84±1,21% com aplicação de GM1 4µg/mL. Nos animais pós-tratados com GM1 o número de células apoptóticas foi reduzido de 13,1±5,0% para 3,8±0,9% para 0h, de 11,5±3,2% para 3,1±0,4% para 8h e de 12,3±2,9% para 4,0±0,8% para 16 horas. **CONCLUSÃO:** A aplicação de GM1 (100ng/mL) duas horas antes da axotomia, ou até 16hs (40ng/mL e 4µg/mL) após a axotomia têm efeitos semelhantes durante as 24 horas pós-cirúrgicas, reduzindo em aproximadamente 60 a 70% o número de células apoptóticas na camada de RGCs. Estes resultados nos permitem sugerir que o GM1 poderia ser utilizado no tratamento pós traumático ou pós cirúrgico do sistema nervoso central, ou no pré-tratamento preventivo de morte neuronal durante cirurgias programadas.

EFEITO DA SEROTONINA NA PROLIFERAÇÃO CELULAR DE LINHAGEM DE NEUROBLASTOMA DE RATO (B103)

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Objetivo:

A serotonina, 5-hidroxitriptamina (5-HT), é descrita por atuar tanto como neurotransmissor quanto neuromodulador no SNC (SUR et al., 1996), entretanto, poucos trabalhos fazem alusão ao seu mecanismo de ação na proliferação de células neoplásicas do sistema nervoso central. Neste contexto, o objetivo deste estudo foi verificar o efeito da 5-HT na proliferação de neuroblastoma de rato (B103).

Materiais e métodos:

As células de neuroblastoma B103 foram cultivadas em meio de cultura DMEM suplementado com 10% de soro bovino fetal (FBS), em estufa a 37°C, com CO₂. Para o experimento cerca de 50.000 células foram mantidas por 48h. Após este período as culturas foram tratadas com 5-HT (50µM e 100µM). A proliferação celular nos grupos controles e tratado foi medida pela taxa de incorporação de [³H]-timidina (1µCi/ml) em um período de 6h. Em seguida, as células foram rompidas com SDS 0,5% e a taxa de proliferação determinada por cintilação líquida. Os valores foram expressos em percentagem do controle.

Resultados:

Observou-se a diminuição da proliferação com a adição de 5-HT à 50µM (20%±4) e à 100µM (40%±8) em relação ao controle.

Conclusões:

Nossos resultados demonstram que a serotonina é capaz de promover uma modulação negativa sobre a atividade proliferativa do neuroblastoma de rato B103 *in vitro*.

Palavras-Chave: neuroblastoma, serotonina, cultura de células

Apoio Financeiro: CNPq/Universal, CAPES

MIRICETIN PREVENTS MeHg-INDUCED IMPAIRMENT OF MICE MOTOR SKILLS

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Objectives: Methylmercury (MeHg), an environmental contaminant, has been shown to cause neurological deficits in both animals and humans. The main explored mechanisms of neurotoxicity are mostly associated with oxidative stress induction. In this context, flavonoids have been shown to possess protective effects against neuronal death and cognitive impairments in neurodegenerative disorders, mainly by their antioxidant activity. Therefore, the objective of the present work was to evaluate the effect of miricetin chronic subcutaneous administration on the behavioral parameters, open field (spontaneous locomotor activity) and rotarod-task (motor performance) in adult Swiss albino male mice exposed to MeHg poisoning.

Methods: Sixty-day-old mice were treated with MeHg (40 mg . L⁻¹) diluted in drinking water freely available, during twenty-one days. Control animals received drinking water *ad libitum*. Miricetin was daily and subcutaneously administered at doses of 3 and 30 mg/Kg.

Results: Results demonstrated that chronic administration of miricetin prevented the MeHg-induced impairment on animal motor skills. The reduction on the spontaneous locomotor activity (up to 80%) elicited in MeHg-exposed animals was significantly prevented by miricetin administration [$F_{(5,29)}=19.44$; $P \leq 0.01$]. Similarly, the motor performance impairment elicited by MeHg poisoning (up to 61%) was partially but significantly prevented by the used of this flavonoid [$F_{(5,25)}=3.36$; $P \leq 0.05$]. These data demonstrated that the deleterious effect of MeHg intoxication on animal motor skills could be prevented by the miricetin administration.

Conclusions: Therefore, the presented results render miricetin as a promising molecule for further pharmacological studies on the search for potential therapeutic agents against MeHg-induced neurotoxicity.

Palavras-Chave: Methylmercury, miricetin, behavioral parameters, neurotoxicity

Apoio Financeiro: CNPq, FAPESC and FINEP-research-grant “Rede Instituto Brasileiro de Neurociência (IBN-Net)” #01.06.0842-00.

OXIDATIVE STRESS AND ENERGY METABOLISM IMPAIRMENT IS INDUCED IN BRAIN FROM STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Oxidative stress appears to play a role in the neuropathogenesis of diabetes mellitus type I. Representative experimental model of this disorder is the streptozotocin (STZ)-induced diabetic rat, in which oxidative stress and energy metabolism parameters were measured. Diabetic rats were induced by a single intraperitoneal STZ injection (80 mg/kg). Four days after STZ injection hyperglycemic animals (glucose > 16 mmol/L) were randomly divided into two groups. One of them remained in a diabetic state through to the 8-week-end-point of our study, and the other received a daily subcutaneous injection of insulin ([Levemir FlexPen 100units/ml](#) insulin, 1UI). Controls received saline solution. Thiobarbituric acid-reactive substances (TBA-RS) and the non proteic sulfhydryl groups (NPSH) were assessed in the rat brain and blood. In addition, the brain activities of complexes I and II of the respiratory chain were also investigated. Rat body weight was controlled throughout the treatment. STZ-induced diabetic rats showed a significant body weight reduction, which was not prevented by insulin administration [$F_{(2,317)} = 154.54$; $P < 0.0001$]. It was also observed, that NPSH levels were markedly reduced in brain (up to 63 %) and blood (up to 26 %) of STZ rats. A partial protection (reduction of 64 %) was observed in brain of STZ + insulin treated animals [brain: $F_{(2,12)} = 9.42$, $P < 0.01$; blood: $F_{(2,12)} = 7.52$, $P < 0.01$]. On the other hand, the lipid peroxidation parameter, TBA-RS measurement, was significantly stimulated in blood (up to 83 %) of STZ-treated rats [blood: $F_{(2,12)} = 17.69$, $P < 0.0001$]. Moreover, insulin treatment partially prevented the increased TBA-RS levels (21 % of reduction). Brain activities of complexes I and II were also severely reduced in brain of STZ (up to 35 %) [complex I: $F_{(2,13)} = 4.07$ $P < 0.05$; complex II: $F_{(2,12)} = 4.58$, $P < 0.05$]. However, insulin administration, at the used doses, did not prevent the alterations observed in the bioenergetic parameters. Therefore, these novel results strongly indicate that oxidative stress and energy metabolism impairment might be involved in the deleterious mechanisms of glucose neurotoxicity.

Palavras-Chave: glucose neurotoxicity, streptozotocin-induced diabetic rats, oxidative stress

Apoio Financeiro: CNPq, FAPESC and FINEP-research-grant “Rede Instituto Brasileiro de Neurociência (IBN-Net)” #01.06.0842-00

INCREASED CREATINE KINASE ACTIVITY IN BRAIN FROM STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Objective: It is believed that energy metabolism impairment plays a role in the neuropathogenesis of metabolic diseases like diabetes mellitus type I. Representative experimental model of this disorder is the streptozotocin (STZ)-induced diabetic rat, in which the activity of creatine kinase (CK) was investigated.

Experimental design: Diabetic rats were induced by a single intraperitoneal STZ injection (80 mg/kg). Four days after STZ injection hyperglycemic animals (glucose > 16 mmol/L) were randomly divided into two groups. One of them remained in a diabetic state through to the 8-week-end-point of our study, and the other received a daily subcutaneous injection of insulin ([Levemir FlexPen 100 U/ml](#) insulin, 1UI). Controls received saline solution.

Methods: CK was measured by enzymologic method.

Results: It was observed that CK activity was markedly increased in brain from STZ rats (up to 136 %) when compared to control animals [$F_{(2,16)} = 38.93$, $P < 0.001$]. Moreover, insulin administration did not prevent the alterations observed in this bioenergetic parameter [controls: $1.8 \pm 0.6 \mu\text{mol} / \text{min} / \text{mg}$ protein; STZ group: $4.3 \pm 0.6 \mu\text{mol} / \text{min} / \text{mg}$ protein; STZ + insulin group: $4.5 \pm 0.8 \mu\text{mol} / \text{min} / \text{mg}$ protein].

Conclusions: Considering the importance of CK activity for cellular energy homeostasis, our results strongly suggest that ATP levels, transporting and buffering might be compromised in brain from diabetic patients.

Palavras-Chave: glucose neurotoxicity, streptozotocin-induced diabetic rats, creatine kinase

Apoio Financeiro: CNPq, FAPESC and FINEP-research-grant “Rede Instituto Brasileiro de Neurociência (IBN-Net)” #01.06.0842-00

Suplementação crônica de vitamina D melhora resposta a testes de reflexo em ratos SHRSP

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Objetivos: Identificar a ação da vitamina D (colecalfiferol) sobre a pressão arterial e se esta seria capaz de prevenir ou reduzir os déficits neurológicos em ratos suscetíveis ao acidente vascular isquêmico (SHRSP).

Métodos: Machos SHRSP (n=12) com 8 semanas e pesando 142 ± 20 g, após período basal de 7 dias, foram divididos em 2 grupos: controle (óleo de coco) e tratado (colecalfiferol a $12,5 \mu\text{g/kg}^{-1}$). A suplementação foi realizada por gavagem orogástrica durante 9 meses. Os animais foram submetidos à avaliação neurológica através de uma bateria de testes sensório-motores e cognitivos, e a pressão arterial aferida por pletismografia 2 vezes por semana. Os dados foram analisados por teste ANOVA-two-way.

Resultados: A pressão arterial foi reduzida significativamente ($p < 0,05$) no grupo tratado: $198,5 \pm 1$ mmHg versus grupo controle: $212,5 \pm 8$ mmHg no 1º mês de tratamento e esse efeito hipotensor prosseguiu por todo ensaio. Quanto aos testes de força e equilíbrio, não foi observada diferença estaticamente significativa entre os grupos, mas a resposta a estes testes foi agravada a partir do 4º mês de ensaio: de $0'14''00 \pm 0'02''65$ para $0'05''81 \pm 0'00''45$ ($p < 0,05$). No teste cognitivo, representado por labirinto, os animais a partir do 6º mês levaram mais tempo para completar o percurso: $0'12''34 \pm 0'04''58$ para $0'30''11 \pm 0'09''46$ ($p < 0,05$). Curiosamente, o grupo tratado teve resposta prolongada no teste termossensório a partir do 4º mês de suplementação de $0'04''61 \pm 0'00''50$ versus $0'03''13 \pm 0'00''15$. Já nos testes de reflexo, foram observadas alterações a partir do 4º mês de ensaio em ambos os grupos, sendo que apenas no 8º mês de suplementação, o grupo tratado teve um score menor que o controle: $0,06 \pm 0,95$ versus $0,25 \pm 0,03$ ($p < 0,05$).

Conclusões: Os achados revelam efeito hipotensor do colecalfiferol mesmo no processo de envelhecimento, além de demonstrarem que vários testes neurológicos simples podem ser usados para avaliar déficits neurológicos e comportamentais. Verificamos também, que o colecalfiferol foi capaz de reduzir a resposta retardada a testes de reflexo.

Apoio: CNPq e CAPES

Palavras-Chave: vitamina D; ratos SHRSP; testes neurológicos

Apoio Financeiro: CNPq e CAPES

C.06.002

Restrição sensório-motora, mas não anóxia, causa diminuição no número de neurônios no córtex somatossensorial de ratos jovens

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O objetivo desse estudo foi investigar os efeitos da anóxia perinatal (AP), da restrição sensório-motora (RSM) e a combinação de ambas, no número de neurônios do córtex somatossensorial (CSS) de ratos machos jovens. Filhotes foram divididos em 4 grupos (n=5 cada): controle, AP, RSM e anóxia combinada à restrição sensório-motora (AP-RSM). Dois episódios de anóxia (12 min cada) foram realizados no 1º e 2º dias pós-natais (P0 e P1) em uma câmara com fluxo de N₂ a 100%. A RSM consistiu em imobilizar os membros posteriores em extensão (16 h/dia do P2 ao P28) com auxílio de um molde adaptado ao tamanho do animal. No P52, os animais foram perfundidos com solução fixadora, os encéfalos foram removidos e pós-fixados, após, seccionados em vibrátomo (20 µm; -2,30 a -3,30 mm do Bregma) e submetidos a técnica de Nissl. Imagens da camada V do CSS na área de representação dos membros posteriores foram obtidas (inicialmente em 20X e amplificadas em 200% para análise estereológica). O número de neurônios foi contado por área (6692,52 µm² cada), em 5 secções, no hemisfério direito e no esquerdo, totalizando 10 campos contados. Os dados foram somados para todas as secções e foi determinado a média e o e.p.m. do número de neurônios / área para cada animal. Os dados foram comparados pela ANOVA de uma via seguida pelo teste post hoc Fisher's LSD. Somente o grupo RSM apresentou diminuição significativa no número de neurônios do CSS em comparação aos grupos controle e AP (6,32±0,33; 8,17±0,25; 7,84±0,33, respectivamente, p < 0,05). O grupo AP-RSM (7,30±0,28) não foi estatisticamente diferente dos grupos controle e anóxia, apesar de haver uma leve diminuição do número de neurônios, tornando esse grupo igual estatisticamente ao grupo RSM. Os dados sugerem que a diminuição do fluxo de informações aferentes causada pela RSM durante o desenvolvimento pode levar a diminuição neuronal no CSS. Além disso, o protocolo de anóxia utilizado pode ter apresentado um efeito neuroprotetor, visto que a anóxia não alterou o número neuronal, entretanto, quando associada à RSM preveniu esta diminuição.

Palavras-Chave: asfíxia perinatal, desuso no desenvolvimento, neurônios piramidais, córtex somatossensorial

Apoio Financeiro: Esse trabalho teve auxílio do CNPq (476880/2007-6), da CAPES e do FINEP/Rede IBN 01.06.0842-00.

C.06.003

NUEVOS PARADIGMAS EN NEUROCIENCIAS: 1. TEORIA NEUROCICLICA 2. CIRCUITO FUNCIONAL BASICO UNIDAD FUNCIONAL DEL SN

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ABSTRACT.- Titulo: NUEVOS PARADIGMAS EN NEUROCIENCIAS: 1. TEORIA NEUROCICLICA 2. CIRCUITO FUNCIONAL BASICO (CFB) UNIDAD FUNCIONAL DEL SN.- El objetivo central del trabajo es demostrar que a través de su estructura cíclica, el SN interviene en la generación de la patología sensorial. Esta es una conclusión derivada de la Investigación Científica Clínica Aplicada (ICCA), apoyada en la biología del comportamiento y en el conocimiento de la psicología del inconsciente. Para ello se tomaron 10 pacientes diagnosticados de Sordera Súbita, que no tuvieran más de 10 días de evolución, se les valoró clínicamente de acuerdo a la psicología del inconsciente y se les sometió a un tratamiento psicológico y medicamentoso cuya acción se basa en el principio cíclico del SN. El resultado obtenido fue la recuperación total de la audición en 8 pacientes (80%) y parcial en los 2 (20%) restantes. Esto solo es posible si la corteza induce a través de la vía eferente esta patología. De igual manera demostrar que a través del CFB el cerebro induce en los órganos periféricos, un grupo de enfermedades que se producen por un exceso o por un defecto de impulsos nerviosos sobre dichos órganos, como los músculos y las glándulas. Esto solo es posible si el cerebro envía sus impulsos a través de un CFB, que es un grupo de tres neuronas apropiadamente ubicadas unas respecto de otras, de tal manera que la neurona efectora tenga la posibilidad de quedar permanentemente inhibida o permanentemente activada, así en el primer evento el músculo se paraliza y en el segundo sufre un espasmo tónico clónico, que son las dos únicas maneras posibles de presentarse la patología muscular. Para ello se tomaron 10 pacientes diagnosticados de parálisis facial con menos de 10 días de evolución, se les valoró clínicamente de acuerdo a la psicología del inconsciente y se les sometió a un tratamiento psicológico y medicamentoso cuya acción se basa en el principio de que la UNIDAD FUNCIONAL DEL SN ES EL CFB y no la neurona aisladamente considerada. El resultado obtenido fue una recuperación total de la función del nervio facial en 7 pacientes (70%) y parcial en los 3 restantes (30%).

Palavras-Chave: INVESTIGACION CIENTIFICA CLINICA APLICADA (ICCA) CIRCUITO FUNCIONAL BASICO (CFB) SN

Apoio Financeiro: RAMIRO M VERGARA

C.06.004

Influência do exercício em esteira sobre a recuperação funcional e sobre a dor em ratos com lesão nervosa periférica

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Objetivos: Esse trabalho teve como objetivos avaliar a melhora funcional e a diminuição da alodinia mecânica em ratos submetidos lesão nervosa periférica e exercício em esteira ergométrica.

Métodos: Ratos Wistar machos adultos (20) foram divididos em 4 grupos, ST-Sham Treino, SS-Sham Sedentário, LT-Lesão Treino, LS-Lesão Sedentário. Ratos dos grupos LT e LS tiveram o nervo ciático direito transecionado, o grupo Sham sofreu todos procedimentos cirúrgicos exceto a transecção. Uma semana após a lesão, os grupos treino iniciaram o exercício em esteira, realizado por quatro semanas. As avaliações foram feitas um dia após a transecção e durante 4 semanas de treino. Para a análise da locomoção utilizou-se o índice de funcionalidade do ciático (IFC) baseado nas marcas dos membros posteriores feitas sobre um papel. O resultado é expresso em unidades onde 0 (± 11) indica normalidade e -100 falta de funcionalidade. Para a análise da alodinia mecânica utilizou-se o *Von Frey*, baseado em respostas nociceptivas para estímulos na superfície plantar da pata direita, com filamentos de diferentes resistências, sendo realizadas 10 repetições para cada filamento.

Resultados: Os grupos foram analisados através da ANOVA 1 via e LSD post hoc.

Os dados são apresentados como média ($\pm$ SEM).

Antes do exercício os grupos LT e LS apresentaram valores piores para o IFC ($-102,46 \pm 7,02$ e $-90,03 \pm 7,35$, respectivamente) do que os grupos ST e SS ($-15,12 \pm 5,4$ e $-15,60 \pm 5,12$, respectivamente; $p < 0,001$). Na 4ª semana o grupo LT apresentou uma melhora significativa no IFC ($-51,10 \pm 13,27$) do que o grupo LS ($-79,01 \pm 5,88$; $p < 0,05$). Os grupos Sham apresentaram valores próximos a -10 considerados normais. Para *Von Frey* antes do exercício os grupos LT e LS apresentaram piores valores para o filamento de maior resistência ($32 \pm 10,95$ e $24 \pm 8,94$, respectivamente) do que os grupos SS e SL ($14 \pm 21,9$ e $22 \pm 13,03$; $p < 0,05$) na 4ª semana o grupo LT apresentou melhora ($6 \pm 5,47$) em relação ao grupo LS ($26 \pm 11,40$; $p < 0,001$).

Conclusão: Esses resultados sugerem que o exercício físico em esteira ergométrica melhora a recuperação funcional e diminui a alodinia mecânica em ratos submetidos a transecção nervosa.

Palavras-Chave: Lesão Nervosa Periférica, Recuperação Funcional, Alodinia Mecânica

Apoio Financeiro: CAPES, CNPQ

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Efeitos do programa de treinamento de equilíbrio e coordenação em ratos após lesão nervosa periférica

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Este trabalho tem como objetivo avaliar a melhora sensoriomotora de ratos submetidos ao treinamento de equilíbrio e coordenação após lesão por esmagamento do nervo ciático.

Ratos Wistar machos adultos, foram divididos nos grupos, Sham (SH, n=5), Sedentário (SE, n=6), Equilíbrio e Coordenação (EC, n=6) e Motor Controle (MC, n=6). O nervo ciático direito dos animais dos grupos SE, EC e MC foi exposto comprimido com uma pinça hemostática e força máxima (30 s). Os animais do grupo SH não se submeteram à compressão do nervo ciático. Após 2 dias os animais EC e MC iniciaram os protocolos de treinamento (4 semanas), sendo que os ratos EC treinaram em uma série de obstáculos elevados como pontes suspensas, pontes de cordas, etc. e o treino dos MC consistiu em os animais atravessarem uma pista plana livre de obstáculos. A distância percorrida pelos animais de ambos os grupos foi a mesma (25 m/dia). Após o período de treino, o *Narrow Beam Test* (NB) e o *Horizontal Ladder Rung Walking Test* (HLRW) foram utilizados para a análise sensoriomotora. Filmou-se os animais atravessando 3 vezes cada aparato de teste, e o número de erros do membro posterior direito foi contado. Para a análise dos dados foi utilizado ANOVA para medidas repetidas e o *post-hoc* de Duncan ($p<0,05$).

Os dados apresentados são a média do número de erros do membro posterior direito por grupo. No NB os grupos SH e EC ($0,5 \pm 0,4$ e $0,7 \pm 0,1$; respectivamente) tiveram significativamente menos erros que os grupos MC e SE ($2,5 \pm 0,5$ e $2,3 \pm 0,6$; respectivamente; $p<0,05$). No HLRW, os grupos SH e EC tiveram significativamente menos erros que o grupo SE ($1,8 \pm 0,3$); entretanto, não houve diferença significativa entre os grupos SH, EC e MC ($0,5 \pm 0,4$; $0,6 \pm 0,2$ e $0,9 \pm 0,3$; respectivamente; $p<0,05$).

Estes resultados sugerem que 4 semanas de treinamento de equilíbrio e coordenação melhoram a performance motora de ratos com lesão do nervo ciático em testes sensoriomotores.

Palavras-Chave: Lesão nervosa periférica, equilíbrio e coordenação, testes sensoriomotores

Apoio Financeiro: CAPES-CNPQ

Behavioral differences on motor performance after three types of global ischemia surgery in *Meriones unguiculatus* evaluated after four and six days

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The aim of this study is analyze the effects of three types of carotid global ischemia surgery in motor performance after four and six days. **Materials and Methods:** Eighty four male naive Mongolian gerbils (*Meriones unguiculatus*; Rodentia; Gerbilidae) weighing 60-80 g were used. The global ischemia was performed after occlusion of external carotid artery. The animals were assigned to seven groups; Non-Ischemic (CC), Unilateral Ischemic with no reperfusion evaluated after 4 days (Uni4nrp), Unilateral Ischemic with no reperfusion evaluated after 6 days (Uni6nrp), Unilateral Ischemic with reperfusion evaluated after 4 days (Uni4rp), Unilateral Ischemic with reperfusion evaluated after 6 days (Uni6rp), Bilateral Ischemic evaluated after 4 days (Bi4) and Bilateral Ischemic evaluated after 6 days (Bi6). All the animals were tested in the Open-field (OF) and Rota rod (RR), in order to evaluate exploratory behavior and motor performance. Data were submitted to one-way variance (ANOVA) and Student Newman Keuls post hoc comparisons. **Results:** All groups showed a significant decrease in motor response (crossing) when compared to the control group (CC), still the groups Uni4rp, Uni6rp, Bi4 and Bi6 showed a decreased motor response when compared the groups Uni4nrp and Uni6nrp ($F_{6,77} = 50,12$; $P < 0,05$) in the OF. The ischemia groups spent less time on the RR ($F_{6,77} = 9,68$; $P < 0,05$), when compared to the control group (CC) with exception of the Uni6nrp. The groups Un4rp and Bi4 showed a significant decrease of spent time when compared to Uni6nrp, Uni4nrp and Bi6 ($F_{6,77} = 9,68$; $P < 0,05$). **Conclusion:** These findings suggest that motor behavior is more affected after bilateral and unilateral carotid ischemia with reperfusion and groups evaluated after 4 days of ischemia showed more deficits when compared after 6 days.

Palavras-Chave: Motor recovery, Global Ischemia, Open-field test, Rota rod test, *Meriones unguiculatus*

Apoio Financeiro: FAPESP (Proc n 2005/02607-3)

Análise comportamental do efeito da eletroestimulação aguda do ponto de acupuntura Zusanli (E36) pós-isquemia cerebral bilateral em gerbilos.

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Objetivo: Verificar o efeito da estimulação aguda do ponto de acupuntura Zusanli (E36) no comportamento motor em gerbilos isquêmicos. **Matérias e Métodos:** Foram utilizados 18 gerbilos, fêmeas, divididos em 3 grupos (n=6): GC (controle), GI (isquemia), e GAC (acupuntura). Nos grupos GI e GAC, os animais foram anestesiados, ambas as artérias carótidas comuns foram expostas e ocluídas por 10 minutos através de um fio de sutura. Apenas no GAC, imediatamente após o evento isquêmico o acuponto E36 foi punterado bilateralmente com uma agulha de acupuntura de 0,18mm de diâmetro e estimulado com um eletroestimulador (WQ-IOD1, Beijing, China) com uma corrente contínua de 20 Hz por 20 minutos. No 3º dia pós-cirúrgico os animais foram avaliados em um monitor de atividade durante 15 minutos. Posteriormente aos experimentos, os animais foram perfundidos e os cérebros retirados para histologia. Os dados foram analisados através de uma análise de variância de uma via (ANOVA) e submetidos ao teste *post-hoc* de Dunnett. **Resultados:** A avaliação comportamental evidenciou um aumento significativo do número de cruzamentos e da distância percorrida do GI em relação aos GC e GAC ($F_{2,15}=24,56$; $p<0,001$) e ($F_{2,15}=22,12$; $p<0,001$), respectivamente. Em relação a permanência dos animais nas laterais do monitor, verificamos um aumento do GI em relação ao GC e GAC ($F_{2,15}=21,68$; $p<0,01$). Por outro lado, o tempo de permanência dos no centro do monitor foi reduzida no GI em relação ao GC e GAC ($F_{2,15}=20,29$; $p<0,01$). Já para os movimentos de rotação no sentido horário e anti-horário, verificamos um aumento significativo da frequência do GI em relação aos GC e GAC ($F_{2,15}=7,49$; $p<0,01$) e ($F_{2,15}=5,91$; $p<0,05$), respectivamente. **Conclusão:** Após o evento isquêmico em gerbilos é bastante conhecido o aumento da atividade exploratória. Em nosso trabalho, a reversão do aumento da atividade exploratória e as modificações dos outros padrões comportamentais, até o presente momento, nos sugerem que o ponto de acupuntura E36 pode interferir nas alterações comportamentais pós-lesão isquêmica cerebral experimental.

Palavras-Chave: Isquemia Cerebral Global, Gerbilo, Eletroacupuntura, Comportamento Motor

Apoio Financeiro: FAEPA

Writer's cramp: Use of Splint in Handwriting Improvement

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Introduction / Objectives

Writer's cramp is a focal hand dystonia, a task specific, characterized by the involuntary contraction in the arm and hand muscles which appears when the person is writing; sometimes can be painful, or normal, in performing other task. The development of focal hand dystonia through repetitive tasks realization happens from degradation of cortical somatosensory representation due to repetitive fast stimulus, and very near to adjacent fingers, sufficient to alter the sensory-motor stimulus harming the motor control. Some patients with writer's cramp when requested to improve the writing velocity, can induce the dystonia although this improvement is not directly proportional to an increase in the number of words. As the pharmacological treatment has not shown the desired response, a study of musculature immobilization damaged by dystonia was performed on a patient with writer's cramp, using splint with the objective of reducing the handwriting alterations.

Participants, Material and Methods

Patient of male gender, 24 years, lawyer, has been presenting for 6 years a progressive difficulty on writing with his right hand and an intense pressure on the paper. A few seconds after beginning the task, an extension of the thumb and metacarpophalangeal of the index finger with displacement of distal phalanx could be noted. This was followed by pain in tenar region. The tools for the evaluation were Burke-Fahn-Marsden Scale, Jedynek's Protocol and Pain Analogic Scale. The rehabilitation training was made throughout eight weeks, in two phases of four weeks each, twice a week for 60 minutes each one. In the first one, the body consciousness with postural correction using relaxing techniques and sensitive information were emphasized, employing the correct reference and the ways to monitor the movement (visual, audition, tactile and proprioceptive stimulus). In the second phase adequate splint was used on the affected musculature of index finger to inhibit the dystonic action. Similar home exercises were daily programmed for 30 minutes.

Results

Upon functional motor reexamination, after the rehabilitation program, i.e. after the eight weeks, the dystonic posture was not found with regard to index and thumb extensions nor the strong pressure to hold the pen, showing a better motor control facilitating the writing and improving legibility, without changing the writing velocity. The pain on tenar region has almost disappeared.

Conclusions

In our study the patient's previous body consciousness in relation to his compensatory maneuvers when writing, resulted that it would not occur during the training period. So, the consciousness, such as the comprehension of disorders caused by writer's cramp, identifying the body compensations to improve the performance, were indispensable factors for satisfactory gains in manual motor ability. The reevaluation of morphofunctional aspects and the hand motor function improvement for writing should be realized once a month, after the rehabilitation program, to verify the maintenance time of positive motor effects acquired during the process. Although a report about gradual worsening of writing after the interruption of daily practice, the maintenance of these benefits, acquired during this conservative training with splints, can remain for an average of six months. It can be concluded, although preliminarily, that the therapeutic response to a non invasive treatment to writer's cramp, through immobilization by splints of the affected musculature is efficient with regards to writing quality, morphofunctional aspects and manual ability.

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Palavras-Chave: writer's cramp; focal hand dystonia; motor rehabilitation; handwriting

C.06.009

INFLUÊNCIA da Exposição PERINATAL ao Bisfenol A sobre A MEMÓRIA ESPACIAL de ratOs
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Objetivos: A ubiquidade de drogas antropogênicas e poluentes no ambiente são ressaltadas cada vez mais por estudos. Alguns destes poluentes interagem com o sistema endócrino e são nomeados de disruptores endócrinos. Bisphenol A (BPA) é um elemento dos policarbonatos utilizado na manufatura de plásticos e resinas-epoxi, o qual é potencialmente ingerido por humanos e é descrito como de baixa atividade estrogênica. O BPA tem sido relacionado com o antecipamento da puberdade, alterações de próstata e mamas, além de interferir na capacidade exploratória dos animais. Este estudo testou o efeito de uma dose do disruptor endócrino BPA, abaixo da considerada nociva (EPA, 1998), na memória espacial de ratos após sua exposição no período perinatal.

Metodologia: Matrizes de ratas Wistar foram expostas a BPA (40 µg/kg/dia) durante a gestação (Grupo pré-natal; PRE), durante a lactação (Grupo pós-natal; POS) ou ao veículo (Grupo controle; CON). Os tratamentos foram efetuados por via oral através de gavagem. Para este estudo foram utilizadas crias machos de 4 meses (n= 72) e crias fêmeas (n=71). Foi utilizado o Labirinto Aquático de Morris (1984) como teste comportamental para avaliação da memória espacial. Os animais mantidos em condições controladas tiveram água e alimento *ad libitum*. O projeto teve aprovação do Comitê de Ética da FURG (CEPAS).

Resultados: Nos machos o grupo POS teve maior tempo de latência ($P<0,05$) durante o treino nos dias 3 e 4 em relação aos grupos CON e PRE, sem diferença ($P>0,05$) no teste final. Nas fêmeas houve variação do tempo de latência nos treinos entre os grupos ($P<0,05$), mas não houve diferença ($P>0,05$) no teste final.

Conclusão: Os resultados sugerem que a exposição perinatal ao BPA influencia a memória espacial dos animais.

Palavras-Chave: Bisphenol A; Water Maze

Apoio Financeiro: FAPERGS

EFEITOS DA MANIPULAÇÃO ARTICULAR VERTEBRAL SOBRE PARÂMETROS DE ESTRESSE OXIDATIVO EM INDIVÍDUOS COM CERVICALGIA.

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Objetivos: A manipulação articular vertebral pode ter efeito hipoalgésico por sua influência sobre mecanismos de controle da sensibilidade dolorosa. Disfunções articulares afetam a função neural pela liberação de agentes pró-inflamatórios, como radicais livres de oxigênio e nitrogênio. O peroxidonitrito (ONOO.) é um potente agente citotóxico e pró-inflamatório. Estudo recente demonstra que a oxihemoglobina é um agente redutor de ONOO.. Uma hipótese plausível para a ação analgésica da manipulação articular vertebral poderia ser o aumento do fluxo sanguíneo regional e diluição dos agentes pró-oxidantes formados localmente. Com este objetivo, o presente estudo demonstrou a possível relação entre lipoperoxidação, mediada por ONOO., e o tratamento por manipulação articular vertebral.

Métodos: Quinze homens com cervicalgia (entre 21 e 50 anos), normotróficos, sedentários, não fumantes, sem patologias associadas e sem tratamento analgésico foram tratados com manipulação articular vertebral por seis sessões. Antes e ao final do tratamento responderam a escala visual de dor e foram submetidos a três coletas de sangue, que ocorreram antes de iniciado o tratamento e após a terceira e a última sessão de manipulação. As amostras foram preparadas para a obtenção dos eritrócitos, que foram submetidos à técnica de quimiluminescência (Free Radic Biol. Med. 10:93, 1991). A hemoglobina foi determinada pelo método de Drabkin. Os dados do sangue foram analisados por ANOVA de medidas repetidas, seguida do pós-teste de Bonferroni, e os qualitativos analisados por teste *T* de Wilcoxon. Os valores de lipoperoxidação sanguínea (expressos em CPS/mg de proteínas – média ± erro padrão): antes: 37273,36 ± 2177,10; durante: 36161,73 ± 3174,78; e após: 34081,28 ± 2013,96, não apresentam diferenças significativas ($P>0,05$), apesar da redução na intensidade de dor ($P<0,05$) percebida pelo paciente. **Conclusão:** A manipulação articular vertebral resultou em analgesia, mas não parece induzir redução na lipoperoxidação de eritrócitos. Contudo, estudos complementares para a determinação dos metabólitos de óxido nítrico e ação de antioxidantes estão sendo realizados para melhor compreensão desta relação.

Palavras-Chave: peroxidonitrito, lipoperoxidação, inflamação, estresse oxidativo, manipulação articular

Apoio Financeiro: CNPq, FAPERGS, CAPES

C.06.011

Mutantes IDH^{NGB2}, MEN^{nnc1} e CAT^{nnc1} de *Drosophila melanogaster* reduzem a atividade motora ao longo do envelhecimento e quando submetidos a dieta hiperlipídica.

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Neste trabalho comparamos o desempenho motor entre uma população selvagem (Oregon-R) e quatro populações mutantes de *Drosophila melanogaster*, (ADP⁶⁰, IDH^{NGB2}, MEN^{nnc1} e CAT^{nnc1}. ADP⁶⁰ é obeso, IDH^{NGB2} tem deficiência de isocitrato desidrogenase, MEN^{nnc1} tem deficiência de enzima málica e CAT^{nnc1} tem deficiência na atividade da catalase) ao longo do envelhecimento e quando submetidos à dieta hiperlipídica.

O teste RING (*Rapid Iterative Negative Geotaxis*) consiste em um aparato de tubos paralelos com 20 animais/tubo. O aparato foi agitado e imagens digitalizadas foram obtidas após 4 segundos (10 repetições). O número de indivíduos encontrados em posição igual ou superior a 8cm foi registrado. Os testes foram realizados em animais com uma semana (1W) e três semanas de vida (3W), animais jovens e de meia idade respectivamente, submetidos a meio alimentar normal (N) e dieta hiperlipídica, normal mais 20% de gordura (DH). Os dados foram analisados por ANOVA de duas vias ($p < 0.05$) (GraphPad 4).

Indivíduos das populações selvagem Oregon-R e ADP⁶⁰ não tiveram variações significativas de desempenho motor em qualquer idade ou meio alimentar. Contudo a atividade de escalada nos mutantes IDH^{NGB2} desenvolvidos em meio padrão declina nos animais de meia idade. Os dados foram expressos em número médio de escaladas $\pm$ DP. (IDH^{NGB2} 1WN= 6.3 ± 1.42 ; IDH^{NGB2} 3WN= 2.2 ± 1.3) ($p < 0.01$). Já nos indivíduos da população MEN^{nnc1} e CAT^{nnc1} a atividade de escalada é diminuída tanto pelo envelhecimento ($p < 0.01$) quanto pela dieta hiperlipídica (MEN^{nnc1} 1WN= 9.2 ± 2.78 ; MEN^{nnc1} 3WN= 1.2 ± 1.1 ; MEN^{nnc1} 1WDH= 3.8 ± 2.1 ; MEN^{nnc1} 3WDH= 0.4 ± 0.55 ; CAT^{nnc1} 1WN= 11 ± 2.4 ; CAT^{nnc1} 3WN= 2.34 ± 1.34 ; CAT^{nnc1} 1WDH= 7.8 ± 2.49 ; CAT^{nnc1} 3WDH= 1.8 ± 0.45) ($p < 0.01$). Os dados obtidos sugerem que determinadas mutações podem reduzir a atividade motora em populações de *Drosophila* ao longo do envelhecimento e/ou submetidas a dietas hiperlipídicas.

Palavras-Chave: *Drosophila melanogaster*, movimento, envelhecimento, dieta hiperlipídica

Apoio Financeiro: CNPQ, PUCRS

**FUNCTIONAL RECOVERY OF WISTAR RATS WITH CONTUSIVE SPINAL CORD INJURY
SUBMITTED TO KINESTHETIC AND PROPRIOCEPTIVE TASKS**

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Objective: Evaluate the functional recovery of Wistar rats with contusive spinal cord injury (SCI) trained in kinesthetic or proprioceptive apparatuses.

Methods: All procedures were approved by the local Ethical Committee and performed in accordance with Mutlicenter Animal Spinal Cord Injury Study. 51 Wistar rats were randomized into five groups: control, maze, ramp, runner and sham. Rats were anesthetized with Diazepam (ip 5mg/ml), Ketamine (ip 100mg/kg) and Xylestesin (local), which was followed by moderate weight drop injuries with NYU-Impactor at T9-T10 levels. After SCI, Cefazolin (ip 0,1ml/100g) was administered. Training in three apparatuses: maze, ramp and runner trace occurred twice a week for two weeks before and five after SCI. Control was not submitted to the training and sham was trained in the maze. Basso, Beattie and Bresnahan (BBB) locomotor test, a 21 point rating scale, was accomplished once a week during five weeks. Rats with complete hindlimb paralysis are scored 0 and those with normal locomotion are scored 21. The motor behaviors analyzed were dorsal or plantar stepping and forelimb-hindlimb coordination. The values obtained were analyzed using ANOVA considering $p < 0.05$ as statistically significant result and correlation between BBB and training.

Results: Maze and ramp groups showed higher motor control development than control and runner ($F_{4,20}=66.55$, $p < 0.0001$). It seems that the training specificity needed in maze and ramp apparatuses, such as limb coordination, is a key factor to develop motor control earlier. There is a positive correlation between plantar stepping and training sessions ($R^2=0.826$). Plantar stepping reflects recovery of coordination and ramp apparatus has a specific stepping requirement, showing a higher and earlier recovery of this behavior ($F_{3,23}=4.32$, $p=0.004$).

Conclusion: The training specificity appears to be an important factor to functional recovery. The ramp meets the necessary features to develop limb coordination that might be due to the effort required. Although training has assisted motor recovery in SCI, this study shows that a specific training seems to be more effective and timeless to reach clinical targets.

Palavras-Chave: spinal cord injury, motor recovery, proprioception and kinesthesia

Apoio Financeiro: CAPES, HCFMUSP

FUNCTIONAL RECOVERY OF LOCOMOTION IN RATS SUBMITTED TO TRAINING IN WATER WITH DIFFERENT TEMPERATURES AFTER SCIATIC NERVE CRUSH

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OBJECTIVE: The objective of this study was to analyze functional recovery of locomotion in rats submitted to swimming training at different temperatures after sciatic nerve crush.

METHODS: Adult male rats were divided into 4 groups: Control-Sham, Sedentary (CSS, n=5); Sedentary-Crush (SC, n= 6); Crush training at 27°C (C27, n= 7); and Crush training at 32°C (C32, n= 6). Animals were anesthetized and the right sciatic nerve was crushed for 30 s. One week after the surgical procedure the C27 and C32 groups began a swimming training program in warm water at 27°C and 32°C ($\pm 1^\circ\text{C}$), respectively. The training was 5 days for week, for 4 weeks, beginning with 10 min on the first day, increasing progressively until 60 min a day at the 4 week. The Sciatic Functional Index (SFI) was applied to evaluate functional status prior to the surgical procedure and, over the training period. The foot-prints were taken from the experimental and normal sides and three footprints from each walking track were analyzed and the average of the measurements was used in SFI calculations. An index score of 0 is considered normal and an index of -100 indicates total impairment. Data were analyzed using ANOVA for repeated measures followed by the *Duncan test* ($p < 0,05$).

RESULTS: Before nerve lesion no significant differences was found in the mean SFI among groups. Subsequently, before the training program, the mean SFI from the crush groups (SC: -58.93 ± 4.21 ; C27: -71.43 ± 8.01 and C32: -73.99 ± 6.45) was different from the CSS (-5.8 ± 2.25) group and this difference remained until 2^a training week. At the 3^a training week, the SFI were similar in all groups ($p > 0.05$). However, the mean SFI value at 4^a week training for C27 (-17.60 ± 3.9) group was similar to CSS (-10.92 ± 1.54) and different from the SC (-25.39 ± 2.30) and C32 (-22.52 ± 4.11) groups.

CONCLUSION: This result provides evidence that nerve injury produced a functional deficit and that swimming in different temperatures does not alter the functional recovery of locomotion. However, the C27 group showed a tendency to be better than C32 and SC groups.

Palavras-Chave: locomotion, sciatic nerve, swimming, different water temperature

Apoio Financeiro: CAPES

C.07.001

Peritraumatic reactions and PTSD severity: the predictive value of tonic immobility

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Introduction: Previous studies suggested the importance of peritraumatic reactions as predictors of PTSD symptoms severity. Despite mounting evidence that tonic immobility occurs under intense life threats its role as predictor of PTSD severity remains by and large understudied.

Objective: To investigate the role of peritraumatic reactions (tonic immobility, panic and dissociation) as predictors of PTSD symptoms severity.

Method: Participants were 32 victims of urban violence with PTSD diagnosed through the Structured Clinical Interview for DSM-IV Axis I Disorders. In order to evaluate PTSD symptoms at baseline, we used the Post-Traumatic Stress Disorder Checklist — Civilian Version (PCL-C). To assess peritraumatic reactions we employed the Physical Reactions Scale (PRS), the Peritraumatic Dissociative Experiences Questionnaire (PDEQ) and tonic immobility questions. Linear regression models were fitted to investigate the magnitude of the association of the three peritraumatic reactions with PTSD symptoms. In order to control for potential confounders, multiple linear regression models were adjusted including the variables sex, time since trauma and negative affect.

Results: Tonic immobility was the best predictor of PTSD symptoms severity being the only one keeping statistical significance when controlled for potential confounders ($\beta = 0.57$, $p = 0.008$).

Conclusions: Tonic immobility surpassed peritraumatic dissociation and panic reactions as predictor of PTSD severity. Our findings should encourage the systematic assessment of tonic immobility in traumatized victims.

Palavras-Chave: posttraumatic stress disorder; predictor; tonic immobility;

Apoio Financeiro: CNPq/PRONEX, CAPES, FAPERJ, IBNNet, Instituto Milênio Saúde Mental e Violência

C.07.002

Chronic Fluoxetine induces a reorganization of central glutamatergic synapses.

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Major depression is a worldwide health problem of high prevalence and costs. However, its underlying neurobiological mechanisms are yet poorly understood. Although antidepressant drugs (ADDs) lead to an immediate rise of monoamines in the synaptic cleft, it is now accepted that the therapeutic effect depends on neuroadaptive mechanisms that occur with a slow time course. We hypothesized that these might involve plastic changes in excitatory forebrain synapses. Objectives: to study changes in glutamate receptor composition and trafficking in rats treated with the ADD Fluoxetine (Flx). Methods: Rats were treated for 2 and 4 weeks with Flx (0.7 mg/kg/day), decapitated and telencephalic subcellular fractions were obtained by differential centrifugation. By Western Blotting of postsynaptic densities (PSDs), we found that the levels of specific glutamate receptor subunits changed in them: While NR2A increased by 32±5% (2 weeks) and 37±5% (4 weeks), NR2B decreased by 20. ±4% (4 weeks) The GluR2 subunit decreased by 20±3% after 2 weeks, but returned to control levels after 4 weeks.

To test whether the changes in the PSD glutamate receptor composition was accompanied by receptor subunit trafficking, we separated by a velocity gradient a subfraction of the microsomal fraction P3 that was immunopositive for intracellular membrane and vesicle markers. The content of NR2A and GluR2 increased selectively in it, suggesting that these subunits are trafficking between cellular compartments. 2D gel analysis and mass spectrometry of the P3 subfractions further indicated substantial differences between the control and Flx condition. Several proteins possibly associated with membrane trafficking were identified. The selective Flx-induced changes may have consequences on the functional properties of extensive neuronal networks and may mediate the delayed antidepressive actions of Flx.

Palavras-Chave: Major Depression, Glutamate Receptors

Apoio Financeiro: Programa Bicentenario de Ciencia y Tecnología, Proyecto Anillo 09-2006 (UW)

C.07.003

Incidence of genetic markers of diurnal preference in a population of bipolar disorders patients

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OBJECTIVES: a) establish the influence of two genetic polymorphisms in genes which are central components of the molecular clock in humans on chronotypes within a local healthy population: 1) variable number of tandem repeats (4 or 5 repeat-alleles; 4R or 5R) in exon 18 of *per3*, and 2) single nucleotide polymorphism C3111T (C or T alleles) in *clock*. b) analyze the frequencies of these polymorphisms and their possible implications in a group of bipolar disorders (BD) patients as related to controls.

METHODS: Chronotypes of healthy individuals (n=38) were determined by the HO test, and the population was divided into three groups according to HO score (morning-types, intermediates and evening-types). DNA from BD patients (n=37) and healthy controls were obtained from blood samples. Genotyping was achieved by PCR followed by MW determination and RFLP. All procedures were approved by a local ethics committee.

RESULTS: In the control group, allele frequency analysis revealed that the 5R allele in *per3* and the C allele in *clock* were sub-represented among evening-type individuals related to intermediates (Fisher Exact Test, $p < 0.05$ for both alleles). Moreover, homocygous for the 4R allele of *per3* had significative lower scores (evening behavior; 2-way AOV, $p < 0.05$) than 5R allele-carriers. Among the BD group, we found that genotype frequencies, according to the presence of 5R or C alleles, were inverted with respect to controls, although not significantly, while allele frequencies did not show any differences.

DISCUSSION: Genetic linkage studies of BD has been relatively elusive in recent years. Due to this fact, the aim of our work was to analyze two genetic markers that could help for a better understanding of the circadian-related clinical features of the pathology. Our results may imply an evening tendency among BD patients, which should be confirmed by activity, sleep or other circadian markers. We will try to establish the relation of these findings with other clinical indexes of BD, including the need for pharmacological treatments of sleep disturbances. **Palavras-Chave:** chronotypes - bipolar disorder - clock genes

Apoio Financeiro: CONICET - ANPCyT - CIC - UNQ

The antidepressant role of dietary long-chain polyunsaturated n-3 fatty acids in two phases in the developing brain

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OBJECTIVES: In this work, we hypothesized if fish oil supplementation, rich in n-3 fatty acids, would present an antidepressant effect on adult rats in two different phases in the developing brain: phase A (supplementation during pregnancy and lactation) and phase B (supplementation during post-weaning until adulthood).

METHODS: In phase A, female rats, used as matrix to obtain male rats, were divided in three groups: FO (fish oil daily supplemented), CF (coconut fat daily supplemented) and C (control not supplemented), and their male offspring was separated in the respective experimental groups (FO n=11, CF n=15 and C n=16). For phase B, 21-year-old male rats were separated in the same experimental groups (n=14/group), following the same supplementation protocol until adulthood was reached. All animals from both phases were submitted to behavioral tests: elevated plus maze, used as a measure of anxiety and exploratory behavior; forced swimming test, to assess animal's depressive state through its immobility time; and the open field, in order to evaluate animals' locomotion.

RESULTS: FO adult rats from both phases demonstrated a significantly decreased immobility time when compared to C and CF groups in the forced swimming test (Phase A ANOVA Kruskal-Wallis, $H(2)=16.37$; $p \leq 0.001$; Phase B ANOVA Kruskal-Wallis $H(2)=11.52$; $p \leq 0.01$). There was no difference in neither motor activity (Phase A ANOVA, $F(2,39)=0.80$, $p > 0.2$ and Phase B ANOVA, $F(2,39)=0.07$, $p = 0.93$) or anxiety behavior in the three groups, excluding false positive results (Phase A $F(2,39)=0.99$, $p=0.37$ and Phase B $F(2,39)=0.97$, $p=0.37$).

CONCLUSION: Our results suggest that fish oil supplementation during Phases A and B had a beneficial effect on preventing depression-like behavior in adult rats. n-3 fatty acids mother supplementation during offspring's critical periods of cortical maturation, synaptogenesis, and myelination could prevent psychopathologies in young adulthood. **Palavras-Chave:** Depression, fish oil, n-3 fatty acids, forced swimming test

C.07.005

THE LATERAL SEPTUM MODULATES HISTAMINE RELEASE IN FREELY MOVING RATS

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The histaminergic neurons localized in the tuberomammillary nucleus of the hypothalamus (TMN) are crucial for maintaining wakefulness during a motivated behavior. During cataplexy animals are highly awake, and TMN neurons, but not other arousal neurons, are very active, and they maintain wakefulness. Also, during cataplexy GABA release in the ventral posterior hypothalamus and in the TMN was reduced compared with waking. We have proposed that GABAergic input could come from lateral septum (LS), and that a reduction in GABAergic input to the TMN should increase vigilance. Using electron microscopy we demonstrated that the LS axons make symmetrical synaptic contacts with TMN proximal dendrites, which strongly suggests that these synapses are GABAergic. In freely moving rats we measured extracellular histamine levels through microdialysis, while modifying LS activity using GABA_A receptor agonist and antagonist muscimol and picrotoxin, respectively. We found that muscimol (0,5nM) injected into LS increased histamine levels in LS. Conversely, picrotoxin (0,1mM) decreased or did not change extracellular histamine levels in LS. The LS controls mood and motivation, TMN is important in motivation too, therefore we propose that the LS controls TMN activity to maintain arousal at levels appropriate for the current behavior.

Palavras-Chave: Tuberomammillary nucleus, lateral septum, histamine, brain

Apoio Financeiro: Fondecyt # 1060476

Avaliação dos Parâmetros Relacionados à Depressão em Ratos Sobreviventes à Sepse

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Objetivos: A Sepse é caracterizada por uma resposta inflamatória decorrente da reação do sistema imunológico à infecção. Esta resposta produzida pode ocasionar disfunção cerebral aguda e déficits a longo prazo (Acta Clin Belg. 5:220, 2006). Estudos mostraram que ratos sobreviventes à sepse apresentaram um aumento do tempo de imobilidade no teste de natação forçada 10 dias após a técnica de ligação e perfuração cecal (CLP) (Braz J Med Biol Res. 40:831, 2007). Neste contexto, o objetivo deste trabalho foi avaliar os parâmetros relacionados a depressão em ratos sobreviventes à sepse.

Métodos e Resultados: Ratos Wistar machos (300-350g) foram submetidos a CLP (grupo sepse; n=15, ressuscitação volêmica + antibióticoterapia) e ao sham (grupo controle; n=15; ressuscitação volêmica). Após 10 dias, o consumo de alimento doce e atividade locomotora foram mensurados no teste de campo aberto. O peso corporal dos animais foi mensurado antes e após os procedimentos. Imediatamente após o teste comportamental, os animais foram anestesiados e o sangue retirado por punção transcardíaca; soro e plasma foram utilizados para verificar os níveis dos hormônios Cortisol e Adrenocorticotrófico (ACTH) respectivamente (Quimiluminescência). Em seguida, foram decapitados e retirado a glândula adrenal para avaliação do peso e o hipocampo para avaliação dos níveis de BDNF (Kit Elisa). Para análise dos dados, foi utilizado teste *t de student* com significância de 0.05. Foi observado que os animais sépticos apresentaram um consumo menor de alimento doce ($t=2.231$; $df=16,090$; $p=0,040$) sem alteração na atividade locomotora e do peso corporal; aumento da glândula adrenal ($t=2.746$; $df=22$; $p=0.012$); aumento dos níveis de Cortisol ($t=2,367$; $df=14$; $p=0,033$) e ACTH ($t=3.035$; $df=4$; $p=0,039$) e diminuição dos níveis de BDNF hipocampais ($t=2.217$; $df=10.926$; $p=0,049$) em comparação com o grupo controle.

Conclusão: Estes resultados suportam a hipótese que ratos sobreviventes à sepse apresentam comportamento relacionado a depressão avaliado pelo consumo de alimento doce (anedonia) e parâmetros relacionados à depressão incluindo a diminuição dos níveis de BDNF hipocampais **Palavras-**

Chave: Sepse, Depressão, BDNF

Apoio Financeiro: CNPq, UNESC, Instituto Cérebro e Mente

ANTIOXIDANT ENZYME ACTIVITIES FOLLOWING ACUTE OR CHRONIC METHYLPHENIDATE TREATMENT IN YOUNG RATS

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Methylphenidate (MPH) is psychostimulant that is used to treat Attention-Deficit/Hyperactivity Disorder and can lead to long-lasting neurochemical and behavioral adaptations in experimental animals. In the present study, the cerebral antioxidant enzymatic system, superoxide dismutase (SOD) and catalase (CAT) was evaluated at different ages following MPH (1, 2 or 10 mg/kg MPH, i.p.) treatment in young rats. Values are expressed as means $\pm$ S.D (n = 5 for each group). *P < 0.05 different from control group (saline). In the acute treatment the SOD activity decreased in the cerebral prefrontal cortex ($f=4.970$, $P<0,05$) with opposite effect in the cerebral cortex ($f=4.560$, $P<0,05$); and the CAT activity decreased in the hippocampus ($f=9.278$, $P<0,05$). In the chronic treatment the SOD activity increased in the hippocampus ($f=12.864$, $P<0,05$) and cerebral cortex ($f= 5.808$, $P<0,05$) and decreased in the striatum ($f=4.088$, $P<0,05$). The observed changes on the enzyme activities in rat brain were dependent on the brain region and duration of treatment with MPH. Probably, the activity of enzymes was not enough to prevent MPH-induced oxidative damage in specific regions of the brain, such as observed for us in another recent study.

Palavras-Chave: Methylphenidate, superoxide dismutase and catalase.

Apoio Financeiro: Cnpq, Instituto Cérebro e Mente, Fapesc

REDUÇÃO DA CATALEPSIA PELA ELEVAÇÃO DO LIMIAR ANAERÓBICO EM RATOS.

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A catalepsia (CT), caracterizada por rigidez muscular flexível que promove a imobilização em posturas bizarras por longo tempo, é uma manifestação associada a alguns transtornos neuro-psiquiátricos e pode ser induzida experimentalmente por diferentes manipulações. A constatação de sua ocorrência após atividade muscular forçada em ratos tratados com lactato, levou o presente estudo a buscar comprovação do envolvimento deste produto do metabolismo anaeróbico da glicose como agente indutor de CT. Para tanto, foi investigado se ela se reduz com a elevação do limiar anaeróbico (capacidade máxima de atividade muscular sem que ocorra elevação da concentração de lactato) proporcionado pelo exercício físico sistemático (condicionamento físico). Oito ratos machos Wistar, com 5 meses de idade, foram submetidos a sessões diárias de 40 minutos de nado forçado, 5 dias por semana, por 4 semanas, sendo a última com sobrecarga progressiva de peso até se atingir 5% do peso corporal (grupo experimental = GE). Oito animais similares, mantidos sedentários, constituíram o grupo controle (GC). A administração ip de 10mM/kg de lactato, seguida de 5 min de atividade muscular forçada manualmente pelo experimentador, 20 min após a injeção, no 29º dia do estudo, mostrou induzir um tempo médio ($\pm$ s.e.) de imobilidade em postura vertical de $3,9 \pm 1,7$ min no GE, que foi menor que o tempo de $29,9 \pm 3,8$ min do GC ($F=37,94$; $p=0,000025$). Os níveis de lactato sanguíneo determinados no 31º dia variaram de 3,7 a 4,0 mmol/L nos dois grupos antes do exercício e assim permaneceram no GE ($3,7 \pm 0,5$ mmol/L), enquanto se elevou significativamente no GC ($5,3 \pm 0,5$ mmol/L) (teste-t; $p<0,05$) após a natação. A CT induzida pelo eletrochoque convulsivo (transauricular, 60 Hz, 1 s, 107 V) foi de $49,0 \pm 10,1$ e de $94,1 \pm 12,1$ min respectivamente nos GE e GC ($F= 8,22$; $p=0,012$). Os dados indicam que o lactato é um agente cataleptogênico eficaz e sua associação com outros indutores merece ser estudada.

Palavras-Chave: catalepsia, limiar anaeróbico, lactato, eletrochoque convulsivo, exercício físico

EFEITOS DO DIÓXIDO DE CARBONO (CO₂) SOBRE OS LIMIARES DAS RESPOSTAS DE DEFESA INDUZIDAS PELA ESTIMULAÇÃO ELÉTRICA DA MATÉRIA CINZENTA PERIAQUEDUTAL DORSAL (MCPD) DE RATOS.

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Cerca de 80% dos pacientes com transtorno de pânico apresentam ataques de pânico durante a infusão endovenosa com lactato de sódio. Uma fração destes pacientes também responde à exposição às concentrações de 5 ou 7% de CO₂. Os comportamentos de defesa produzidos pela estimulação elétrica da MCPD têm sido propostos como um modelo experimental de ataque de pânico. **Objetivo:** Este estudo verificou se estes comportamentos são facilitados pela exposição ao CO₂. **Metodologia:** Ratos Wistar albinos machos (n = 20) adultos foram implantados com eletrodos na MCPD e estimulados antes e durante a exposição ao CO₂ nas concentrações de 8% e 13%. Os limiares das respostas de defesa foram avaliados por análise logística de limiares e testes do χ^2 da razão de verossimilhanças. **Resultados:** O CO₂ produziu aumentos significantes sobre os limiares das respostas de exoftalmia (8%, DI₅₀= 15), imobilidade (8%, DI₅₀= 21; 13%, DI₅₀= 23), trote (8%, DI₅₀= 21; 13%, DI₅₀= 39), galope (8%, DI₅₀= 31; 13%, DI₅₀= 58). Contudo, enquanto a micção foi facilitada por ambas as concentrações de CO₂ (8%, DI₅₀= -69; 13%, DI₅₀= -54), a defecação somente foi facilitada pela concentração de 8% (DI₅₀= -53). Por fim, o CO₂ não teve efeitos sobre os limiares de salto. **Conclusão:** Com exceção da defecação e micção, o CO₂ não teve efeitos facilitadores sobre as respostas de defesa, nem mesmo sobre o galope, o qual tem sido sugerido como o melhor representante dos ataques de pânico. Contudo, observamos que os ratos Wistar apresentam extrema resistência à anóxia e hipercapnia, uma característica certamente devida ao processo de seleção em habitat subterrâneo. Portanto, nossos resultados não excluem a participação da MCPD nos ataques de pânico da clínica. Apoio: FAPES, CNPq.

Palavras-Chave: Transtorno do Pânico, Matéria Cinzenta Periaquedutal, Dióxido de Carbono, Comportamento de Defesa, Estimulação Elétrica.

EFEITOS DA ESTIMULAÇÃO DA HABÊNULA SOBRE AS RESPOSTAS DE DEFESA INDUZIDAS POR ESTIMULAÇÃO ELÉTRICA DA MATÉRIA CINZENTA PERIAQUEDUTAL (MCPD) DE RATOS

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Introdução: Várias evidências sugerem que a serotonina (5HT) inibe os ataques de pânico via projeções do núcleo dorsal da rafe (NDR) à MCPD. Por sua vez, a habênula (Hb) é a principal fonte de aferências do NDR. **Objetivos:** Examinar se a estimulação elétrica da Hb inibe a reação de defesa induzida por estimulação elétrica da MCPD. **Materiais e Métodos:** Ratos Wistar machos adultos (n=16) foram implantados com 2 eletrodos, um dirigido à Hb e outro à MCPD. Nas sessões de estimulação, pulsos senoidais (60 Hz, 30 s, 0-55 μ A c.a.) eram aplicados em passos de 5 μ A, a cada uma das estruturas, bem como às duas estruturas simultaneamente. Os limiares de resposta foram avaliados por análise logística de limiares e testes do χ^2 da razão de verossimilhanças ao nível descritivo de 5%. **Resultados:** Comparados aos limiares da estimulação isolada da MCPD, a sessão com estimulação concomitante da Hb e MCPD apresentou limiares mais elevados para as respostas de exoftalmia (ΔI_{50} = 48%; χ^2 =21.9; 1 g.l.; P<0,0001), trote (ΔI_{50} = 37%; χ^2 = 14.1; 1 g.l.; P<0,0005), galope (ΔI_{50} = 30%; χ^2 = 7.1; g.l.; P<0,01) e salto (ΔI_{50} = 52%; χ^2 = 9.5; 1 g.l.; P<0,005). Os limiares de defecação e micção não foram alterados. **Conclusões:** Nossos dados sugerem que a Hb inibe as respostas de defesa induzidas pela estimulação da MCPD, presumivelmente, mediante projeções da Hb ao NDR.

Palavras-Chave: Matéria Cinzenta Periaquedutal, Habênula, Estimulação Elétrica, Reação de Defesa, Pânico

AVALIAÇÃO COMPORTAMENTAL DE UMA LINHAGEM SEMI-CONGÊNICA (GERAÇÃO N8) PARA UM *LOCUS* NO CROMOSSOMO 4 DO RATO COM EFEITO SOBRE A REATIVIDADE EMOCIONAL

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Introdução: Um inter cruzamento entre as linhagens isogênicas de ratos Lewis (LEW) e espontaneamente hipertensos (SHR), possibilitou a identificação de um QTL (*Quantitative Trait Locus*) chamado *Ofil1* (no cromossomo 4) com influência na locomoção central do campo aberto, um índice experimental de ansiedade (Molecular Psychiatry. 4: 453,1999). Construção de linhagens congênicas, a partir do cruzamento da linhagem LEW (doadora) e SHR (receptora), está sendo realizada para refinar o estudo do locus *Ofil1*. **Objetivo:** Caracterizar o perfil comportamental da geração N8 da linhagem congênica em desenvolvimento, nos testes de ansiedade, campo aberto (CA) e caixa branca/preta (CBP). **Materiais e Métodos:** Ratos da geração N8 foram genotipados para três marcadores moleculares microssatélites (D4Mgh6, D4Rat76 e D4Mgh11), localizados na região genômica do *Ofil1*. Machos e fêmeas heterozigotos (LEW-SHR) e homozigotos/controles (SHR-SHR) para os três marcadores foram testados no CA e CBP e os dados analisados por ANOVA de duas vias (sexo e genótipo). **Resultados:** Ratos com genótipo LEW/SHR para *Ofil1*, apresentaram maior locomoção central ($p=0.128$) no CA (21.318 ± 2.001) quando comparados com ratos homozigotos SHR/SHR (17.185 ± 1.868). Na CBP, os ratos LEW/SHR permaneceram mais tempo no compartimento branco ($p<0.05$) (55.227 ± 6.608) quando comparados com ratos SHR/SHR (36.888 ± 4.641). A ANOVA também revelou um efeito sexo no CA ($p<0.0001$) e na CBP ($p<0.01$). Fêmeas de ambos os genótipos, no teste do CA, exibiram maior locomoção central (25.952 ± 1.949) e periférica (56.095 ± 4.302) em comparação aos machos (13.857 ± 1.236), (35.964 ± 2.479), respectivamente. Na CBP, as fêmeas realizaram maior número de transições (3.231 ± 0.181) em comparação aos machos (2.464 ± 0.181). **Conclusão:** Os resultados sugerem que o alelo LEW para o *Ofil1* induz a exploração de áreas aversivas no OF e na CBP. Ao final do desenvolvimento da linhagem congênica (N=10), espera-se que animais homozigotos para o alelo LEW revelarão uma diferença significativa entre os dois genótipos contrastantes (LEW/LEW e SHR/SHR), permitindo assim, avaliar efeitos comportamentais e neurobiológicos do *Ofil1* em processos relacionados à ansiedade.

Palavras-Chave: Linhagem congênica, SHR, LEW, *Ofil1*, emocionalidade, ansiedade

Apoio Financeiro: CNPq, FAPESC

Behavioral Evaluation in young and adult rats after chronic treatment with methylphenidate

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Methylphenidate (MPH) is the most prescribed medication for children and adolescents with attention-deficit hyperactivity disorder. When administered repeatedly, it can result in persisting cellular and behavioral adaptations. However, it has been shown to vary in their pharmacokinetics and their efficacy throughout the light/dark cycle with fluctuations in dopamine levels. The present study investigated whether differences in the chronic MPH administration in young and adult rats at the beginning of the light or dark phase may cause changes in its effect on behavior evaluated by the elevated plus-maze and forced swimming test. Animals (young (P25; 75-85 g) and adults (P60; 250-300 g) received (light or dark cycle) IP injections of MPH Hydrochloride (2 mg/kg) or saline once a day for consecutive 28 days. Differences among experimental groups were determined by Student t test. p values less than 0.05 were considered to indicate statistical significance. Two hours after the last injection animals were submitted to the behavioral tests. In the elevated plus-maze test, young rats treated with MPH at light cycle, but not at dark cycle, showed an increase in the time spent in enclosed arms; while adult rats did not have behavioral alterations at both light and dark cycle. In the forced swimming test, we did not find any alterations in both young and adult rats at both light and dark cycle. In conclusion, these results indicate that chronic exposure to MPH during preadolescence lead to behavioral alterations related to an enhancement in anxiety that endure into adulthood only in light cycle protocol. These results highlight the need for further research to better understand the effects of stimulants on the developing nervous system and the potential enduring effects resulting from early-life drug exposure.

Palavras-Chave: Methylphenidate, light cycle, dark cycle and behavioral alterations

Apoio Financeiro: CNPq, FAPESC, Instituto Cérebro e Mente and UNESC

C.07.013

Antidepressant-like effects of N-acetyl-L-cysteine in rats

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Oxidative stress disturbances have been reported in depressed patients and in animals submitted to stress. Recent evidence suggests that antidepressants may have antioxidant properties. However, the therapeutic potential of antioxidants as antidepressant drugs has not been systematically investigated. Objectives: Therefore, the present study tested the hypothesis that N-acetyl-L-cysteine (NAC), a cysteine prodrug with powerful antioxidant activity, would possess antidepressant-like properties. Methods: Male Wistar rats were treated with NAC (5; 15; 50; 150 and 250 mg/Kg), imipramine (15mg/Kg) or vehicle and submitted to the forced swimming test (FST). Results: Treatment with NAC (at the doses of 15, 50 and 150 mg/Kg) or imipramine induced a significant decrease in immobility time (at the doses of 15, 50 and 150 mg/Kg; $F_{(6,35)} = 6.856$, $P = 0.0001$; Duncan test $P < 0.05$), without significant effects on exploratory behavior in an open arena ($F_{(6,39)} = 1.804$, $P = 0.247$). Conclusions: These results suggest that NAC can produce antidepressant effects.

Palavras-Chave: N-acetyl-L-cysteine, depression-like behavior, forced swimming test

Apoio Financeiro: FAPESP; CNPq; FAEPA

CANABIDIOL APRESENTA EFEITO PRÓ-DEPRESSIVO, VIA ATIVAÇÃO DE RECEPTORES CB1, EM CAMUNDONGOS SUBMETIDOS AO ESTRESSE CRÔNICO MODERADO.

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Objetivos: Relatos recentes da literatura mostram que a manipulação farmacológica do sistema endocanabinóide apresenta efeito antidepressivo, neuroprotetor e aumenta a neurogênese em roedores. Portanto, o objetivo deste trabalho é de investigar um possível efeito antidepressivo do canabidiol, um fitocanabinóide, em camundongos submetidos ao estresse crônico moderado.

Métodos: Camundongos machos suíços (90 dias, 40 g) foram submetidos ao estresse crônico moderado (ECM), durante 5 semanas. Grupos controles foram mantidos em suas respectivas caixas moradias, sem ter contato com os estímulos estressores. Após o período do ECM, os animais foram tratados diariamente, durante 14 dias, com canabidiol (10 mg/Kg, i.p.) ou veículo. A fim de se investigar a influência dos receptores CB1 no efeito do canabidiol, um grupo adicional de animais submetido ao ECM receberam AM251 (1 mg/Kg, i.p.) antes da administração de canabidiol. Os animais controles foram tratados de forma similar com veículo e canabidiol. Após 48 h do término do tratamento, os animais foram submetidos ao teste do nado forçado, sendo avaliado o tempo em posição de imobilidade durante 5 minutos de teste. Os dados obtidos foram analisados utilizando-se ANOVA de uma via e Duncan post hoc, considerando-se diferença estatística quando $P < 0,05$.

Resultados: Foi encontrada diferença significativa no tempo de imobilidade entre os grupos experimentais $F_{(5,24)} = 9,77$ $P < 0,01$. O grupo submetido ao ECM e tratado com veículo apresentou um aumento do tempo de imobilidade quando comparado ao grupo controle com tratamento similar ($159,33 \pm 12,13$; $79,25 \pm 14,99$, respectivamente) ($P < 0,01$). A administração de canabidiol aumentou o tempo de imobilidade nos animais submetidos ao ECM ($228,61 \pm 9,92$), quando comparado ao grupo submetido ao ECM e tratado com veículo ($P < 0,05$). No entanto, o canabidiol não alterou o tempo de imobilidade nos animais controle ($111,67 \pm 21,43$). Conforme esperado, a administração de AM251 aumentou o tempo de imobilidade nos animais submetidos ao ECM ($237,2 \pm 6,27$). Curiosamente, quando administrado previamente ao canabidiol, o AM251 reverteu o aumento do tempo de imobilidade induzido pelo canabidiol nos animais submetidos ao ECM ($185,1 \pm 27,49$).

Conclusão: O canabidiol, na dose utilizada no presente estudo, apresenta um efeito pró-depressivo no modelo do ECM. Este efeito está relacionado, ao menos em partes, com a ativação dos receptores CB1, haja vista que o pré-tratamento com AM251 reverteu este efeito pró-depressivo.

Palavras-Chave: Sistema Endocanabinóide; Depressão; Estresse Crônico Moderado; Plasticidade Neuronal; Camundongos

Apoio Financeiro: FAPESP

C.07.015

Mice lacking p35 exhibit hyperactivity, paradoxical response to psychostimulants and dopamine signaling deregulation: A potential mouse model of Attention Deficit with Hyperactivity Disorder?
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Attention deficit/hyperactivity disorder (ADHD) is the main psychiatric disorder affecting the 12% worldwide scholar age children. These kids display inappropriate levels of inattention, hyperactivity, and impulsivity that cause impairment in intellectual functioning. Drugs that regulate dopamine (DA) transmission, like psychostimulants (methylphenidate, d-amphetamine) are the usual and effective medicaments in ameliorate the behavioural symptoms. DA and components of catecholamine signalling are the first candidates in the ADHD aetiology. Considering that cdk5/p35 complex participates in membrane trafficking, endocytosis, drug addiction, and plays a critical role in dopaminergic transmission, we were encouraged to think a possible association of cdk5/p35 with ADHD. **Objective:** Investigate a possible role for cdk5/p35 in ADHD neurobiology. **Methods:** Transgenic mice deficient in p35 (p35KO) and WT were tested in an open field, treated or not with psychostimulants, and elevated plus maze. Expression levels of Tyrosine hydroxylase (TH), DA transporter (DAT) and D1 receptor were determinate in striatum and prefrontal cortex by western blot (WB). DA levels and its metabolites were quantified by liquid chromatography in these brain tissues. TH immunohistochemistry in brain sections were performed in both genotypes. **Results:** P35KO exhibited spontaneous hyperactivity and paradoxical response to psychostimulants. In the plus maze, p35KO mice did not manifest any behavioral sign of approach/avoidance conflict, entering indiscriminately into either protected or unprotected areas, indicating a deficit on proper behavioral inhibition. WB analysis revealed a significant increase of TH in striatum compared with WT, as well as a strong immunoreactivity in this area of brain sections, and raised levels of DA and its metabolites. Different expression levels of D1 and DAT were found between genotypes. **Conclusions:** These results suggests a strong association of cdk5/p35 with ADHD. We propose p35KO mouse as a novel animal model of ADHD, that fulfills many of the validation criteria for assessing ADHD models and they may also provide some useful insights into the basis neural pathways that subserve ADHD-like behaviors.

Palavras-Chave: ADHD, cdk5/p35, TH, DAT, D1, amphetamine, methylphenidate, dopamine

Apoio Financeiro: CONICET (PIP 6485) and FONCyT (PICT 05-38084)

Estudo do efeito da desnutrição protéica sobre o estímulo doloroso e o nado forçado em ratos Wistar.

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A desnutrição protéica precoce está relacionada com alterações comportamentais, morfológicas e neuroquímicas no SNC. Há poucos estudos sobre alterações comportamentais, em modelos animais, induzidas pela desnutrição protéica na fase adulta. O objetivo foi avaliar o efeito da desnutrição sobre a resposta ao estímulo doloroso no teste da formalina e sobre o tempo de imobilidade no teste do nado forçado. Ratos Wistar foram submetidos à desnutrição protéica pré e pós-natal (7%; controle 25%). Aos 4 meses de idade, os animais foram colocados em cilindros com água a 25°C durante 15 minutos no teste do nado forçado, foi anotado o tempo em segundos de imobilidade, após 24 horas os animais foram submetidos ao reteste durante 5 minutos. No teste da formalina, os animais receberam 50ul de formalina (1% em salina) intraplantarmente na pata esquerda; igual volume de solução salina foi injetado na pata contralateral. Foi avaliado o tempo gasto (segundos) pelos animais em lamber e/ou manter a pata erguida de 0-5 min (primeira fase, nocicepção neurogênica) e 5-30 min (segunda fase, nocicepção inflamatória) após injeção de formalina. Os resultados foram expressos como média ($\pm$ DP). Houve diferença (Teste t, $p < 0,0001$) entre os pesos dos animais normonutridos ($309,8 \pm 16,9$ g) e desnutridos ($149,3 \pm 8,4$ g). Os desnutridos apresentaram maior tempo de imobilidade no teste do nado forçado (controle 753 ± 56 ; desnutrido 798 ± 39 seg; Teste t, $p < 0,05$). A desnutrição interferiu significativamente na resposta ao estímulo nociceptivo em ambas as fases do teste da formalina. Na primeira fase, os desnutridos gastaram maior tempo de resposta nociceptiva (controle = $83,5 \pm 77,4$ seg; desnutridos = $251,2 \pm 71,0$ seg, Teste t, $p < 0,01$). Na segunda fase, também houve diferença significativa entre os grupos (controle = $430,8 \pm 510,0$ seg; desnutridos = $1124 \pm 256,0$ seg, Teste t, $p < 0,05$). No teste do nado forçado, um modelo animal de depressão, os animais desnutridos aumentaram o tempo de imobilidade. O estado nutricional alterou a resposta ao estímulo doloroso em ratos no teste de formalina, os animais submetidos à dieta hipoprotéica apresentaram um perfil hiperalgésico. **Palavras-Chave:** desnutrição protéica; nado forçado; teste da formalina; ratos

Apoio Financeiro: PIBIC CNPq/UFRGS; BIC/FAPERGS, BIC/UFRGS

Efeito da vitamina E em modelos animais preditivos de ação antidepressiva: evidência do envolvimento do sistema noradrenérgico

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O α -tocoferol (α -T), forma mais abundante da vitamina E, é o principal antioxidante lipossolúvel do organismo (FASEB J. 13:1145, 1999). Pacientes com depressão apresentam baixos níveis séricos de vitamina E, porém existem dúvidas se esta deficiência contribui para a patogênese da doença (Eur J. Clin. Nutr. 59:304, 2005). A depressão está associada à hipofunção do sistema noradrenérgico e muitos antidepressivos aumentam a disponibilidade sináptica de noradrenalina (Cell Signal. 17:549, 2005). Este estudo investigou o efeito do α -T no teste do nado forçado (TNF) e no teste de suspensão da cauda (TSC) e o envolvimento do sistema noradrenérgico neste efeito. Camundongos Swiss foram utilizados. O α -T foi administrado nas doses de 10-300 mg/kg, p.o. e 0.01-10 nmol/sítio, i.c.v e os animais foram posteriormente submetidos ao TNF, TSC ou teste do campo aberto (TCA). Para investigar o envolvimento do sistema noradrenérgico na ação antidepressiva do α -T, os animais foram pré-tratados com veículo, prazosim (1 mg/kg, i.p.) ou ioimbina (1 mg/kg, i.p.) e tratados com veículo ou α -T (100 mg/kg, p.o.). Adicionalmente, um possível efeito sinérgico entre doses sub-ativas de fenilefrina (5 mg/kg, i.p.), clonidina (0,06 mg/kg, i.p.) ou desipramina (8 mg/kg, p.o.), e uma dose sub-ativa de α -T (10 mg/kg, p.o.) foi investigado no TNF. O tratamento oral com α -T reduziu o tempo de imobilidade nas doses de 30 e 100 mg/kg no TNF e no TSC (87.04 $\pm$ 1.70%, 73.89 $\pm$ 1.41% e 61.53 $\pm$ 6.10%, 68.64 $\pm$ 4.89% em relação ao grupo controle). A administração i.c.v de α -T também reduziu o tempo de imobilidade no TNF nas doses de 0.1 e 1 nmol/sítio (63.40 $\pm$ 5.47% e 73.31 $\pm$ 7.76%) e no TSC na dose de 0.01 nmol/sítio (74.60 $\pm$ 5.47%). O tratamento com α -T não alterou a locomoção dos animais no TCA. A ação antidepressiva do α -T no TNF foi prevenida pelo pré-tratamento com prazosim (88.61 $\pm$ 4.42%) ou ioimbina (98.84 $\pm$ 2.86%). Houve um efeito sinérgico entre α -T e fenilefrina (75.08 $\pm$ 2.27%), clonidina (89.37 $\pm$ 1.35%) ou desipramina (78.46 $\pm$ 4.14%). A administração de α -T por via oral ou central produziu efeito antidepressivo específico em camundongos, o qual parece ser mediado por uma interação com receptores α_1 e α_2 -adrenérgicos.

Palavras-Chave: Vitamina E, depressão, modelos animais de depressão, sistema noradrenérgico

Apoio Financeiro: CAPES, CNPq, IBNET

EFEITOS DA OVARECTOMIA PRECOCE NA MEMÓRIA AVERSIVA E DE RECONHECIMENTO DE RATAS IDOSAS

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Objetivo: Dados da literatura sugerem que a curto prazo a remoção das glândulas gonadais prejudica a memória em ratos. Porém, pouco é sabido sobre os efeitos da ovariectomia (OVX) a longo prazo na memória de ratas idosas. O presente trabalho visa avaliar os efeitos da OVX precoce em ratas idosas sobre a memória aversiva e de reconhecimento de objetos.

Materiais e Métodos: Foram utilizadas ratas Wistar adultas (6 m) e idosas (18 m). As ratas foram divididas em grupos (n=12/grupo): sham adulta, sham idosa e OVX idosa, sendo a OVX realizada no 3º mês de vida. Para avaliação da memória aversiva, as ratas foram treinadas no teste da esquiava inibitória, onde o tempo gasto para descer da plataforma foi registrado em 2 sessões: 1) treino, onde as ratas receberam um choque após descer da plataforma, e 2) teste: 24 h após. A memória de reconhecimento de objetos foi avaliada em uma arena através do tempo de exploração de 2 objetos idênticos (A + A; treino) e 2 objetos diferentes (A + B, teste) 24 h após.

Resultados: De modo geral, as ratas idosas sham apresentaram prejuízo cognitivo comparado com ratas adultas. No teste de reconhecimento de objetos, as ratas idosas sham não apresentaram diferença no índice de reconhecimento comparado à sessão de treino [adultas sham: 0.60(0.49-0.65) vs. 0.78(0.66-0.85)*, idosas sham: 0.55(0.42-0.66) vs. 0.68(0.54-0.75), idosas OVX: 0.42(0.32-0.51) vs. 0.67(0.64-0.70)*, *P<0.05 vs. treino], sugerindo que a OVX atenua os déficits cognitivos causados pelo envelhecimento. No teste da esquiava inibitória, ratas idosas OVX não apresentaram diferença na latência para descer da plataforma comparado com as ratas idosas sham [adultas sham: 45(18-180), idosas sham: 5(2-7)*, idosas OVX: 10(5-27)*, *P<0.05 vs. adultas sham], sugerindo que a OVX não altera os déficits cognitivos causados pelo envelhecimento. Por fim, não houve diferença na atividade locomotora entre os grupos analisados.

Conclusões: Os dados sugerem que a longo prazo, a OVX pode atenuar, ao menos, o déficit de memória de reconhecimento causado pelo envelhecimento. Estes dados contribuem para o entendimento do papel dos hormônios ovarianos nos prejuízos cognitivos causados pelo envelhecimento. **Palavras-Chave:** ratas, ovariectomia, memória, esquiava inibitória, reconhecimento de objetos.

Apoio Financeiro: CNPq (Brasil), IBRO (Return Home Fellowship) e UNESC

A ADMINISTRAÇÃO DE LÍCIO E VALPROATO PREVINEM O EFEITO HIPERLOCOMOTOR DO NEUROPEPTÍDEO S EM CAMUNDONGOS

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Objetivos: O neuropeptídeo S (NPS) é um peptídeo formado por 20 aminoácidos e é o ligante endógeno de um receptor acoplado a proteína G denominado de NPSR. Diversos estudos demonstraram que a administração de NPS aumentou a atividade locomotora de roedores. Fármacos psicoestimulantes têm sido empregados na pré-clínica como modelos animais para estudo da mania. Diante disso, o presente estudo tem por objetivo avaliar os efeitos do pré-tratamento com lítio e valproato, fármacos estabilizadores do humor, no efeito hiperlocomotor do NPS.

Metodologia: Camundongos CF-1 machos (35-40 g) foram utilizados neste estudo. Os animais foram intraperitonealmente pré-tratados com lítio 100 mg/kg ou valproato 200 mg/kg 25 min antes do tratamento com NPS. O NPS foi injetado na dose de 0.1 nmol/camundongo por via ICV 5 min antes da avaliação da atividade locomotora. A locomoção dos animais foi efetuada em uma arena automática, sendo a distância percorrida (cm) e o número de rearings avaliados por um período de 30 min.

Resultados: Nossos resultados demonstraram que o NPS 0.1 nmol causou hiperlocomoção em camundongos comparado ao grupo controle. O tratamento com lítio e valproato per se não alterou a distância percorrida pelos camundongos quando comparado ao controle. O pré-tratamento com lítio e valproato preveniu o aumento da distância percorrida pelos animais tratados com NPS 0.1 nmol comparados com os que receberam apenas NPS 0.1 nmol (Salina + salina: 3523 ± 434; salina + NPS: 5783 ± 655[#]; lítio + salina: 2566 ± 484; lítio + NPS: 3543 ± 562^{*}; valproato + salina: 2422 ± 324; valproato + NPS: 2715 ± 541^{*}; [#]P<0.05 vs. salina + salina; ^{*}P<0.05 vs. salina + NPS). Nenhuma diferença significativa no número de rearings foi encontrada entre os grupos estudados.

Conclusão: Em conclusão, o nosso estudo demonstra que os fármacos estabilizadores do humor (lítio e valproato) preveniram o efeito hiperlocomotor induzido pelo NPS em camundongos. Este estudo propõe a administração de NPS como um novo modelo animal de mania.

Palavras-Chave: Neuropeptídeos; Valproato; Lítio; Atividade Locomotora

Apoio Financeiro: CNPq(Brasil), IBRO (Return Home Fellowship) e UNESC.

TRATAMENTO AGUDO COM IMIPRAMINA E FLUOXETINA NÃO ALTERA A DENSIDADE DE RECEPTORES 5-HT_{1A} DA MATÉRIA CINZENTA PERIAQUEDUTAL DE RATOS.

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Objetivo: Investigar se o tratamento agudo com imipramina ou fluoxetina altera a densidade de receptores 5-HT_{1A}, presentes na matéria cinzenta periaquedutal (MCP) de ratos expostos previamente ao Labirinto em T elevado (LTE).

Métodos: Ratos Wistar machos (220-240 g, n= 8-10) receberam imipramina (15 mg/kg, i.p.), fluoxetina (10 mg/kg, i.p.), midazolam (2 mg/kg, i.p.; grupo controle positivo) e salina (NaCl a 0,9%; grupo controle) e, 20 minutos após, foram submetidos ao teste no LTE conforme descrito por Graeff et al (1). Terminado o teste, os animais foram sacrificados, seus encéfalos retirados e as MCP dissecadas foram submetidas à eletroforese em gel de poliacrilamida e transferidas eletroforéticamente para membranas de nitrocelulose, que posteriormente foram submetidas à técnicas de revelação e quantificação de proteínas (Western Blotting).

Resultados: De acordo com a ANOVA unifatorial, as porcentagens (em relação ao grupo salina) de receptores 5-HT_{1A} dos grupos tratados com imipramina (127,47 ± 16,35), fluoxetina (116,63 ± 21,30) e midazolam (120,02 ± 23,15) não foram estatisticamente diferentes da porcentagem de receptores do grupo salina (100,00 ± 5,35) (F3,38 = 2,02, p = 0,65).

Conclusão: O tratamento agudo com imipramina e fluoxetina não alterou a densidade de receptores 5-HT_{1A} presentes na MCP de ratos. Tais resultados corroboram a ausência de efeitos com o tratamento agudo desses antidepressivos sobre o comportamento defensivo avaliado no LTE e na ansiedade clínica. Faz-se necessário investigar os efeitos do tratamento crônico com esses fármacos sobre a densidade de receptores 5-HT_{1A} na MCP de ratos.

Referências: Braz. J. Med. Biol. Res. 26:67, 1993.

Palavras-Chave: Labirinto em T elevado, Western Blotting, Matéria Cinzenta Periaquedutal, Imipramina, Fluoxetina

O SINERGISMO ENTRE OS ACUPONTOS E36 E BP6 É CRUCIAL PARA O EFEITO ANTIDEPRESSIVO DA ELETROACUPUNTURA EM RATOS SUBMETIDOS AO TESTE DO NADO FORÇADO.

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OBJETIVO: Em um estudo prévio verificamos que a estimulação conjunta dos pontos E36 e BP6 apresentou efeito antidepressivo em ratos submetidos ao teste do nado forçado e desamparo aprendido. No entanto, não se sabe se o efeito encontrado foi decorrente de um sinergismo entre os acupontos ou de um dos acuponto em específico. Neste trabalho comparamos o efeito da estimulação isolada do ponto E-36 e BP-6 com a estimulação concomitante destes pontos em ratos submetidos ao teste do nado forçado.

MÉTODOS: Ratos Wistar EPM-1, machos (90 dias, 250g), foram submetidos a três procedimentos de EA. No grupo AC1+2 foi avaliado o efeito da estimulação concomitante dos acupontos E36 e BP6, enquanto que, nos grupos AC1 e AC2 foi verificado, respectivamente, o efeito da estimulação específica de cada acuponto. Grupos adicionais nos quais foram realizados EA em regiões não-ponto serviram como Sham (Sham1+2, Sham1, Sham2). O estímulo utilizado para a EA consistiu de 7 aplicações em dias alternados, de 2Hz e 50µA, sendo que cada sessão durou 20 min. Ainda, outros dois grupos controle foram adicionados ao experimento: Naïve e Anestesiado. Após 24h do último tratamento, os animais foram submetidos a uma sessão pré-teste de 15min de nado forçado e no dia seguinte ao teste do campo aberto e logo em seguida a sessão teste do nado forçado, ambos com 5 min de duração. Durante os 5 min de cada teste foi avaliado o número de quadros explorados no campo aberto e tempo de permanência em posição de imobilidade no nado forçado. Os dados obtidos foram analisados por ANOVA de uma via, seguida de Tukey *post hoc*, considerando-se diferença estatística quando $p < 0,05$.

RESULTADOS: Somente o grupo EA1+2 apresentou uma diminuição significativa do tempo de imobilidade quando comparado ao grupo Naïve e Anestesiado [$F(7,56)=4,97$ $p < 0,05$]. Ainda, os grupos experimentais não diferiram entre si quanto à atividade locomotora no teste do campo aberto [$F(7,56)=2,16$ $p=0,08$].

CONCLUSÃO: O efeito antidepressivo verificado pela estimulação concomitante dos acupontos E36 e BP6 é decorrente do efeito sinérgico entre estes pontos e não de um efeito específico de um dos pontos utilizados. Nossos dados encontram-se de acordo com a visão da Medicina Tradicional Chinesa, que define que cada acuponto apresenta funções específicas, no entanto quando utilizados de forma isolada, os efeitos terapêuticos são limitados.

Palavras-Chave: Acupuntura; antidepressivo; ratos;

Apoio Financeiro: FAPESP; CAPES; CNPq

EFEITOS DA CETAMINA EM RATOS SUBMETIDOS AO PROTOCOLO DE ESTRESSE CRÔNICO E VARIADO

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Objetivos: Estudos recentes demonstraram que a administração aguda e crônica de cetamina produziu ação antidepressiva em animais no teste do nado forçado, porém apenas o tratamento agudo aumentou significativamente os níveis de BDNF hipocampais. Com isso este estudo objetivou avaliar os efeitos da cetamina em ratos submetidos ao protocolo de estresse crônico e variado (ECV).

Métodos e Resultados: Ratos Wistar adultos (250-350 g) divididos em (n=15): Controle+Sal, Controle+Cetamina Agudo, Controle+Cetamina Crônico, ECV+Sal, ECV+Cetamina Agudo, ECV+Cetamina Crônico, foram submetidos ao protocolo de ECV por 40 dias. Após, foi avaliado a anedonia, atividade locomotora e o tratamento intraperitoneal com salina ou cetamina (15 mg/kg) 60 min antes dos testes comportamentais. O tratamento consistiu em sete dias: no tratamento agudo, a cetamina foi administrada no primeiro dia do teste comportamental e nos dias subsequentes receberam salina e no tratamento crônico, receberam cetamina em todos os dias. O peso corporal dos animais foram mensurados antes e após os procedimentos. Após os testes comportamentais, os animais foram anestesiados e o sangue retirado para verificar os níveis de Cortisol e do hormônio adrenocorticotrófico (ACTH). A glândula adrenal foi retirada para avaliação do peso e o hipocampo para avaliação dos níveis de BDNF. Foi utilizado teste ANOVA *post-hoc* Tukey com significância de 0.05. Os grupos Controle+Cetamina Crônico e ECV+Cetamina Crônico apresentaram um maior consumo de alimento doce quando comparados aos grupo Controle+Sal e ECV+Sal ($p<0.05$) e nenhum grupo demonstrou alteração na atividade locomotora; não houve aumento de peso corporal no grupo ECV+Sal ($p>0.05$); houve aumento significativo da glândula adrenal; dos níveis de Cortisol e ACTH no grupo ECV+Sal ($p<0.05$) e não houve aumento significativo dos níveis de BDNF hipocampais nos grupos avaliados em comparação com os grupos controles ($p>0.05$).

Conclusão: Estes resultados suportam a hipótese que ratos submetidos ao protocolo ECV e tratados cronicamente, mas não agudamente com Cetamina apresentaram diminuição dos parâmetros relacionados à depressão, porém, nenhum dos tratamentos aumentou os níveis de BDNF hipocampais.

Palavras-Chave: Cetamina, modelo animal de estresse crônico variado, BDNF, depressão

Apoio Financeiro: Instituto Cérebro e Mente, UNESC, FAPESC, CNPq

ESTRESSE CRÔNICO VARIADO AUMENTA O ESTRESSE OXIDATIVO EM PARTICULAS SUBMITOCONDRIAS EM CÉREBRO DE RATOS

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Objetivo: Recentemente, foi evidenciado que espécies reativas de oxigênio (ERO) têm uma participação importante na patologia de muitas doenças neurológicas e psiquiátricas. O objetivo desse estudo foi avaliar os parâmetros comportamentais, fisiológicos e a geração de ERO nas partículas submitocondriais de ratos expostos a um modelo de estresse crônico e variado (ECV).

Métodos e Resultados: Ratos Wistar machos (3-4meses, 220-310g) foram dividido em dois grupos de quinze animais cada. Um grupo de animais foi exposto durante 40 dias a um modelo de estresse crônico e variado (ECV) e o outro grupo não foi submetido ao modelo (controle). Após os 40 dias, o consumo de alimento doce, atividade locomotora e o peso corporal foram avaliados para verificar comportamento anedônico. Além disso, foram avaliados a geração de ERO nas partículas submitocondriais (Superóxido e peroxidação lipídica –TBARS) em hipocampo, pré-frontal e córtex após o protocolo. A análise dos dados foi feita pelo teste *T de Student* com significância de 0,05. Os resultados demonstraram uma diminuição da ingestão de alimentos doces em ratos submetidos ao procedimento de ECV comparado ao controle ($t=3,978$; $df=21,979$; $P=0,001$), diminuição do peso corporal ($t=5,899$; $df=28$; $P=0,0001$) foi observada em ratos estressados. Atividade locomotora não foi afetada em ratos estressados. A geração de ERO em partículas submitocondriais no cérebro foram: aumento da geração de superóxido no pré-frontal ($t=6,160$; $df=8$; $P=0,0001$), córtex ($t=8,087$; $df=8$; $P=0,0001$) e hipocampo ($t=4,747$; $df=8$; $P=0,001$), e aumento na peroxidação lipídica no córtex ($t=4,747$; $df=8$; $P=0,001$).

Conclusão: Estes resultados suportam a hipótese que ratos submetidos ao modelo de ECV apresentaram dano oxidativo em todas as estruturas avaliadas e que este modelo animal pode ser utilizado para investigar alterações energéticas cerebrais relacionados à depressão.

Palavras-Chave: Espécies reativas de oxigênio, estresse crônico variado, depressão

Apoio Financeiro: UNESC, CNPq, Instituto Cérebro e Mente

CORRELAÇÕES COMPORTAMENTAIS NA LINHAGEM DE RATOS ESPONTANEAMENTE HIPERTENSOS (SHR) : IMPLICAÇÕES PARA TRANSTORNOS PSIQUIÁTRICOS.

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Objetivos: A esquizofrenia e o transtorno de déficit de atenção e hiperatividade (TDAH) compartilham diversas alterações comportamentais. Todavia, as possíveis intersecções entre esses transtornos não estão bem estabelecidas. A linhagem de ratos SHR tem sido utilizada para o estudo do TDAH. Paralelamente, dados de nosso laboratório mostram que o déficit de medo condicionado ao contexto (MCC) apresentado por animais SHR é revertido por neurolépticos (utilizados na esquizofrenia), mas não por psicoestimulantes (utilizados no TDAH). O objetivo deste trabalho foi caracterizar e correlacionar alterações comportamentais relacionadas à esquizofrenia e/ou ao TDAH apresentadas pela linhagem SHR.

Métodos e Resultados: Ratos Wistar e SHR adultos (n=28) foram avaliados quanto à locomoção basal e habituação em campo aberto, interação social, esQUIVA discriminativa em labirinto em cruz elevado (EDLCE); MCC e locomoção induzida pelo efeito agudo de anfetamina e sua sensibilização. O teste de Pearson mostrou uma correlação positiva entre locomoção basal ($95,1 \pm 4,4$), locomoção na habituação ($59,17 \pm 3,9$), locomoção induzida pela anfetamina ($123,82 \pm 8,4$) e a porcentagem de tempo nos braços abertos da EDLCE ($9,9 \pm 2,3$) na linhagem Wistar. As correlações observadas para a linhagem SHR não seguem esse padrão: o aumento de locomoção basal ($142,3 \pm 5,2$) correlaciona-se com o MCC ($17,4 \pm 4,1$) e a locomoção sensibilizada ($210,2 \pm 8,7$).

Conclusão: Nossos dados sugerem que os ratos Wistar, embora menos ativos que os ratos SHR, têm seus parâmetros de atividade motora correlacionados entre si. As correlações observadas na linhagem SHR não estão relacionadas à atividade motora dos animais, mas sim a comportamentos relacionados a transtornos psiquiátricos. Esse padrão pode indicar associações entre esses sintomas, sugerindo essa linhagem como um modelo útil tanto para a identificação precoce de transtornos psiquiátricos, quanto para a avaliação do prognóstico e de melhores estratégias terapêuticas.

Palavras-Chave: Esquizofrenia, Transtorno de Déficit de Atenção e Hiperatividade, Ratos Espontaneamente Hipertensos, Modelo Animal

Apoio Financeiro: CAPES, CNPq

A participação de vias de sinalização celular no efeito antidepressivo do ácido fólico no teste do nado forçado

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Objetivos: O ácido fólico (AF) é uma vitamina do complexo B que está envolvido na patogênese da depressão em humanos, uma vez que foi relatado que pacientes com depressão apresentam baixas concentrações plasmáticas e eritrocitárias de AF e estes pacientes não respondem tão bem ao tratamento com antidepressivos (Am J Psychiatry 154:426, 1997; Nutrition 16:544, 2000). Demonstramos recentemente que a administração de AF produz efeito antidepressivo no teste do nado forçado (TNF) em camundongos (Neuropharmacology 54:464, 2008). Tendo em vista que vários trabalhos têm mostrado que os antidepressivos ativam as vias proteína cinase dependente de AMPc (PKA), da proteína cinase C (PKC) e modulam as vias da proteína cinase II dependente de Ca²⁺/calmodulina (CaMKII) e das proteínas cinases ativadas por mitógenos (MAPK) e das proteínas cinases reguladas por sinal extracelular (ERK) (J Neurochem. 74:21, 2000; Cell Signal. 17:549, 2005). O objetivo deste trabalho foi investigar a participação das vias de sinalização intracelular dependentes de PKA, MAPK/ERK, CaMKII e PKC na ação antidepressiva do AF no TNF.

Métodos e Resultados: Foram utilizados camundongos *Swiss* adultos de ambos os sexos, que foram pré-tratados com veículo ou com os seguintes compostos: H-89 (um inibidor da PKA, na dose de 1 µg/sítio, i.c.v.), PD98059 (um inibidor da MAPK/ERK cinase, na dose de 5 µg/sítio, i.c.v.), KN-62 (um inibidor da CaMKII, na dose de 1 µg/sítio, i.c.v.), queleritrina (um inibidor da PKC, na dose de 1 µg/sítio, i.c.v.). Depois de 15 minutos, os camundongos receberam AF (10 nmol/sítio, i.c.v.) e decorridos 15 minutos, eles foram submetidos ao TNF. O efeito antidepressivo do AF (62% ± 8,57) em relação ao tempo de imobilidade do grupo controle (100%) no TNF foi completamente prevenido pelos pré-tratamentos dos camundongos com H-89 (101% ± 4,98) e com KN-62 (88,2% ± 7,1).

Discussão: Estes resultados indicam que o AF exerce seu efeito antidepressivo no TNF por modular vias de sinalização celular (PKA e CaMKII) que regulam a neuroplasticidade e a sobrevivência celular o que poderia explicar, pelo menos em parte, a neuroproteção ocasionada pelo AF nos modelos de depressão.

Palavras-Chave: depressão; sinalização celular; teste do nado forçado

Apoio Financeiro: CNPq e CAPES

**INVESTIGAÇÃO DO EFEITO DO ÁCIDO ASCÓRBICO EM MODELOS ANIMAIS
PREDITIVOS DE ATIVIDADE ANTIDEPRESSIVA**

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O L-ácido ascórbico (AA; vitamina C) está presente em altas concentrações no encéfalo, sob a forma de ascorbato e, além de seu papel como antioxidante (neuroprotetor), pode atuar como neuromodulador dos sistemas dopaminérgico e glutamatérgico (Prog. Neurobiol. 43:537, 1994; Trends Neurosci. 23:209, 2000), os quais estão relacionados com a patofisiologia da depressão (Eur. J. Pharmacol. 375:31, 1999; Nat. Rev. Neurosci. 2:343, 2001). Este trabalho objetiva investigar a ação antidepressiva do AA no teste do nado forçado (TNF) e no teste da suspensão da cauda (TSC), dois modelos utilizados na pesquisa de compostos com possível ação antidepressiva. Camundongos Swiss (30-45g) foram utilizados. A fim de investigar a ação antidepressiva do AA no TNF e no TSC, os animais foram tratados (p.o.), com veículo ou AA (0,1- 100 mg/kg) 60 min antes do TNF, TSC ou do teste do campo aberto. Para elaboração da curva tempo-resposta do AA, os animais foram tratados com veículo ou AA (1 mg/kg, p.o.), 30, 60, e 120 min antes do TSC. Adicionalmente, um possível efeito sinérgico entre doses sub-ativas de fluoxetina (1 mg/kg, p.o.), imipramina (1 mg/kg, p.o.) ou bupropiona (1 mg/kg, p.o.) e uma dose sub-ativa de AA (0,1 mg/kg, p.o.) foi investigado no TSC. O tratamento com AA, reduziu o tempo de imobilidade no TSC (1 e 10 mg/kg), ($57,11 \pm 6,19\%$ e $70,31 \pm 6,49\%$ respectivamente, em relação ao grupo controle= 100%), sem alterar a atividade locomotora. O AA (1 mg/kg), reduziu o tempo de imobilidade dos animais quando administrado 60 min antes do TSC ($58,48 \pm 5,84\%$), mas não após 30 e 120 min. A administração de fluoxetina, imipramina ou bupropiona em combinação com AA (0,1 mg/kg) reduziu ($54,31 \pm 3,08\%$, $70,12 \pm 5,88\%$ e $79,98 \pm 6,59\%$ respectivamente, em relação ao grupo controle) o tempo de imobilidade dos animais no TSC, sem alterar a atividade locomotora. Os resultados indicam que o AA tem propriedades antidepressivas quando administrado por via oral, sendo seu efeito potencializado por antidepressivos clássicos.

Palavras-Chave: Ácido ascórbico, depressão, teste da suspensão da cauda, vitamina C

Apoio Financeiro: CAPES, CNPq, IBN-Net (01.06.0842-00)

ENVOLVIMENTO DOS SISTEMAS SEROTONINÉRGICO, NORADRENÉRGICO E DOPAMINÉRGICO NA AÇÃO ANTIDEPRESSIVA DA BETA-GLUCAN NO TESTE DO NADO FORÇADO

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Objetivos: A beta-glucan é um polímero de glicose presente em leveduras e fungos. Vários estudos têm mostrado que a beta-glucan pode modular a função imune e também apresenta uma ação antioxidante (Neurosurg. Rev. 28:298, 2005; Eur. J. Pharmacol. 542:170, 2006). Recentemente demonstramos que a beta-glucan apresenta efeito antidepressivo no teste do nado forçado (TNF) em camundongos (XXII Reunião Anual da FeSBE, nº 13.005, 2007). Este estudo tem por objetivo investigar o envolvimento dos sistemas serotoninérgico, noradrenérgico e dopaminérgico na ação antidepressiva da beta-glucan no TNF.

Métodos e Resultados: Camundongos Swiss (30-40 g, n= 6/grupo) de ambos os sexos foram utilizados. O tempo de imobilidade no teste do nado forçado foi o parâmetro utilizado para avaliar a atividade antidepressiva. O pré-tratamento dos animais com cetancerina (5 mg/kg i.p., antagonista de receptor 5-HT_{2A}) ou NAN-190 (antagonista de receptor 5-HT_{1A}) reverteu de maneira significativa (100,7±1,4% e 99,2±1,1% em relação ao grupo controle, respectivamente) o efeito antidepressivo da beta-glucan (0,1 mg/kg, p.o.) (80,6±2,1% em relação ao grupo controle). Além disso, o pré-tratamento dos animais com prazosim (1 mg/kg, i.p., antagonista α_1 adrenérgico) ou ioimbina (1 mg/kg, i.p., antagonista α_2 adrenérgico) também reverteu de maneira significativa (97,2±1,0% e 97,5±2,0% em relação ao grupo controle, respectivamente) o efeito antidepressivo da beta-glucan (0,1 mg/kg, p.o.) (80,6±2,1% em relação ao grupo controle). O pré-tratamento dos animais com sulpirida (50 mg/kg i.p., antagonista D₂ dopaminérgico) ou SCH-23390 (antagonista D₁ dopaminérgico) também causou reversão de maneira significativa (103,2±0,7% e 101,2±1,0% em relação ao grupo controle, respectivamente) do efeito antidepressivo da beta-glucan (0,1 mg/kg, p.o.) (80,6±2,1% em relação ao grupo controle) no TNF. Os dados foram analisados por ANOVA de uma via, seguido pelo teste de Tukey's HSD (dados significantes quando P<0,05).

Conclusão: Estes resultados sugerem o envolvimento dos sistemas serotoninérgico, noradrenérgico e dopaminérgico na ação antidepressiva da beta-glucan no TNF.

Palavras-Chave: Beta-glucan, teste do nado forçado, sistema serotoninérgico

Apoio Financeiro: CNPq, PROCAD-CAPES, IBNET

Behavioral syndrome in omega-3 deficient-rats: hyperactivity, anxiety-like behavior and impaired long-term memory.

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Introduction and objective: Polyunsaturated omega-3 fatty acids (w3PUFAs) are very important to brain development. Many studies related w3PUFAs deficiency with behavior abnormalities in animal models. The present study aimed to investigate the influence of w3PUFAs in behavior in adult rats.

Methods: Wistar male rats were divided in two groups: one received a diet with w3PUFAs (w3-diet) and other received a w3 deficient-diet (w6-diet), modeled after a Japanese or Western diets, respectively, since the conception to 60 days post-natal. To evaluate hyperactivity behavior it was used open field task; to evaluated anxiety-like behavior it was used elevate plus maze task; and inhibitory avoidance was used to evaluated short-term memory (STM, 1.5h after trail) and long-term memory (LTM, 24h and 7 days after trail).

Results: Rats that received w6-diet showed higher locomotor activity in open field than w3-diet group (107.5 ± 5.07 ; 89.28 ± 4.47 squares; $p < 0.05$), which characterizes hyperactivity behavior. They also stayed less time (w3-diet 29.83 ± 8.44 ; w6-diet 10.06 ± 3.06 s) and presented less number of entries in open arms (w3-diet 2.44 ± 0.54 ; w6-diet 0.94 ± 0.26 ; $p < 0.05$) showing anxiety-like behavior. In inhibitory avoidance, animals remembered the shock and did not step down in 1.5 and 24h after trail. But w6-diet group did not remember the shock 7 days after trail, showing impair in LTM.

Conclusion: Chronic omega-3 deficient-diet during development was capable to cause behavior abnormalities in rats. Western diet is very poor in w3PUFAs and the consumption of these compounds has to be stimulated to prevent possible brain developmental damages and possible consequences in adulthood.

Palavras-Chave: omega-3, behavior, memory, hyperactivity, anxiety-like behavior

Apoio Financeiro: CNPq/Brazil, CAPES, IBNnet, FAPERGS

Fluoxetina afecta diferencialmente las conductas de desesperanza aprendida y ansiedad inducidas por distintos modelos de estrés crónico en ratas.

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La depresión es una patología psiquiátrica caracterizada por un número variable de síntomas cognitivos, emocionales y neurovegetativos. El estrés crónico es uno de los modelos animales de depresión más utilizados, ya que altera conductas como la desesperanza aprendida y ansiedad semejantes a los observados en pacientes deprimidos y provoca cambios adaptativos que se previenen mediante el uso de fármacos antidepresivos (FADs). Fluoxetina, un inhibidor de la recaptación de serotonina, es uno de los antidepresivos más usados en la actualidad. Se han descrito una gran cantidad de modelos animales de estrés crónico que tienen como característica la reproducción parcial de los signos y síntomas de la enfermedad. La mayoría de estos trabajos evalúan el efecto agudo del fármaco, lo que no se relaciona con el retardo en la mejoría clínica observada después del uso crónico de estos fármacos. Esto nos sugiere que existen fenómenos neuroadaptativos que se establecen por el uso crónico del fármaco y que permiten el desarrollo del efecto antidepresivo. Por otra parte, históricamente han utilizado dosis altas de fluoxetina, subtóxicas para las ratas. Además el efecto antidepresivo del fármaco ha sido evaluado principalmente en ratas “*nativas*” controles, que no presentan la enfermedad previamente al comienzo de la terapia. Sin embargo, es sabido que los fármacos antidepresivos solo tienen efectos sobre los pacientes diagnosticados con depresión no a sí sobre sujetos sanos. Todas estas limitaciones hacen necesario una mejor comprensión de los distintos modelos en animales de depresión. Para esto se establecieron dos modelos de depresión inducida por estrés crónico en ratas: la restricción de movimiento en jaulas y la inmovilización en bolsas. Se evaluó el efecto de la administración crónica de dosis bajas de fluoxetina (0,7 mg/kg) en cuatro grupos experimentales: Control + Veh (C), Control+Flx (CF), Estrés + Veh (E), Estrés +Flx (EF). Se determinó los niveles de ansiedad mediante el test de la supresión en la ingesta de alimento y el laberinto en cruz elevado en oscuridad y se evaluó la conducta de desesperanza aprendida mediante el test de la suspensión de la cola. La administración de fluoxetina revierte el aumento en los niveles de ansiedad inducido por el modelo de estrés crónico por restricción de movimiento, sin afectar los niveles de ansiedad inducidos por el modelo de la inmovilización de movimiento. De la misma manera, dosis bajas fluoxetina aumentan las conductas activas en ratas sometidas al test de nado forzado solo en ratas sometidas a estrés crónico por restricción de movimiento. Este trabajo permitirá comprender mejor los cambios que induce la depresión, una patología de altísima prevalencia y costo para nuestra sociedad. **Palavras-Chave:** Behavior, Stress, fluoxetine.

C.07.030

O TESTE VISOMOTOR DE BENDER COMO INSTRUMENTO DE DIAGNOSTICO DO TDAH: UM ESTUDO EM AMAZONAS

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RESUMO

O transtorno de déficit de atenção e hiperatividade (TDAH) é comum em crianças na idade escolar, 3% a 7% da população infantil, se intensifica com a presença de comprometimento orgânico e caracteriza-se por ter diminuída a capacidade de atenção, impulsividade e hiperatividade, (APA, 2003)

OBJETIVOS

Correlacionar as variáveis comorbidade e TDAH a través das respostas obtidas no teste Visomotor de Bender.

METODO

Estudo correlacional analítico realizado numa amostra de 10 alunos de ambos sexos de 10 a 12 anos da Escola estadual Jose Carlos Mestrinho da cidade de Itacoatiara-interior do Estado do Amazonas no ano de 2007, utilizou-se como instrumentos de avaliação o teste Visomotor de Bender e o questionário de Entrevista clinica para identificar outros transtornos.

RESULTADOS

A prevalência encontrada de déficits segundo o teste de Bender na percepção visual, na memória e nos conceitos espacial foi 38,6%. Sendo que a presença de comorbidade, transtorno de ansiedade, e tics intensificaram a 80% a prevalência dos sintomas de TDAH como desatenção, impulsividade, dificuldade para organizar tarefas e atividades. encontrando-se maior prevalência em homens (70%) em contraposição (30%) em mulheres.

CONCLUSÃO

O teste de Bender constitui um ótimo Instrumento no diagnostico de TDAH, (Cunha & col.(1993). Em relação aos dados obtidos prevalência de diagnostico de TDAH em homens, justifica-se que na maioria dos estudos publicados a proporção de TDAH é maior entre crianças do sexo masculino do que entre aquelas do sexo feminino, (Vasconcelos.2003). A presença de comorbidade intensifica a probabilidade de um prognóstico desfavorável no futuro (Souza, Serra, Mattos & Franco, 2001)

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Palavras-Chave: TDAH, Teste de Bender, transtornos de ansiedade

Apoio Financeiro: FAPEAM/CAPES

**ESCALAS DE AUTO-AVALIAÇÃO DIFERENCIAM ANSIEDADE DE DEPRESSÃO? -
RESULTADOS DE UM ESTUDO PRELIMINAR**

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Objetivos: Diferentes estudos têm questionado a capacidade de instrumentos de avaliação de diferenciar pacientes com transtornos de ansiedade e depressão. Muitos destes estudos, porém, têm sido influenciados por comorbidades, variações nos critérios diagnósticos utilizados, ou uso de medicamentos. Sendo assim, o objetivo do presente trabalho foi avaliar a subescala Traço do Inventário de Ansiedade Traço-Estado (IDATE-T) e a Escala Hospitalar de Ansiedade e Depressão (HAD), em relação à capacidade de discriminação entre pacientes com transtornos de ansiedade e depressão, de acordo com o DSM-IV-R, livres de comorbidades e sem tratamento.

Métodos e Resultados: Os sujeitos do estudo foram 6 pacientes com Depressão Maior (DM) e 10 pacientes com Transtorno de Ansiedade Generalizada (TAG), aos quais foram aplicados o IDATE e a HAD, logo após o diagnóstico por um psiquiatra. Um grupo de 8 voluntários saudáveis (VS), com a mesma faixa etária, serviu de controle. As comparações entre grupos mostraram que os pacientes com DM eram significativamente diferentes dos pacientes com TAG ($p < 0,05$), de acordo com a HAD (média $\pm$ dp: HAD-Ansiedade: DM = $16,3 \pm 2,3$; TAG = $11,1 \pm 4,9$; VS = $4,1 \pm 0,8$ / HAD-Depressão: DM = $14,2 \pm 3,6$; TAG = $9,3 \pm 2,7$; VS = $4,5 \pm 1,8$), mas não de acordo com o IDATE-T (média $\pm$ dp: HAD-Ansiedade: DM = $53,2 \pm 3,4$; TAG = $52,0 \pm 3,8$; VS = $46,4 \pm 5,2$). Os voluntários saudáveis diferiram dos pacientes em ambas as escalas.

Conclusões: Apesar do pequeno tamanho da amostra, os resultados preliminares aqui apresentados sugerem que a HAD, mas não o IDATE-T, é capaz de diferenciar entre pacientes com transtornos de ansiedade e depressão.

Palavras-Chave: Transtornos de ansiedade, depressão, IDATE-T e HAD.

CONTINUITY BETWEEN DREAMING AND WAKING IN PSYCHOTIC PATIENTS

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OBJECTIVES: Kant, Schopenhauer, Maury and Bleuler associated schizophrenia with the pathological-like mental state of dreaming. Freud discovered that dreams reflect previous waking events ("day residue"). He also postulated that dreams are similar to psychotic hallucinations.

Neuropsychological studies of patients with localized brain lesions show that mesolimbic dopaminergic pathways necessary for dreaming are also involved in the generation of psychotic symptoms. The purpose of the present study is to assess the prevalence of psychopathological elements on the dream reports of psychotic patients. **METHODS:** For this preliminary report, 31 medicated psychotic patients (24 schizophrenics and 7 bipolar) were submitted to a clinical interview (DSM-IV), and to the BPRS and PANSS, two standards questionnaires for the quantification of psychotic symptoms. Patients had recent waking and dream narratives digitally recorded, transcribed and subjected to a quantitative analysis of changes in the psychopathological content of the discourse. Four major categories of psychopathological elements were considered, comprising delusions, hallucinations, disruptive behavior and negative symptoms. Speech dynamics were plotted as temporal graphs across those categories. Dream-waking continuity was defined as the occurrence of psychopathological symptoms on dream narratives.

RESULTS: We found a significant positive relationship between dream-waking continuity and BPRS in schizophrenic patients (mean BPRS $\pm$ SEM in narratives with dream-waking continuity: $16,63 \pm 1,34$; without dream-waking continuity: $12,33 \pm 1,57$; Kruskal-Wallis followed by Mann-Whitney, $p < 0,05$). The effect did not reach significance in bipolar patients (with dream-waking continuity: $12,7 \pm 1,61$; without dream-waking continuity: $8,9 \pm 1,96$; $p > 0,05$). **CONCLUSIONS:** Schizophrenic patients reported more psychopathological symptoms on dream narratives when their BPRS scores were higher, i.e. when they were more psychotic. Quantification of the bipolar group of patients requires a larger sample.

Palavras-Chave: dream, psychosis, schizophrenia

Apoio Financeiro: AASDAP

EFEITOS DA ANFETAMINA SOBRE A INIBIÇÃO PROMOVIDA POR UM PRÉ-PULSO DA RESPOSTA DE SOBRESSALTO INDUZIDA POR UM PULSO ("PREPULSE INHIBITION OF STARTLE") NA LINHAGEM DE RATOS ESPONTANEAMENTE HIPERTENSOS SHR: IMPLICAÇÕES PARA O ESTUDO DA ESQUIZOFRENIA

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Objetivos: Déficits do processo de filtro sensorio-motor, que se refletem em uma diminuição da inibição promovida por um pré-pulso da resposta de sobressalto induzida por pulso ("prepulse inhibition of startle" - PPI), estão associados a diferentes distúrbios psiquiátricos. Dentre eles, destacam-se a esquizofrenia e o transtorno de déficit de atenção e hiperatividade (TDAH). O objetivo do presente trabalho foi verificar os efeitos da anfetamina - uma droga tanto utilizada para o tratamento do TDAH quanto capaz de induzir ou agravar psicoses - sobre a resposta de PPI na linhagem SHR.

Métodos: Ratos Wistar (W) ou SHR, adultos, foram distribuídos em 4 grupos (n= 7 -10), tratados com salina ou 1,0; 2,5 e 5,0 mg/kg de anfetamina (ANF). Quinze minutos após, os animais foram submetidos ao teste de PPI. Nesse teste, foram apresentados 3 tipos de estímulos por 10 vezes, distribuídos randomicamente em intervalos médios de 20 segundos: 1) pulsos de 120 dB por 40 ms (capaz de produzir uma resposta de sobressalto); 2) pré-pulsos de 65, 70 ou 75 dB por 20 ms apresentados 80 ms antes do pulso; 3) ausência de estímulo. No início de cada sessão, 10 pulsos foram apresentados para a habituação dos animais. A média da amplitude de sobressalto após as sessões de pulso (P) bem como a média da amplitude da resposta de sobressalto após as sessões de pré-pulso - pulso (PP) foram calculadas para cada animal com base na seguinte equação: inibição por pré-pulso (%) = $100 - [(PP / P) \times 100]$

Resultados: A anfetamina promoveu uma diminuição da resposta de PPI somente na linhagem SHR. Essa diminuição foi observada com as 3 intensidades de pré-pulso (65, 70 ou 75 dB) para a dose de 5,0 mg/kg de anfetamina (SHR SAL=45,12 ± 4,36; 41,55 ± 4,6; 35,74 ± 5,07; SHR ANF 5,0=23,84 ± 2,7; 24,26 ± 4,24; 21,51 ± 2,14, respectivamente)

Conclusão: Uma vez que a anfetamina (capaz de agravar sintomas psicóticos em indivíduos portadores de esquizofrenia além de promovê-los em indivíduos normais) promoveu um prejuízo no funcionamento do filtro sensorio-motor somente na linhagem SHR, nossos resultados indicam que o processamento de informações nessa linhagem seja mais sensível a manipulações "pró-esquizofrênicas".

Palavras-Chave: esquizofrenia, ratos espontaneamente hipertensos, prepulse inhibition of startle, modelos animais

Apoio Financeiro: FAPESP, CNPq

CURVA DOSE-EFEITO MELATONINA NO TESTE DE NATAÇÃO FORÇADA VERSÃO MODIFICADA.

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Objetivo: avaliar o efeito de diferentes doses de melatonina (1, 5, 25, 50, 100 mg/kg) sobre o teste de natação forçada versão modificada.

Métodos e Resultados: 70 ratos machos foram divididos nos seguintes grupos: controle (n=10), melatonina (M 1, 5, 25, 50, 100 mg/kg) (n=10) e imipramina 25 mg/kg (n=10). Todos os animais receberam três injeções destas drogas nos seguintes tempos: 1h e 8h depois da sessão treino, e 1h antes da sessão teste. O experimento consistiu em duas sessões: sessão treino e 24h depois a sessão teste. Inicialmente cada animal foi exposto ao aparato durante 15 minutos (min). Concluída a sessão treino, os animais foram tratados conforme o grupo e o esquema de administração citada acima. Vinte e quatro horas após a sessão treino os grupos foram novamente expostos ao teste e avaliados durante 5 min. Os parâmetros utilizados para avaliação foram: tempo de imobilidade, tempo de natação e tempo de escalada. Os dados foram analisados por ANOVA seguida do teste de Tukey-Kramer. Os resultados mostraram que o grupo M1 e imipramina exibiram significativa redução no tempo de imobilidade (47.0 ± 12.6 ; 56.6 ± 7.3 ; $p < 0.05$) quando comparados aos grupos controle (108.0 ± 15.9), M25 (110.1 ± 16.8), M50 (138.37 ± 13.3) e M100 (155.6 ± 14.0). Não foi observada diferença estatisticamente significativa entre os grupos imipramina e M1. No parâmetro tempo de natação, o grupo M100 apresentou redução no tempo de natação (97.6 ± 11.0 ; $p < 0.05$) quando comparado aos grupos tratados com M1 (202.2 ± 11.2) e M5 (187.3 ± 10.5), não diferindo, entretanto, dos grupos controle (149.2 ± 15.45), M25 (147 ± 18.6), M50 (139.4 ± 17.2) e imipramina (148.3 ± 12.4). No último parâmetro, o grupo imipramina apresentou aumento do tempo de escalada (95.0 ± 10.0 ; $p < 0.05$) quando comparado aos grupos controle (48 ± 12.2), M1 (50.7 ± 11.3), M5 (44.7 ± 8.8), M25 (42.8 ± 7.8), M50 (24.1 ± 8.2) e M100 (46.7 ± 8.4).

Conclusão: O presente estudo sugere que a melatonina na dose de 1 mg/kg apresentou efeito antidepressivo, sendo este efeito comparável ao da imipramina.

Palavras-Chave: Melatonina, imipramina e efeito antidepressivo.

Apoio Financeiro: CAPES e CNPq.

EFEITOS ANSIOLÍTICOS DA MANIPULAÇÃO PRECOCE EM RATOS SUBMETIDOS À HIPÓXIA-ISQUEMIA ENCEFÁLICA NEONATAL

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Objetivo: O presente trabalho objetiva investigar os efeitos sobre o nível de ansiedade de ratos adultos submetidos à hipóxia-isquemia encefálica neonatal e da separação materna de curta (SM) e de longa duração (PM). **Metodologia:** Foram utilizados ratos Wistar, machos e fêmeas. Grupos: controles sem separação materna (CTNSM, n=10); com separação materna de 15min. (CTSM, n=9); e com privação materna de 180min (CTPM, n=11); HINSM, n= 9; HISM, n=10; HIPM, n=8. No 7º dia pós-natal, os ratos foram submetidos à isquemia pela oclusão permanente da artéria carótida comum direita, sendo após expostos à uma atmosfera hipóxica (90min-8%O₂; 92%N₂). No 90º dia avaliou-se o grau de ansiedade através do teste de claro-escuro (durante 10 min), onde era observado o tempo que permanecia em um dos lados (claro ou escuro), o número de cruzamentos em cada um dos lados e de “rearing”. Foi realizado também o teste de comportamento alimentar, onde foi avaliado a motivação para a ingestão de alimento palatável (número de pellets doces). **Resultados:** Quanto ao comportamento alimentar, a análise estatística indicou que no teste alimentado, os animais do grupo NSM (0,8±0,2) consumiram menos alimento doce que os SM (1,1±0,2) e os PM (1,5±0,2). Existem dados da literatura que indicam que este aumento do consumo de alimento doce não se relaciona com aumento nos níveis de ansiedade. No teste claro-escuro, os animais do grupo SM e PM apresentaram um aumento no “tempo claro” (SM=134,2±21,8; PM=126,3±19,4) comparado com o grupo NSM (67,2±14,9). Houve também aumento no “cruzamento claro” dos grupos SM e PM (SM=56,9±10,7; PM=50,2±8,1) em relação ao NSM (22,5±5,1) e aumento no “rearing claro” dos grupos SM (22,5±2,7) e PM (23±4,3) em contraste com o NSM (9,3±2,7). A hipóxia-isquemia não resultou em diferenças significativas entre os grupos nos testes realizados. **Conclusão:** Os animais submetidos à separação materna de curta e longa duração apresentaram um menor nível de ansiedade e um maior consumo de alimento palatável (doce). O aumento do cuidado maternal nas ninhadas com separação materna pode ser uma hipótese para as mudanças nos níveis de ansiedade e no comportamento alimentar dos ratos.

Palavras-Chave: hipóxia-isquemia encefálica neonatal , separação materna, ansiedade

LACK OF HYPOTHALAMUS-PITUITARY-ADRENAL AXIS ACTIVATION DURING THE PERIAQUEDUCTAL GRAY MATTER-EVOKED DEFENCE REACTION

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Ever since the seminal studies of Hans Selye, activation of hypothalamus-pituitary-adrenal (HPA) axis is emblematic of stress. Consequently, the lack of HPA axis responses following the undisputable psychological stress of a panic attack stands out as one of the most intriguing findings of contemporary psychiatry. On the other hand, the defensive behaviors and aversive emotions produced by stimulation of the dorsal periaqueductal gray matter (DPAG) have been proposed as a model of panic attacks. Therefore, we examined whether the plasma levels of 'stress hormones' corticotropin and prolactin show any change following the DPAG-evoked freezing and flight behaviors of the rat. Rats bearing an electrode into the DPAG and an intra-atrial catheter were stimulated at 9:00 a.m., 18-24 h after the catheter implantation. Blood samples were withdrawn just before 1-min stimulation of DPAG, immediately after (5 or 15 min) and throughout 3 to 27 h following stimulation. In another experiment, samples were withdrawn either before or following a prolonged stimulation (5 min) of the DPAG with flight threshold intensity. Hormones were measured by either chemiluminescent or double-antibody immunoassays. Hormone plasma levels following freezing and flight behaviors were compared to those of resting or restraint-stressed rats. Data show that stress hormones remain unaltered following the DPAG-evoked defensive behaviors. Not even the 5-min stimulation of DPAG with the flight threshold intensity changed corticotropin plasma levels significantly. As far as we known, this is the first demonstration of the lack of stress hormone responses following the intense emotional arousal and physical exertion of a fear-like behavior in rats. Data add new evidence of DPAG involvement in spontaneous panic attacks. **Palavras-Chave:** Periaqueductal Gray Matter, Panic, Stress, ACTH, Corticotropin, Prolactin

Apoio Financeiro: AFIP-UNIFESP

OCORRÊNCIA DE IMOBILIDADE TÔNICA EM RATOS DURANTE A CATALEPSIA INDUZIDA PELO ELETROCHOQUE CONVULSIVO.

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A imobilidade corporal permeia diversos comportamentos defensivos das espécies. A catalepsia (CAT), que se caracteriza por um quadro de rigidez muscular e imobilidade duradoura, garante a sobrevivência com menor gasto de energia no perigo prolongado. A imobilidade tônica (IT), por sua vez, possibilita defesa antipredatória por “fingir-se de morto”. Embora um mecanismo neural comum de comando das imobilidades tenha sido postulado, os ratos não manifestam a IT, mesmo que a CT seja facilmente induzida. O presente estudo demonstrou que a IT ocorre em ratos quando estão em CT. Eletrochoque convulsivo para induzir CT foi aplicado em 20 ratos machos Wistar, com cinco meses de idade. Cinco minutos após, a instalação da CT foi avaliada por 300s, para então testar a ocorrência de IT por 15 minutos. Ao final, a preservação da CT foi avaliada por mais 300s. A CT foi avaliada pelo tempo de manutenção da postura vertical com apoio dos membros anteriores nas paredes laterais do canto de uma gaiola de grades metálicas. A IT foi avaliada pelo tempo de permanência em decúbito dorsal com o corpo longitudinalmente encaixado em uma canaleta. Os animais controles não apresentaram IT, tampouco CAT. O eletrochoque induziu CT em 19 animais (95%), dos quais 12 (60%) apresentaram IT. O tempo médio $\pm$ sd de IT foi de 13,75 $\pm$ 2,86 minutos. Todos os 10 ratos com IT com duração de 15 min mostraram CT na reavaliação. Conclui-se que: a) existem susceptibilidades individuais e intensidades diferentes de CT; b) a postura que se força para avaliação da IT é altamente estressante ao rato; c) a ocorrência de IT na CT corrobora a existência de um mecanismo central comum de comando.

Palavras-Chave: Eletrochoque convulsivo; Catalepsia; Imobilidade tônica; Ratos

Apoio Financeiro: FAPESP

**AUTONOMIC AND NEUROENDOCRINE CHANGES BEFORE AND AFTER
PSYCHOTHERAPY: A PTSD CASE STUDY**

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Post-traumatic stress disorder (PTSD) has been associated with dysregulation of the neuroendocrine and autonomic system. Our aim was to investigate the effects of Cognitive Behavioral Therapy (CBT) on the physiological (Heart Rate, Cardiac Vagal Tonus, Skin Conductance) and neuroendocrine (Cortisol and Dehydroepiandrosterone (DHEA)) variables and psychometric self-report measures (negative affect, resilience, PTSD symptoms, depression, anxiety and social support). The patient was a 45-year-old man who had suffered three assaults and failed to respond adequately to pharmacological treatment with selective serotonin reuptake inhibitors. His physiological and psychometric responses at rest were measured before and after four months of CBT. CBT led to reduction of heart rate (3.2%), skin conductance (19.6%), and cortisol (5.4%) as well as increase of cardiac vagal tone (40.6%) and DHEA (118.2%). Furthermore, CBT promoted reduction of PTSD symptoms (19.6%), depression (15.8%), anxiety (28.6%) and negative affect (40.0%) scores and enhancement of resilience (23.1%) and social support scores (33.3%). These results suggest that the dysregulation of neuroendocrine and autonomic system at rest may have been normalized after successful CBT. Furthermore, our data also suggest, that these same treatment have promoting effects on positive emotions, behaviours and beliefs. We can call on increasing evidence that treatments are not only 'anti' pathology but 'pro' wellness in nature. Our study, shows the need for further study of the therapeutic enhancement of health traits and related biological underpinnings.

Palavras-Chave: POST-TRAUMATIC STRESS DISORDER, AUTONOMIC, NEUROENDOCRINE, PSYCHOTHERAPY

Apoio Financeiro: CNPq

EFEITOS DA ATIVAÇÃO DO CÓRTEX PRÉ-FRONTAL PROMOVIDA PELA VERATRIDINA SOBRE A RESPOSTA DE MEDO CONDICIONADO AO CONTEXTO NAS LINHAGENS WISTAR E SHR (SPONTANEOUSLY HYPERTENSIVE RATS)

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Objetivos: Recentemente verificamos que a linhagem de ratos SHR apresenta um déficit na tarefa de medo condicionado ao contexto que é especificamente revertido por neurolépticos (drogas utilizadas na terapêutica da esquizofrenia). Nesse sentido, déficits de entendimento de contextos emocionais estão presentes em diversos transtornos psiquiátricos, como o transtorno de déficit de atenção e hiperatividade e a esquizofrenia. O córtex pré-frontal, dentre outras estruturas, participa da regulação de respostas emocionais. Assim, o objetivo do presente estudo foi verificar os efeitos da ativação do córtex pré-frontal, pela veratridina, sobre o medo condicionado ao contexto nas linhagens Wistar e SHR.

Métodos e Resultados: Animais Wistar (n=7) e SHR (n=7) foram submetidos à cirurgia estereotáxica para implantação de cânulas na região pré-límbica do córtex pré-frontal. Três dias após a cirurgia, os animais receberam uma injeção local de 1µl de veratridina (vt) (1µg/µl) ou solução salina em cada hemisfério. Trinta minutos após, os animais foram submetidos à sessão de treino da tarefa de medo condicionado ao contexto, na qual os animais receberam choques de 5 seg., de 0.4 mA a cada 30 seg. até completar 5 min. Vinte quatro horas após, todos os animais foram observados no mesmo contexto para a quantificação do tempo de congelamento. Observamos que: 1) ratos da linhagem SHR tratados com salina apresentaram diminuição do tempo de congelamento quando comparados com os ratos Wistar tratados com salina (M±EP: Wistar- 148,5±17,4 X SHR- 36,6±13,0); 2) ratos Wistar tratados com vt apresentaram uma resposta de congelamento diminuída em relação seu controle (13,8±4,8); e 3) a injeção de vt promoveu um aumento do tempo de congelamento apresentado pela linhagem SHR (77,5±17,4).

Conclusão: A ativação do córtex pré-frontal promove, em ratos Wistar, um déficit de resposta de medo condicionado ao contexto de mesma magnitude do déficit observado espontaneamente na linhagem SHR. Na linhagem SHR, esta ativação promove uma reversão do déficit de resposta de medo condicionado ao contexto. **Palavras-Chave:** Ratos SHR; medo condicionado ao contexto; veratridina; córtex pré-frontal

Apoio Financeiro: CNPq

Effects of chronic treatment with imipramine on Brain-Derived Neurotrophic Factor levels in the dorsal periaqueductal gray matter and hippocampus

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Introduction: Antidepressant drugs have been successfully used for the treatment of mood and anxiety pathologies such as depression and panic disorder. Different hypotheses have been formulated to explain the mechanisms by which drugs like imipramine and fluoxetine exert their antidepressive effects. Based on a wealth of experimental evidence, it has been suggested that these compounds act by increasing the synthesis and signaling of neurotrophic proteins, mainly BDNF (brain-derived neurotrophic factor), in key depression-associated areas such as the hippocampus and frontal cortex [1]. BDNF is the most abundant and widely distributed neurotrophin in the central nervous system. It is now believed that the main function of BDNF in the adult is to regulate synaptic plasticity. Once the main neurotransmitter involved in panic disorder is serotonin (5-HT) and, like BDNF, alterations in 5-HT signaling have demonstrable effects on synaptic plasticity and adult neurogenesis, specially in the hippocampus. Substantial evidence suggests that BDNF promotes the development and function of serotonergic neurons. BDNF and its receptor (TrkB) are co-expressed in serotonergic neurons within the dorsal raphe and median raphe. It is presently unknown if interference with the neurotrophic factors may also be of relevance for the antipanic effect caused by these compounds.

Objective: In the current study we compared the effect of chronic administration of imipramine on BDNF levels in the rat dorsal periaqueductal gray matter (DPAG) and hippocampus. The DPAG in the brainstem has been considered a critical structure in the mediation of panic attacks [2]. It has been also shown that BDNF injection in midbrain areas enhances the synthesis of tryptophan hydroxylase, an enzyme involved in the first step in the serotonin biosynthetic pathway [3]. Facilitation of 5-HT neurotransmission in the DPAG has been associated with the antipanic effect of different classes of drugs, including antidepressants [2]

Methods: Male Wistar rats, chronically treated (21 days, i.p.) with imipramine (10 mg/kg, i.p.) or saline (n = 6/group), were housed in group with free access to food and water. Three hours after the last injection, the animals were deeply anesthetized with chloral hydrate, decapitated and brains were removed on ice. The DPAG and hippocampus were isolated, the tissues homogenated, centrifuged and the supernatant kept frozen for later analysis. BDNF levels were determined by ELISA (BDNF Emax; Promega, USA) and values obtained were expressed as a ratio of the total of protein present in each area.

Results: Animals treated with imipramine caused a significant ($p < 0.05$) increase in BDNF levels both in the DPAG [Mean $\pm$ SEM of pg BDNF/ μ g total protein – saline = 0.692 ± 0.038 ; imipramine = 0.923] and hippocampus [saline = 1.425 ± 0.066 ; imipramine = 2.127 ± 0.219].

Conclusions: Chronic treatment with imipramine enhances BDNF levels not only in brain areas associated with depression such as the hippocampus, but also in a panic-related structure, the DPAG. This latter effect may be of importance for the drug well-known panicolytic activity.

References:

- 1 - Biol Psychiatry 59. 1116-1127, 2006.
- 2 - Neuropharmacology.52. 1188-1195, 2007.
- 3 - J Neurosci Res. 52. 149-158, 1998.

Palavras-Chave: antidepressant, bdnf, periaqueductal gray

Apoio Financeiro: Fapesp e CNPq

RELAÇÃO ENTRE O NÍVEL DE ATIVIDADE FÍSICA, DEPRESSÃO, ANSIEDADE E QUALIDADE DE VIDA EM HOMENS ACIMA DE 65 ANOS DE IDADE.

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OBJETIVO: Verificar a associação entre o nível de atividade física com a depressão, ansiedade e qualidade de vida (QV) em idosos. **MÉTODO:** A amostra foi composta por 66 homens saudáveis e funcionalmente independentes com idade acima de 65 anos de idade. Foram aplicados os seguintes questionários: questionário internacional de atividade física (IPAQ) versão curta, escala geriátrica de depressão (EGD), IDATE traço (IT) e IDATE estado (IE) para ansiedade e SF-36 para avaliação da qualidade de vida. **RESULTADOS:** Houve correlação positiva entre a frequência de caminhada (FC) e o SF-36 nos domínios capacidade funcional $r=0,37$, aspecto físico $r=0,32$, dor $r=0,34$, vigor $r=0,30$, aspecto emocional $r=0,49$, saúde mental $r=0,37$ e média de todos os domínios $r=0,47$ e a frequência de atividade moderada com SF-36 nos domínios vigor $r=0,32$ e média de todas as dimensões $r=0,24$, sugerindo que quanto maior a frequência de caminhada e a duração das atividades moderadas melhor os escores de QV. Foram encontradas correlações negativas entre a FC, o total de caminhada (TC) e EGD $r=-0,32$ e $r=-0,25$ respectivamente, sugerindo que quanto maior a frequência de caminhada diária ou o total semanal menor os escores de depressão. Houve correlações negativas entre a FC, a duração da caminhada e o TC com IT $r=-0,38$, $r=-0,25$ e $r=-0,30$ respectivamente mostrando que quanto maior a frequência, a duração e o total da caminhada menor os escores de ansiedade. Todas as correlações foram significativas (Pearson $p \leq 0.05$). **CONCLUSÃO:** De acordo com os dados analisados, parece haver uma associação entre o nível de atividade física e os escores de depressão, ansiedade e qualidade de vida em homens acima de 65 anos, demonstrando que os indivíduos que apresentaram maiores níveis de atividades físicas tiveram maiores escores de qualidade de vida e menores escores de depressão e ansiedade.

REFERÊNCIAS: Int J Geriatr Psychiatry 21:410, 2006; Med Sci Sports Exerc 39:1401, 2007.

Palavras-Chave: Atividade Física - Depressão - Ansiedade - Qualidade de vida - Idoso

Apoio Financeiro: Afip, Cepid, Cepe, Fapesp e Capes.

Investigação do efeito da agmatina em um modelo de Parkinson induzido por reserpina em camundongos

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A agmatina é uma amina catiônica presente no sistema nervoso central de mamíferos, onde atua como um neuromodulador/neurotransmissor (Tr. Pharmacol. Sci. 21:187, 2000). Estudos pré-clínicos têm demonstrado que a administração de agmatina produz efeito neuroprotetor, anti-amnésico, ansiolítico, anticonvulsivante, antinociceptivo e antidepressivo (Brain Res. 1084:210, 2006; Neurobiol. Learn. Mem. 78:465, 2002; Eur. J. Pharmacol. 550:112, 2006; Neurosci. Lett. 390:129, 2005; Neuropharmacology 48:1021, 2005; Neuroreport 13:387, 2002.). Adicionalmente, a administração de agmatina após o tratamento com MPTP, atenua os efeitos neurotóxicos deste composto (Neurosci. Lett. 296:97, 2000). Este trabalho objetiva investigar os efeitos da agmatina na hipolocomoção induzida por reserpina em camundongos, um modelo animal para a Doença de Parkinson. Camundongos Swiss machos (30-40 g) foram utilizados. A fim de investigar o efeito na agmatina na hipolocomoção induzida por reserpina, os animais foram pré-tratados por via i.p. com reserpina (1 mg/kg) ou veículo (1 ml/kg). Após 30 minutos os animais foram tratados com doses crescentes de agmatina (1, 10 ou 100 mg/kg, i.p.) ou veículo. Após 24 h os animais foram submetidos ao teste do campo aberto para a avaliação dos seguintes parâmetros: número de cruzamentos, número de *rearing*, número de respostas de auto-limpeza, tempo de imobilidade, tempo de latência para sair do primeiro quadrante e quantidade de bolos fecais. A administração de reserpina diminuiu a atividade locomotora ($31.2 \pm 7.1\%$) e o número de *rearing* ($29.3 \pm 5.9\%$) em relação ao grupo controle=100%, mas não alterou as respostas de auto-limpeza, a defecação e o tempo de latência dos animais. Além disso, houve um aumento do tempo de imobilidade (*freezing*) ($904.9 \pm 260.9\%$) em relação ao grupo controle=100%. O tratamento com agmatina (10 mg/kg) reverteu significativamente a hipolocomoção ($78.0 \pm 5.08\%$) e o aumento do tempo de imobilidade ($202.4 \pm 50.2\%$) induzidos pela administração de reserpina. Os resultados indicam que a agmatina previne os efeitos induzidos pela reserpina em um modelo animal de Doença de Parkinson em camundongos, o que corrobora a sua ação neuroprotetora.

Palavras-Chave: Agmatina, doença de Parkinson, hipolocomoção, reserpina, teste do campo aberto.

Apoio Financeiro: CAPES; CNPq, IBN-Net (01.06.0842-00) e UFSC.

Efeito antidepressivo da atividade física na roda de corrida voluntária

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A atividade física proporciona muitos benefícios à saúde, incluindo ações antidepressivas em pacientes com depressão maior (BMJ 322:763, 2001). Corroborando com estes achados alguns estudos pré-clínicos também reportam o robusto efeito antidepressivo da atividade física (Brain Res 1199:148, 2008; Nat Med 13:1476, 2007), entretanto os mecanismos biológicos responsáveis por tais efeitos ainda permanecem desconhecidos. Este estudo avaliou o efeito antidepressivo da atividade física no teste da suspensão da cauda (TSC) e no teste do nado forçado (TNF), testes preditivos de atividade antidepressiva, em camundongos Swiss machos adultos (35-45g) expostos a 21 dias de livre acesso a roda de corrida voluntária. Camundongos expostos a roda de corrida voluntária apresentaram redução do tempo de imobilidade no TSC e no TNF ($73,9 \pm 3,4\%$ e $79,8 \pm 3,8\%$, respectivamente, comparado aos respectivos grupos controle = 100%), sem alterar a atividade locomotora no teste do campo aberto. Devido à depressão estar correlacionada com uma disfunção no balanço oxidativo, investigamos o efeito da atividade física no perfil antioxidante. A atividade física aumentou significativamente os níveis de tióis não protéicos ($130,7 \pm 7,8\%$, comparado ao respectivo grupo controle), sem alterar a atividade das enzimas antioxidantes glutathione peroxidase e glutathione reductase no cérebro anterior. Além disso, a atividade física não alterou os níveis de substâncias reativas ao ácido tiobarbitúrico (marcador de lipoperoxidação). É descrito que mais de 90% dos tióis não protéicos são glutathione (GSH), a partir disto verificamos o efeito antidepressivo da administração i.c.v. de GSH no TNF e no TSC. A administração de GSH produziu uma significativa resposta antidepressiva nas doses de 100-1000 e 300-3000 nmol/ sítio, i.c.v., no TSC ($66,9 \pm 6,8\%$; $68,0 \pm 2,8\%$; e $71,0 \pm 1,5\%$ em relação ao controle), e no TNF ($69,8 \pm 1,6\%$, $66,2 \pm 2,0\%$; e $62,2 \pm 1,3\%$ em relação ao controle), respectivamente, sem alterar a locomoção no teste do campo aberto. Os resultados sugerem que a atividade física pode promover um efeito antidepressivo, cujos mecanismos poderiam ser dependentes, ao menos em parte, da GSH. **Palavras-Chave:** Atividade Física, Depressão, Glutathione, Teste do Nado forçado, Teste da Suspensão da Cauda

Apoio Financeiro: CAPES; CNPq; IBN-Net (01.06.0842-00); UFSC

Subchronic inhibition of nitric oxide (NO) synthesis prevents learned helplessness development and enhances hippocampal BDNF expression in rats

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Objectives: Acute administration of NO synthase (NOS) inhibitors induces antidepressant-like effects in the forced swimming test. However, these effects lack further investigation in other animal models of depression. Moreover, the mechanisms and brain regions that mediate these effects are unknown. NO decreases BDNF expression *in vitro*, a neurotrophin that is required for antidepressant-induced behavioral effects. Therefore, the aim of this study was to investigate the effects induced by subchronic treatment with 7-NI, a neuronal NOS inhibitor, on helpless development and on hippocampal BDNF expression in rats.

Methods: Male Wistar rats were submitted to pretest (PT) session with inescapable footshocks (IS, 40, 1 mA, 10s) or no shocks (NS). Six days later, the animals were submitted to the test with escapable footshocks (T, 30, 0.8mA, 10s). Immediately after PT, rats received the first of 7 (one/day) ip injection of vehicle, 7-NI (30, 60 mg/Kg) or imipramine (15 mg/Kg). The last injection was administered 1h before T. Animals that failed to avoid/escape the shocks during test in more than 10 trials were considered helpless. Another group of rats received 1 daily injection/7days of the same treatments and was sacrificed 1h40min after the last injection for hippocampal dissection and BDNF quantification (ELISA).

Results: Pre-exposure to IS increased the percentage of helpless rats (70% vs NS: 28.6; χ^2 , $p < 0.05$). Imipramine and 7-NI (30mg/Kg) treatment reduced the percentage of helpless rats in IS group (40% and 45% respectively; χ^2 , $p < 0.05$). No significant effects were observed in NS group. Moreover, subchronic treatment with imipramine or 7NI (30 mg/kg) increased hippocampal BDNF expression (78% and 31%, respectively; $F_{2,16} = 9.3573$; $p = 0.0020$).

Conclusions: Subchronic treatment with 7-NI induces antidepressant-like effect in the learned helplessness model and increases hippocampal BDNF expression. These effects are similar to that induced by the prototype antidepressant imipramine. Therefore our results support the hypothesis that NO may be involved in the neurobiology of depression and that this could involve modulation of hippocampal BDNF expression. **Palavras-Chave:** antidepressant, nitric oxide, BDNF, learned helplessness

Apoio Financeiro: FAPESP, CNPq, FCFRP-USP, FAEPA

EFEITOS DO METILFENIDATO SOBRE A TAREFA DE MEDO CONDICIONADO AO CONTEXTO EM ANIMAIS WISTAR E SHR JOVENS.

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Objetivos: A linhagem de ratos espontaneamente hipertensos (SHR) tem sido sugerida como um modelo para o estudo do transtorno de déficit atencional e hiperatividade (TDAH), uma patologia que acomete principalmente crianças. Contudo, recentemente verificamos que animais adultos dessa linhagem apresentam um déficit de medo condicionado ao contexto que é especificamente revertido por neurolépticos (drogas utilizadas na terapêutica da esquizofrenia) e potencializado por psicoestimulantes, como o metilfenidato (drogas utilizadas na terapêutica do TDAH). O objetivo do presente trabalho foi verificar o efeito de diferentes doses de metilfenidato sobre o medo condicionado ao contexto em animais Wistar e SHR jovens.

Métodos e Resultados: Animais Wistar (n=39) e SHR (n=38), machos, de trinta dias, foram tratados com salina ou metilfenidato (0,25, 0,5 ou 1,0 mg/kg, i.p.). Quinze minutos após, os animais foram submetidos à sessão de treino da tarefa de medo condicionado ao contexto, na qual os animais receberam choques de 5 seg., de 0.4 mA a cada 30 seg. até completar 5 min. Vinte quatro horas após, todos os animais foram observados no mesmo contexto para a quantificação do tempo de congelamento por 5 min. Observamos que: 1) Ratos SHR tratados com salina apresentaram diminuição do tempo de congelamento quando comparados com os ratos Wistar tratados com salina (M±EP: SHR – 28,6±9; Wistar 68,2±18,9); 2) O tratamento com metilfenidato não modificou a resposta de medo condicionado ao contexto em nenhuma das doses administradas tanto nos ratos SHR (M±EP: SHR-0.25 – 15,2±3,1; SHR-0.5 – 23,5±4,9; SHR-1.0 – 25±8,9) quanto nos ratos Wistar (M±EP: Wistar-0.25 – 39,6±15,1; Wistar-0.5 – 64,1±11,9; Wistar-1.0 – 66,7±13,6).

Conclusão: O déficit de medo condicionado ao contexto apresentado por ratos SHR jovens não parece ser mediado por alterações relacionadas com o TDAH, uma vez que o metilfenidato não foi capaz de revertê-lo. **Palavras-Chave:** ratos espontaneamente hipertensos, transtorno de déficit de atenção e hiperatividade, metilfenidato, medo condicionado ao contexto

Apoio Financeiro: CNPq

THE NEURONAL ARCHITECTURE OF TELENCEPHALIC VOCAL CONTROL AREAS OF PARROT FAMILY

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The high cognitive capacity and intelligence of parrots are quite obvious, as they possess the ability to develop a vocabulary, and oratory skill. Parrots can acquire and use human speech (Interspecies communication) and now believed to have the intelligence and emotional makeup up to 3-4 years old child.

However parrots brain is organized very differently from that of mammals. They are able to perform extremely complex behavior, emotions and cognition. The purpose of the present study is to apply Golgi impregnation method in an attempt to describe and classify the neuronal architecture of those regions of telencephalon which are responsible for vocal learning. We have studied nine separate discrete regions in parrot brain, which has been previously identified as vocal control nuclei. The result showed that all vocal control nuclei have interneuronal variability, on the basis of morphological structure. Neurons have been distinguished on the basis of soma size & shape; dendrites tree pattern, presence & absence of spines on dendrites. Different types of neurons are classified in different vocal control regions according to morphological variability.

Hv (Hyper striatum ventrale) nucleus consist of mainly pyramidal cell type neurons, these neurons are further subdivided into two classes (a) Thick dendrites with densely packed spines and (b) Filliform dendrites with loosely packed spines. Neurons with multipolar cell type have also been encountered in this region. These neurons are also of two type multipolar spinous and multipolar aspinous.

The Hvo regional (Hvc complex) is consist of bitufted neurons with aspinous thick dendrites.

Neostriatum region of parrot telencephalon possesses typical pyramidal neurons with spinous and aspinous filliform dendrites. It has also been observed that dense spinous dendrite from this region extends up to NAO (oval nucleus of the anterior N within NAO complex), and NAOc (vocal anterior neostriatal field including and surrounding NAO). Our findings are an attempt to understand the neural mechanisms involved in vocal learning or language. We also review and discuss origin and evolution of language.

Palavras-Chave: vocal control, parrot, emotion , cognition, neuroarchitecture, telencephalon

Apoio Financeiro: nil

Interaction of Zinc with Antidepressants in the Tail Suspension Test

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Zinc is a transition metal that is present at high concentrations in the central nervous system. It has been shown to produce antidepressant-like effects in several animal models of depression (Behav Brain Res 144: 87, 2003; Pharmacol Rep 57: 713, 2005), which suggests that it has a significant role in the pathophysiology of depression. In this study, zinc chloride (ZnCl₂) was given alone or in combination with several classes of antidepressants by oral route (p.o.) to male adult Swiss mice (35-45g) and the behavioral response in the tail suspension test (TST), a predictive test of the antidepressant activity, was investigated. ZnCl₂ administered at a dose of 10 and 30 mg/kg reduced the immobility time in the TST ($80.1 \pm 5.9\%$ and $76.0 \pm 5.2\%$ respectively as compared to the control group=100%), without affecting the locomotor activity in open-field test. The antidepressants fluoxetine, paroxetine, imipramine, desipramine and bupropion produced a significant reduction in the immobility time in TST at the doses of 10, 1, 1, and 10 mg/kg, p.o., respectively ($79.9 \pm 3.9\%$; $63.4 \pm 5.1\%$; $76.8 \pm 6.1\%$; $73.8 \pm 6.4\%$; $72.6 \pm 7.4\%$, respectively as compared to the control group). The combined treatment of sub-effective doses of ZnCl₂ (1 mg/kg) with sub-effective doses of fluoxetine (5 mg/kg), paroxetine (0.1 mg/kg), desipramine (0.1 mg/kg), imipramine (0.1 mg/kg) or bupropion (1 mg/kg) induced a significant reduction in the immobility time in the TST when compared with the control group ($72.5 \pm 6.3\%$; $61.9 \pm 5.9\%$; $77.0 \pm 2.9\%$; $65.5 \pm 4.5\%$; $56.2 \pm 4.2\%$, respectively). The treatment with sub-effective doses of ZnCl₂ and antidepressants alone or in combination did not affect the locomotion in open-field test, except that desipramine alone reduced the ambulation ($52.15 \pm 15.93\%$ compared to the control group). The results first indicate that ZnCl₂ administered by p.o. route produces an antidepressant-like effect in the TST. Moreover, synergistic effects of zinc with conventional antidepressants were shown in the TST, suggesting that an improvement in the response to the antidepressant therapy occurs when zinc is combined with several classes of antidepressants. **Palavras-Chave:** Depression, Zinc, Antidepressants, Tail Suspension Test

Apoio Financeiro: CAPES; CNPq, IBN-Net (01.06.0842-00) and UFSC

O CONSUMO DE ETANOL EM “BINGE” AUMENTA O TEMPO EM IMOBILIDADE EM RATOS ADOLESCENTES SUBMETIDOS AO TESTE DO NADO FORÇADO

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OBJETIVO: Sabe-se que o etanol provoca perda de neurônios e diminui a neurogênese hipocampal. Levando-se em consideração a importância desta estrutura na fisiopatogenia dos transtornos do humor, o objetivo deste trabalho foi avaliar o efeito da intoxicação crônica de etanol em “binge” (padrão similar ao utilizado pelos adolescentes brasileiros) em ratos adolescentes submetidos ao teste do nado forçado.

MATERIAL E MÉTODOS: Ratos Wistar machos receberam etanol (2,5 ou 5g/kg, v.o.) em dias alternados, entre o 30º e o 60º dia de vida (adolescência). Animais controle receberam tratamento similar com solução salina. Utilizou-se um N=10 por grupo. Após 48h, os mesmos foram confinados por 15 min em um cilindro contendo água (pré-teste do nado-forçado). No próximo dia, os animais foram submetidos a 5 min de teste do campo aberto (a fim de avaliar o número de quadros explorados e tempo em que o animal permaneceu parado) e logo em seguida a 5 min ao mesmo cilindro no qual foi exposto no dia anterior (sendo registrado o tempo em imobilidade durante o teste). Os dados obtidos no teste do campo aberto e nado forçado foram avaliados utilizando-se ANOVA de uma via, seguida de teste de Tukey post hoc quando necessário. Foi considerada diferença estatística quando $P < 0,05$. **RESULTADOS:** Os animais tratados com etanol na dose de 5 g/Kg apresentaram um aumento no tempo de imobilidade quando comparado aos animais tratados com a dose de 2,5 g/Kg e salina $F(2,27)=13,32$ $P < 0,01$ ($146,8 \pm 9,4$; $107,6 \pm 6,9$; $85,8 \pm 8,6$; respectivamente). Ainda, o etanol na dose de 5 e 2,5 g/Kg diminuiu o número de quadros explorados no campo aberto quando comparados ao grupo controle $F(2,27)=23,27$ $P < 0,01$ ($56,6 \pm 6,1$; $58,2 \pm 5,4$; $81,1 \pm 9,1$; respectivamente), no entanto não aumentou o tempo em que o animal permaneceu parado na arena $F(2,27)=0,53$ $p=0,60$ ($113,2 \pm 17,9$; $131,1 \pm 13,2$; $112,7 \pm 11,8$; respectivamente).

CONCLUSÃO: O modelo utilizado no presente trabalho, a fim de mimetizar o padrão de consumo de álcool entre os adolescentes brasileiros, sugere que o padrão de consumo “binge” promove um comportamento depressivo em ratos adolescentes.

Palavras-Chave: álcool, depressão, binge

Apoio Financeiro: APOIO FINANCEIRO: FAP - FCMSCSP

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Efeito do tamoxifeno, da queleritrina e da medroxiprogesterona na hiperlocomoção induzida por anfetamina, um modelo animal de mania

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Objetivos

Avaliar o efeito do tamoxifeno, da queleritrina e da medroxiprogesterona em modelo animal de mania.

Métodos

Camundongos Swiss adultos (60-90 dias) machos. Mediu-se a atividade locomotora durante 20 min em uma caixa automatizada com sensores fotoelétricos, sendo número de cruzamentos considerado como índice de atividade locomotora. O aumento desta atividade após anfetamina foi indicativo de efeito excitatório e o bloqueio deste efeito foi considerado indicativo de efeito antimaníaco. No dia do teste os animais foram tratados com drogas experimentais ou seus veículos, e 15 minutos depois tratados com anfetamina (3mg/kg) ou seu veículo. A validação do método foi feita com carbonato de lítio (droga padrão para transtorno bipolar) 15 minutos antes da administração de anfetamina. A seguir foram testadas drogas inibidoras da PKC (tamoxifeno e queleritrina), a medroxiprogesterona (MPA), droga com ação somente antiestrogênica.

Resultados

Os resultados foram analisados por ANOVA de uma via, seguidos pelo teste post-hoc de Newman-Keuls. O lítio, nas doses de 100 e 150 mg/kg, foi capaz de bloquear a hiperlocomoção produzida pela anfetamina (n=6/grupo, $F(5,30)=3,737$, $P<0,01$). O tamoxifeno 1 mg/kg (mas não na dose de 0,5 mg/kg) foi capaz de bloquear a hiperlocomoção induzida pela anfetamina (n=6/grupo, $F(3,20)=13,75$, $P<0,0001$). A queleritrina, 1 mg/animal, icv, foi capaz de bloquear a hiperlocomoção produzida pela anfetamina (n= 4-5/grupo, $F(3,14)=47,56$, $P<0,001$). A dose intermediária de MPA (3 mg/kg) reduziu parcialmente a hiperlocomoção provocada pela anfetamina (n=5/grupo, $F(7,48)=24,06$, $P<0,0001$), o que não se obteve com as doses de 1 e 6 mg/kg.

Conclusões

O tamoxifeno inibiu a hiperlocomoção produzida pela anfetamina, o que demonstra um possível efeito antimaníaco. Pelos resultados obtidos com as demais drogas, sugere-se que o principal mecanismo de ação deste efeito seria a inibição da via da PKC, podendo haver uma participação de ação antiestrogênico.

Palavras-Chave: tamoxifeno, mania, PKC, antiestrogênico

Apoio Financeiro: CNPq

Brain-derived neurotrophic factor levels of rats submitted to forced swimming test and learned helplessness: effects of stress and imipramine treatment

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Change in brain derived neurotrophic factor (BDNF) expression has been implicated in the development of depression. Restraint stress exposure can decrease the BDNF levels in the hippocampus whereas chronic (10-14 days) antidepressant treatment increases these levels. Moreover, BDNF injections into the hippocampus or brainstem produce antidepressant-like effects. The aims of the present study were to verify if the stress induced by forced swimming test (FST) or by footshocks in the learned helplessness (LH) model would modify the hippocampal BDNF levels and if these levels were restored by imipramine treatment. Male Wistar rats received i.p. injections (24, 19 and 1h before the test, subchronic treatment) of vehicle or imipramine (15 mg/kg) and were submitted to FST. An independent group received daily imipramine (15 mg/kg) for seven days (chronic treatment) and was submitted to the LH. Non-stressed animals were submitted to same pharmacological treatments. Immediately after the test the rats were sacrificed under deep urethane anesthesia. Their hippocampi were dissected and BDNF levels were measured by ELISA. Chronic but not the subchronic treatment with imipramine increased BDNF levels in non-stressed group (BDNF(pg)/total protein(microg), mean±sem: subchronic treatment, vehicle: 2.16±0.40, imipramine: 2.10±0.25, n=4, p=0.9; chronic treatment, vehicle: 0.98±0.07, imipramine: 1.24±0.07, n=7, p=0.03, unpaired t-test). However, in the stressed groups, neither imipramine (subchronic or chronic treatment) nor stress modified BDNF levels (subchronic treatment, FST-vehicle: 2.09±0.34, FST-imipramine 1.75±0.27, naïve: 1.85±0.09, n=6, p=0.7; chronic treatment, LH-vehicle: 1.63±0.32, LH-imipramine 1.74±0.32, naïve 2.02±0.25, n=6-11/group, p=0.7, one-way ANOVA). The present results indicate that BDNF levels increase after chronic (7 days) treatment with imipramine but this effect disappears when the animals are submitted to the LH model. They suggest, therefore, that antidepressant and stress exposure interacts to modulate BDNF expression. **Palavras-Chave:** BDNF, depression

Apoio Financeiro: CAPES, FAEPA, FAPESP

C.07.051

Zinc prevents behavioral changes induced by mercury.

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Objectives: Considering the neurotoxicity of mercury (Hg) and that several biochemical alterations induced by this metal are prevented by zinc pre-exposure in young rats, we investigated the effects of zinc on possible behavioral alterations induced by HgCl₂ on performance of rats during a phase of high susceptibility of CNS to external insults.

Methods: Wistar rats were subcutaneously injected with saline or ZnCl₂ (27 mg/kg/day) from 3rd to 7th postnatal day and with saline or HgCl₂ (5 mg/kg/day) on five subsequent days. The rats were submitted to negative geotaxis task on 3, 5, 7, 9, 11 and 13; tail immersion on 13, 20 and 27; beaker test from 17 to 20; rotarod test on 25 and 30; and open field task on 30 and 31 days old.

Results: HgCl₂ treatment induced significant reduction in the weight gain from 12 until 33 days of age ($p < 0.05$); increase in the latency of negative geotaxis reflex in the sessions 5 (59%; $p < 0.05$) and 6 (64%; $p < 0.05$); increase in the latency to refuge access in the sessions 1 (9.6%; $p < 0.05$) and 4 (97%; $p < 0.05$) and in the percentage of fall in session 4 (233 %; $p < 0.05$) in the beaker test; decrease in the rearing (36%; $p < 0.05$) and crossing number (37%; $p < 0.05$) and increase in the exit latency of the initial area (66%; $p < 0.05$) in session 1 in the open field task. The performance of rats in the other tasks was not altered by treatments. The ZnCl₂ pre-treatment prevented ($p < 0.05$) the reduction in the weight gain, the increase in the latency of negative geotaxis reflex, the increase in the latency to refuge access in the session 4 and in the percentage of fall in the beaker test, and the diminution of crossing number in the open field task.

Conclusion: Exposure to Hg caused impairment in weight gain, motor function, muscular strength and locomotor activity and Zn prevented these alterations induced by Hg on performance of rats in behavioral tasks. **Palavras-Chave:** mercury, zinc, behavioral changes, weight gain

Apoio Financeiro: CAPES, CNPq, FIPE

Aumento en los niveles de ansiedad en el laberinto elevado en forma de “+”, en un modelo de diabetes tipo II en ratas.

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Poco se ha estudiado acerca de la relación diabetes-ansiedad en animales y humanos y los estudios existentes muestran resultados controversiales. Los estudios publicados en modelos animales de diabetes tipo I en roedores, muestran un incremento de los niveles de ansiedad en animales hechos diabéticos por la inyección de estreptozotocina, con respecto a sus controles [1,2]. El objetivo de este trabajo es determinar si en un modelo de diabetes tipo II existen también incrementos en los niveles de ansiedad en las ratas diabéticas, al ser evaluadas en un modelo animal de ansiedad: el laberinto elevado en forma de “+” (LE+). Para lograr este objetivo se administró a un grupo de animales una solución de sacarosa al 20% durante 6 meses (n=16). Bajo estas condiciones, desde los primeros dos meses de tratamiento las ratas presentaron cambios similares a los que se observan en el síndrome metabólico de los humanos y signos que semejan a la diabetes tipo II al final del tratamiento. A los animales control (n=16) se les administró agua en lugar de sacarosa. Las pruebas conductuales en el LE+ se realizaron a los 3 y 6 (3M y 6M) meses de tratamiento. Los resultados obtenidos indican que las ratas diabéticas permanecen menos tiempo en los brazos abiertos del LE+, en comparación con los controles (Control 3M:22.6 ±7.5; Control 6M:13.4 ±4.4; Sacarosa 3M:24±6.1, Sacarosa 6M:4.9±2.8; p<0.005) y un menor porcentaje en el número de entradas a los mismos brazos (Control 3M: 13.2 ±4.1, Control 6M: 7.2 ±0.5; Sacarosa 3M:18.4±4.2 Sacarosa 6M:6.4±1.3; p<0.0004), indicando un incremento en sus niveles de ansiedad. Los datos de locomoción medidos en el campo abierto no mostraron diferencias significativas. Con base en estos resultados y a lo reportado en la literatura, se puede sugerir que los niveles de ansiedad observados en estos animales podrían estar relacionados con la aparición de cambios metabólicos congruentes con la presencia de un cuadro de diabetes tipo II. Los mecanismos subyacentes a esta relación permanecen por ser dilucidados.

Referencias:

[1] Psychopharmacology. 135:361. 1998

[2] Jpn J Pharmacol. 86:47. 2001

Palavras-Chave: ansiedad, diabetes tipo II, laberinto elevado en forma de “+”

Apoio Financeiro: CONACYT

EFEITO ANTIDEPRESSIVO DO EXTRATO HIDROALCOÓLICO DE *Rosmarinus Officinalis* L. EM CAMUNDONGOS: EVIDÊNCIAS DO ENVOLVIMENTO DO SISTEMA MONOAMINÉRGICO

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O *Rosmarinus officinalis* L. (Labiatae) possui diversos usos na medicina popular, incluindo o tratamento de transtornos de humor (J Ethnopharmacol 107:157, 2006). Neste estudo, o efeito do extrato hidroalcoólico desta planta foi examinado no teste do nado forçado (TNF) e teste da suspensão da cauda (TSC), testes preditivos de atividade antidepressiva em camundongos Swiss. O extrato causou um efeito antidepressivo em ambos modelos, pois reduziu significativamente o tempo de imobilidade no TNF (100 mg/kg, p.o.) e TSC (10 e 100 mg/kg, p.o.), sendo a % de redução: 50,3±2,8 %; 77,1±4,3 % e 71,94±3,0 % em relação aos grupos controle =100%, respectivamente. O extrato não alterou significativamente a locomoção e o número de “rearings” no teste do campo aberto. O efeito anti-imobilidade do extrato (10 mg/kg, p.o.) no TSC, foi prevenido pelo pré-tratamento dos camundongos com *p*-chlorofenilalanina (100 mg/kg, i.p., inibidor da síntese de serotonina, por quatro dias consecutivos; 86,2±2,9 %), NAN-190 (0.5 mg/kg, i.p., antagonista de receptor 5-HT_{1A}; 99,5±6,4%), cetanserina (5 mg/kg, i.p., antagonista de receptor 5-HT_{2A}; 100,9±3,2 %), prazosina (1 mg/kg, i.p., antagonista de receptor α_1 adrenérgico; 99,9±3,2%), SCH23390 (0,05 mg/kg, s.c., antagonista de receptor dopaminérgico D₁; 100,8±4,6 %) ou sulpirida (50 mg/kg, i.p., antagonista de receptor dopaminérgico D₂; 102,7 ±7,5 %). O pré-tratamento com ioimbina (1 mg/kg, i.p., antagonista de receptor α_2 adrenérgico) não foi efetivo em prevenir o efeito anti-imobilidade do extrato. O tratamento dos camundongos com doses sub-ativas de MDL72222 (0.1 mg/kg, i.p., antagonista de receptor 5-HT₃) e do extrato (1 mg/kg, p.o.) resultou em efeito sinérgico no TSC (70,4±5,6 % em relação ao grupo controle). Os resultados do presente estudo sugerem que o extrato hidroalcoólico de *Rosmarinus officinalis* L. apresenta efeito antidepressivo específico (não é um mediado por um efeito psicoestimulante), o qual é dependente da interação com o sistema monoaminérgico (via receptores 5-HT_{1A}, 5-HT_{2A}, 5-HT₃, α_1 adrenérgico, dopaminérgico D₁ e D₂) podendo ser utilizado como uma nova alternativa terapêutica para o tratamento da depressão.

Palavras-Chave: depressão, serotonina, noradrenalina, dopamina, *Rosmarinus officinalis*, teste do nado forçado, teste da suspensão da cauda.

Apoio Financeiro: FAPESC-SC, CAPES, CNPq, UFSC, IBN-Net (01.06.0842-00).

EFEITOS DA OVARECTOMIA PRECOCE NO COMPORTAMENTO DE RATAS JOVENS E IDOSAS SUBMETIDAS AO LABIRINTO EM CRUZ ELEVADO E AO TESTE DA NATAÇÃO FORÇADA

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Objetivo: Estudos clínicos sugerem que as mulheres na fase peri-menopausa mostram maiores chances de desenvolver transtornos afetivos, o que foi atribuído às alterações hormonais. Poucos estudos pré-clínicos avaliaram os efeitos a longo prazo da privação hormonal no comportamento. O presente trabalho tem por objetivo avaliar os efeitos da ovariectomia (OVX) precoce em ratas jovens e idosas submetidas ao teste da natação forçada (TNF) e ao labirinto em cruz elevado (LCE).

Metodologia: Foram utilizadas ratas Wistar jovens (6 meses) e idosas (18 meses). As ratas foram divididas em grupos (n=10/grupo): sham jovem, OVX jovem, sham idosa e OVX idosa, sendo a OVX realizada no terceiro mês de vida. As ratas foram submetidas ao LCE, no qual por 5 min registrou-se a frequência de entradas e o tempo de permanência nos braços abertos do aparelho. O TNF foi realizado em um cilindro com 30 cm de água a 25 °C. Este teste se dividiu em duas sessões: treino (15 min) e, após 24 h, teste (5 min), sendo desta vez registrado o tempo de imobilidade (s) das ratas.

Resultados: Os resultados demonstraram que a retirada precoce dos ovários, seja em ratas jovens que em idosas, causou redução significativa do tempo (SHAM jovem: 27.9±7,8; OVX jovem: 10.6±2.7*; SHAM idosas: 37.0±7.9; OVX idosas: 12.4±3.7*; *P<0.05 vs. sham) e da frequência de entradas nos braços abertos do LCE quando comparado com as ratas sham jovens e idosas, respectivamente. No TNF, as ratas idosas, sham ou OVX, apresentaram tempo de imobilidade superior em relação às ratas jovens sham ou OVX (SHAM jovem: 89±10; OVX jovem: 81±11; SHAM idosas: 133±12*; OVX idosas: 118±9*; *P<0.05 vs. jovens), respectivamente. Não foi observada diferença na locomoção avaliada no teste do campo aberto nas ratas empregadas neste estudo.

Conclusões: Estes dados sugerem que a OVX precoce causa alterações persistentes nos níveis de ansiedade das ratas, sendo estas alterações visíveis até idade avançada. Por outro lado, a idade, mas não a OVX, induz um comportamento do tipo depressivo em ratas. Estes achados são de grande relevância para o entendimento do papel dos hormônios ovarianos e do envelhecimento nos transtornos afetivos em fêmeas.

Palavras-Chave: ovariectomia; depressão; ansiedade; labirinto em cruz elevado; natação forçada

Apoio Financeiro: CNPq (Brasil), IBRO (Return Home Fellowship) e UNESC

Efeitos da exposição crônica a efluentes de curtume sobre parâmetros comportamentais de ansiedade e depressão em camundongos

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Sugere-se que a exposição à poluição industrial vem alterando o comportamento dos indivíduos. Recentemente, resultados prévios em nosso laboratório mostraram que o tratamento crônico de camundongos a efluentes de curtume induziu alterações importantes no estado oxidativo celular no sistema nervoso central. O objetivo deste trabalho foi avaliar aspectos comportamentais, em modelos animais de ansiedade e depressão, após exposição crônica de camundongos a efluentes de curtume bruto (ECB) e tratado (ECT). Foram utilizados efluentes de curtume brutos (ECB) e tratados (ECT) com um processo de voltametria cíclica nos ensaios de fotodegradação eletroquímica (radiação UV e potencial controlado). Foram utilizados 40 camundongos albinos, linhagem CF1, machos (3 meses, pesando entre 30 e 40 g), expostos durante 17 dias aos efluentes com ou sem tratamento fotoeletroquímico (0,1% e 1%) na água de beber. No 15º dia, os animais foram submetidos ao labirinto em cruz elevado, onde foram anotados o tempo gasto e o número de entradas nos braços abertos e fechados. No 17º dia, os animais foram submetidos ao teste de nado forçado, sendo observado o tempo de imobilidade. O ECB 0,1% e 1% aumentaram significativamente de forma dose-dependente o tempo gasto no braço fechado (controle 165 ± 99; ECB 0,1% 232 ± 48; ECB 1% 270 ± 32; ECT 0,1% 225 ± 55; ECT 1% 200 ± 67 segundos; ANOVA seguida de Duncan, $p < 0,05$). Além de que, houve uma tendência à redução no tempo gasto no braço aberto ($p < 0,0513$). ECB e ECT não alteraram os níveis de locomoção, avaliados pelo número total de entradas em ambos os braços. A exposição aos efluentes (ECB e ECT) não alterou o tempo de imobilidade no teste do nado forçado. Os resultados sugerem que a exposição ao efluente bruto induz um estado de ansiedade em camundongos, enquanto que o efluente tratado não alterou os estados de ansiedade e de depressão. Os dados indicam que estudos de avaliação de risco da exposição a contaminantes à saúde mental devem ser implantados e confirmam a importância do tratamento de efluentes industriais.

Palavras-Chave: efluentes de curtume, tratamento fotoeletroquímico, nado forçado, labirinto em cruz elevado, camundongos

Apoio Financeiro: UNIVATES

EFFECTS OF SUBCHRONIC LEAD ESPOSURE ON BEHAVIOR OF ADULT SWISS MICE IN FORCED SWIMMING TEST AND OPEN FIELD.

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Objective: The lead acetate exposure for 7 days affect the behavior of adult mice on the forced swimming test (FST) and do not affect the ambulation in open-field (OF). In the present work, we investigated the effects of longer lead exposure on behavior of adult mice on FST and OF.

Methods: At postnatal day 30, Swiss mice were randomly assigned to receive 540ppm of lead acetate in distilled water via their ad-lib water supply (Pb-TREATED) or tap water (CONTROL) for at least 60 days (average exposure period = 80.3 days, SD = 8.8, highest exposition time = 90 days). During the last 5 days of exposure, mice were submitted to OF (CONTROL: 8 females and 12 males, Pb-TREATED: 9 females and 12 males) or FST (CONTROL: 12 females and 8 males, Pb-TREATED: 9 females and 11 males). The immobility time, the number of 30° turns and the number of spontaneous alterations in turning biases were recorded in 3 FST sessions (test time = 5min, test-retest time interval = 48h). The ambulation in center and in periphery was recorded in 1 OF session (test time = 5min). After behavioral tests, blood samples were collected to blood-lead analysis using an Atomic Absorption Spectrometry. The relationship between blood-lead levels (Pb-B) and behavioral variables were evaluated using Pearson correlation coefficient ($P < 0.05$, two-tailed). Univariate and repeated measures ANOVAs were performed, respectively, for OF and FST data.

Results: In CONTROL Group, 67.5% of mice presented detectable Pb-B, that ranged from 0.6 to 4.2 µg/dL (mean = 1.1; SEM = 0.2). In PB-TREATED Group, the Pb-B presented a great individual variability ranging from 52.0 to 106.9 µg/dL (mean = 75.7; SEM = 2.0). No differences were observed between CONTROL and PB-TREATED groups in OF. On FST, PB-TREATED mice made more 30° turns ($F = 6.9$, $df = 1.0$, $P = 0.01$) and presented less spontaneous alterations ($F = 4.6$, $df = 1.0$, $P < 0.05$) than CONTROL ones. In PB-TREATED group, immobility times were inversely related to Pb-S ($r = 0.54$, $df = 21$, $P = 0.01$).

Conclusions: Our data suggest that FST is a useful tool to investigate the effects longer lead exposure in adult mice.

Palavras-Chave: Lead exposure, Behavior, Forced swimming, Open-field

Apoio Financeiro: Financial support: SR2 / UERJ

INVESTIGAÇÃO DO POTENCIAL ANTIDEPRESSIVO DO EXTRATO HIDROALCOÓLICO DE *Rosmarinus Officinalis* L. EM CAMUNDONGOS SUBMETIDOS À BULBECTOMIA OLFATÓRIA

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A ablação bilateral dos bulbos olfatórios resulta em alterações comportamentais compatíveis ao que é observado em pacientes com depressão. Desta forma, a bulbectomia olfatória (B.O.) em roedores tem sido proposta como um modelo animal de depressão ([Exp. Neurol.204:20, 2007](#)). Este trabalho investigou o efeito do extrato hidroalcoólico de *Rosmarinus officinalis* L. (Labiatae) em camundongos Swiss submetidos ao modelo de bulbectomia olfatória bilateral. Após 14 dias pós-operatório os grupos experimentais (SHAM e B.O.) foram avaliados quanto a atividade locomotora e exploratória no teste do campo aberto (TCA), e submetidos a 14 dias de tratamento com o extrato (10 e 100 mg/kg, p.o.) ou com o antidepressivo fluoxetina (10 mg/kg, p.o., controle positivo) e seus respectivos veículos. Decorridos os 14 dias pós-tratamento, os animais foram avaliados em diversos testes comportamentais: TCA, “*splash test*” e teste da suspensão da cauda (TSC). Os resultados indicaram primeiramente um aumento significativo na atividade locomotora e exploratória (“*rearings*”) nos animais bulbectomizados, sem alterar o número de “*groomings*” e bolos fecais, quando avaliados no TCA 14 dias pós-operatório ($132,3 \pm 6,4\%$; $150,8 \pm 7,6\%$), % em relação aos grupos controle SHAM=100%, respectivamente. A B.O. também induziu a uma perda de peso nos animais bulbectomizados observado em 7 e 14 dias P.O. ($94,4 \pm 1,3\%$; $94,4 \pm 1,21\%$). O tratamento dos animais com extrato (10 e 100 mg/kg, p.o.; $41,1 \pm 7,1\%$, $51,9 \pm 9,6\%$) e fluoxetina (10 mg/kg, p.o., $43,3 \pm 7,7\%$) reduziu o tempo de latência da auto-limpeza no “*splash test*” e o tempo de imobilidade no TSC ($47,3 \pm 5,5\%$; $55,5 \pm 5,6\%$ respectivamente), porém não foi capaz de reduzir a hiperatividade locomotora dos animais bulbectomizados (resultados expressos em % em relação ao grupo BO-veículo). O efeito antidepressivo do extrato (100 mg/kg, p.o.; $77,6 \pm 8,3\%$) e fluoxetina (10 mg/kg, p.o., $61,9 \pm 4,8\%$) também foi evidente no grupo SHAM após o tratamento repetido de 14 dias no TSC, % em relação ao grupo controle=100%. Concluimos que o extrato hidroalcoólico de *Rosmarinus officinalis* apresenta efeito antidepressivo no modelo da B.O. similar ao produzido pela fluoxetina.

Palavras-Chave: depressão, bulbectomia olfatória, *Rosmarinus officinalis*, teste do campo aberto, teste da suspensão da cauda, “*splash test*”

Apoio Financeiro: FAPESC-SC, CAPES, CNPq, UFSC, IBN-Net (01.06.0842-00)

EFFECTS OF NEONATAL ETHANOL EXPOSURE IN the BEHAVIOR of adolescent SWISS MICE tested in the plus-maze discriminative avoidance task

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Objectives: Approximately 9% of pregnant women report drinking alcohol with 3% reporting binge alcohol use. There are several studies about the use of alcohol during pregnancy and its consequences. However, little is known about the alterations in behavior in individuals whose mothers reported alcohol consumption during the third trimester of gestation. Hence, we assessed behavior in the Plus-Maze Discriminative Avoidance Task (PMDAT) of adolescent Swiss mice that were neonatally exposed to ethanol.

Methods: Animals in the ETOH group received i.p injections of ethanol (5g/Kg) from the 4th to 7th postnatal day (PN4 to PN7; the third trimester equivalent of human gestation); a period in which the brain is highly vulnerable to the damaging effects of ethanol. The VEH group received saline solution instead. The animals were tested in the PMDAT. The apparatus is a modified elevated plus-maze. It has 2 enclosed arms opposite to 2 open arms, elevated from the floor. One of the enclosed arms has 3 aversive stimuli (loud sound, intense light and strong air flow). Animals were tested on PN30 and PN31. On PN30, the test lasted 10 min and the aversive stimuli were turned on whenever the animals entered the aversive enclosed arm. On PN31, the test lasted 3 min and the aversive stimuli were no longer employed.

Results: There were no significant differences between groups in the first test. In the second test, ETOH mice spent less time in aversive arm (ETOH: 20.5±8.5s; VEH: 57.5±12.1s; ANOVA: F=5.23; p=0.02) and more time in the enclosed arm than VEH mice (ETOH: 144.7±10.1s; VEH: 106.5±12.9s; ANOVA: F=4.73; p=0.03). Furthermore, ETOH mice entered the aversive arm less frequently (ETOH: 9±4%; VEH: 24±3%; ANOVA: F=10.70; p=0.003) and the enclosed arm more frequently than CONTROL mice (ETOH: 71±9%; VEH: 39±6%; ANOVA: F=8.53; p=0.006). Finally, ETOH mice were less active than VEH mice (ETOH: 3.9±1.1; VEH: 8.9±1.7; ANOVA: F=5.07; p=0.03).

Conclusion: These results suggest that, in mice, exposure to ethanol during the neonatal period causes persistent alterations in behavior, particularly in anxiety levels.

Palavras-Chave: neonatal exposure, ethanol, adolescence, anxiety, development

Apoio Financeiro: Financial support: CNPq, FAPERJ, SR2-UERJ.

TÔNUS VAGAL E SINTOMAS DO TRANSTORNO DE ESTRESSE PÓS-TRAUMÁTICO

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Estudos recentes observaram associações entre a atividade autonômica parassimpática e a capacidade de regulação emocional. Estudos mostram que pacientes com transtorno de estresse pós-traumático (TEPT) apresentam sintomas de revivescência, evitação, entorpecimento emocional e hiperexcitabilidade. O objetivo do presente estudo é investigar a associação entre a atividade parassimpática sobre o ritmo cardíaco e sintomas de estresse pós-traumático em pacientes diagnosticados com TEPT. A aquisição do sinal eletrocardiográfico e a aplicação de um questionário que mede sintomas de TEPT, foi realizada em vinte pacientes com diagnóstico do transtorno. A atividade autonômica parassimpática (tônus vagal) foi extraída através da análise espectral da variabilidade dos intervalos dos batimentos cardíacos em alta frequência. O tônus vagal cardíaco mostrou correlação inversa com escores de sintomas de entorpecimento emocional ($r = -0.50$, $p = 0.04$) e sintomas de evitação ($r = -0.57$, $p = 0.02$), porém não foi encontrada nenhuma associação entre a atividade parassimpática e os sintomas de revivescência ($r = -0.2$, $p = 0.4$) e hiperexcitabilidade ($r = -0.007$, $p = 0.9$). Através desses resultados, pode-se concluir que o tônus vagal reduzido, um índice que vem sendo associado a desregulação emocional, se correlacionou com uma maior gravidade de sintomas de entorpecimento emocional e evitação. Esses resultados contribuem para o melhor entendimento das bases psicofisiológicas dos sintomas de TEPT e podem contribuir para novas formas de intervenção terapêutica.

Palavras-Chave: Transtorno de Estresse Pós-Traumático, Tonus vagal, Entorpecimento Emocional, Psicofisiologia

Apoio Financeiro: CNPq

EXPOSIÇÃO AO PESTICIDA ORGANOFOSFORADO METAMIDOFÓS INDUZ COMPORTAMENTO ASSOCIADO À DEPRESSÃO EM CAMUNDONGOS

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Introdução: Estudos epidemiológicos indicam uma associação entre depressão e exposição a pesticidas. Para avaliar esta associação, quantificamos o tempo de imobilidade (indicador de comportamento associado à depressão) em camundongos expostos ao pesticida metamidofós, um inibidor da acetilcolinesterase (AChE).

Metodologia: Camundongos Suíços machos adultos foram tratados com metamidofós por 29 dias, nas concentrações de 0,5mg/kg (2,5% DL50) ou 2mg/kg (10% DL50) na água que bebiam. Animais controle receberam veículo. Os testes foram realizados ao final do período de exposição (grupo DUR) ou dez dias após a exposição (grupo TARDIO). Os animais foram submetidos ao nado forçado e o tempo de imobilidade foi registrado em 5 intervalos de 2min. A seguir, os animais foram submetidos ao teste da suspensão pela cauda por 6min e o tempo de imobilidade foi quantificado. Para avaliar alterações motoras, foi realizado o teste de locomoção forçada (Rotarod), sendo registrada a latência para a queda dos animais e, também, o teste de esforço, no qual os animais eram colocados em um recipiente com água com pesos presos às suas caudas até permanecerem imersos por 5s, sendo registrado o tempo de permanência na superfície. Adicionalmente, a atividade da AChE foi avaliada por método colorimétrico.

Resultados: No teste do nado forçado, houve um aumento do tempo de imobilidade no grupo 2mg/kg-TARDIO em relação ao grupo controle no quarto ($13,3 \pm 2,9$ vs. $4,6 \pm 1,2$; $p=0,004$) e quinto ($18 \pm 3,5$ vs. $5,7 \pm 1,5$; $p=0,002$) intervalos de teste. Observamos um aumento de imobilidade também no teste da suspensão pela cauda, no grupo 0,5mg/kg-DUR em relação ao grupo controle ($182,6 \pm 8,2$ vs. $135,9 \pm 11$; $p=0,04$). A atividade da AChE foi diminuída em relação ao controle no grupo 2mg/kg-DUR ($0,8 \pm 0,04$ vs. $1,4 \pm 0,04$; $p<0,0001$) e 2mg/kg-TARDIO ($1,2 \pm 0,1$ vs. $1,5 \pm 0,08$; $p=0,008$). Não houve diferenças significativas no Rotarod e no teste de esforço.

Conclusão: Nossos resultados indicam que metamidofós induz comportamento associado à depressão. Esta conclusão é corroborada pela não alteração do desempenho motor. O aumento de imobilidade mesmo na ausência de inibição da AChE sugere que outros mecanismos estão envolvidos **Palavras-Chave:** organofosforado; metamidofós; depressão; comportamento animal

Apoio Financeiro: Faperj, SR-2 UERJ, CNPq.

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Transcriptional Response to Lithium in Mouse Astrocytes.

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Objectives: Astrocytes are a sub-type of glial cells which own an active carbohydrate metabolism and act, among others, as a metabolic support for neurons in the brain. They metabolize glucose that can be used for their own support or they can export lactate to feed neurons (1). The goal of this study is to investigate the effects of lithium, a drug used to treat bipolar disorder, in astrocyte's biological processes.

Methods: In order to identify differentially expressed genes in astrocytes treated with 2mM lithium for three days, a microarray approach was used. After RNA isolation, cDNA was synthesized and labeled with Cy3-dCTP or Cy5-dCTP. The labeled cDNA was hybridized with a microarray library from mouse genes that contains 20,000 genes of known function or with homology to known genes. Differential expression of genes was analyzed with genArise software where we calculated the Z-score and identified genes which expression was changed. Afterwards genes were classified using FATIGO and Kegg's metabolic pathway software. To confirm our results, astrocytes were kept for 2 hours in a medium without glucose to deplete the glycogen content. Then glucose was added to the medium at 5mM final concentration with lithium 2mM or insulin 1mM. The effect of lithium in insulin-like response was investigated by glucose release. The extinction was measured at 340nm.

Results: Our results show that lithium increases the expression of 7 genes involved in the insulin pathway from a total of 696 genes used for the analysis. It is the fifth more altered pathway from the up-regulated pathways. To corroborate our result, we saw that lithium tends to increase the content of glycogen when astrocytes are treated during 2 hours with 2mM LiCl.

Conclusions: We saw that lithium 2mM has an insulin-like effect in the glycogen content in astrocytes. Maybe it occurs because lithium inhibits the enzyme glycogen sintase kinase 3 (2), and then, phosphorylation and regulation of glycogen synthase is abolished.

References: 1- Neurochemistry Intertional 48(6-7):616-22, 2006; 2- European Journal of Biochemistry 270(18):3829-38, 2003. **Palavras-Chave:** Astrocytes; Lithium; Microarray.

Apoio Financeiro: Supported by CNPq, FAPERJ.

EFFECTS OF NEONATAL MALNUTRITION PROGRAMMING ON LEARNING/MEMORY, NOVELTY-SEEKING AND ANXIETY-LIKE BEHAVIOURAL TRAITS IN ADULT MALE RATS

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OBJECTIVE: Hypoprolactinemia induced by maternal bromocriptine (BRO) treatment at the end of lactation programmes for higher catecholamine and corticosterone levels. These hormones are known to be associated with behaviour. Hence, in the present study we investigated the long-term behavioural effects of maternal hypoprolactinemia associated with novelty-seeking, anxiety and learning/memory in adult male rats. **METHODS:** Lactating Wistar rats either received bromocriptine (BRO; 1mg/twice a day i.p.) or saline (C) at the postnatal (PN) days 18, 19 and 21. Forty four BRO and 34 C rats were tested at adulthood according to the following protocol: *Experiment 1:* Learning/memory were assessed at PN172 in the radial arm water maze (RAWM). Each animal was tested 4 times a day for 5 consecutive days. They were allowed 2 min to explore and find the hidden escape platform. The latency to find the platform was noted. *Exp. 2:* At PN180, animals were tested in the hole board arena (HB) in order to assess novelty-seeking behaviour. Animals were allowed 10 min to explore. The number of explored holes (head-dips) was noted. *Exp. 3:* At PN182, anxiety levels were assessed in elevated plus-maze (EPM). Time spent in the open arms and total number of arm entries (open + closed) were recorded over a period of 10 min. **RESULTS:** *Exp 1:* C rats reduced the latency to find the platform significantly faster (repeated measures ANOVA: $F=3.6$, $df=3$, $P=0.014$) than BRO ones; *Exp 2:* There were no significant differences (ANOVA: $P>0.05$) between groups regarding the number of head dips (BRO: 14.5 ± 1.1 ; C: 15.1 ± 1.6); *Exp 3:* BRO rats ($8.8\pm3.3s$) spent significantly less time (Mann-Whitney: $Z=2.4$, $P=0.018$) in the open arms than C ones ($27.0\pm8.2s$). Furthermore, BRO rats (15.7 ± 2.1 entries) had a significantly smaller number of total arm entries (ANOVA: $F=15.9$, $df=1$, $P<0.001$) than C ones (27.6 ± 2.1 entries). **CONCLUSIONS:** Neonatal malnutrition (a consequence of bromocriptine induced maternal hypoprolactinemia) results in animals with deficits in learning/memory, higher levels of anxiety and lower levels of activity.

Palavras-Chave: Bromocriptine, Hypoprolactinemia, behaviour, Learning/memory, anxiety, novelty-seeking

Dosis moderadas de alcohol durante la gestación tardía son suficientes para reproducir conductas características del Síndrome de Hiperactividad y Déficit de la Atención en ratas periadolescentes.

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El Trastorno por Hiperactividad y Déficit de la Atención (ADHD) es uno de los desordenes psiquiátricos de mayor incidencia en niños de edad escolar, con una prevalencia del 8 al 12%. Se caracteriza por impulsividad, períodos atencionales breves e hiperactividad (*Biol Psychiatry* 57:1239, 2005). Su tratamiento se basa en el uso de psicoestimulantes (principalmente Metilfenidato) que “paradójicamente” disminuyen estos síntomas (*Neurosci Biobehav Rev* 24:27, 2000). La exposición prenatal al alcohol afecta al desarrollo neurobiológico produciendo, en determinadas condiciones, alteraciones similares a las descritas para este síndrome (*Lancet* 366:237, 2005). Objetivos: Determinar si la exposición prenatal a una dosis moderada de alcohol genera alteraciones características del ADHD, las cuáles puedan ser revertidas con Metilfenidato (MPH). Métodos: Ratas Wistar periadolescentes (24 días), provenientes de hembras administradas (i.g.) con alcohol (3 g/Kg) o agua, durante los DGs 17 al 20 fueron evaluadas en un campo abierto (Open Field) y en el laberinto en cruz elevado (Plus Maze). Resultados: Animales prenatalmente expuestos al alcohol mostraron mayor actividad locomotora en el Open Field y permanecieron más tiempo en los brazos abiertos del Plus Maze, que sujetos controles. En un segundo experimento, animales prenatalmente expuestos al etanol o agua, fueron administrados (i.p.) con MPH (0, 0.5, 1 o 1.5 mg/Kg), previo a la evaluación en el Open Field. Los animales controles aumentaron significativamente los niveles de actividad locomotora, conforme al aumento en la dosis de MPH. La actividad de los sujetos tratados con alcohol no mostró diferencias significativas entre el vehículo o las dosis de esta droga. Conclusiones: Este conjunto de resultados indican que la experiencia prenatal con una dosis etílica moderada, durante un periodo gestacional muy acotado, incrementa los patrones de actividad e impulsividad semejantes a los descritos en los modelos animales de ADHD. Si bien las dosis de MPH utilizadas no revirtieron el efecto del etanol, se observó que estos animales no aumentaron su actividad con respecto a animales sin experiencia etílica prenatal.

Palavras-Chave: ADHD, exposición prenatal, alcohol, metilfenidato.

Apoio Financeiro: CONICET y Agencia Córdoba Ciencia.

EFFECTS OF NEONATAL NICOTINE PROGRAMMING ON LEARNING/MEMORY, ANXIETY and novelty-seeking behavioural traits IN ADULT MALE RATS

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OBJECTIVE: Maternal nicotine exposure during lactation programmes for hyperleptinemia and hypothyroidism in adult offsprings. Changes in the levels of these hormones are known to affect behaviour. Hence, here we investigated the long-term behavioural effects of neonatal nicotine exposure in adult male rats. **METHODS:** Lactating Wistar rats implanted with minipumps either containing nicotine (NIC=6mg/Kg/dia) or a solution with equivalent concentration of sodium bitartrate (C) during the first 14 postnatal (PN) days. Twenty six NIC and 25 C rats were tested at adulthood according to the following protocol: **Experiment 1:** Learning/memory was assessed at PN150 in the radial arm water maze (RAWM). Each animal was tested 4 times a day for 5 consecutive days. They were allowed 2 min to find the hidden escape platform. The latency to find the platform was noted. **Exp. 2:** At PN160, anxiety levels were assessed in elevated plus-maze (EPM). Entries into each area of the maze (open and closed arms and centre square) were recorded over a period of 10 min (subdivided into four 2.5min segments: S1 to S4). **Exp. 3:** At PN162, animals were tested in the hole board arena (HB) in order to assess novelty-seeking behaviour. Animals were allowed 10 min to explore (subdivided into four 2.5min segments: S1 to S4). The number of explored holes was noted. **RESULTS: Exp 1:** No TREATMENT effect or interactions were observed in the RAWM (ANOVA: $p>0.10$) **Exp 2:** A significant difference (ANOVA: $F=2.5$, $df=3$, $p=0.06$) between groups was observed regarding the pattern of entries into the centre square throughout the test: Only C rats increased the number of entries into this area (C: from 2.1 ± 0.5 in S1 to 4.3 ± 1.0 in S4; NIC: from 3.6 ± 0.7 in S1 to 3.6 ± 0.8 in S4). **Exp 3:** An interaction (ANOVA: $F=5$, $df=3$, $p=0.003$) between TREATMENT and SEGMENT was observed regarding the number of explored holes: Only NIC rats reduced the number throughout the test (NIC: from 7.2 ± 0.9 in S1 to 1.9 ± 0.4 in S4; C: from 4.5 ± 0.7 in S1 to 4.0 ± 1.0 in S4). **CONCLUSIONS:** Neonatal nicotine affects novelty-seeking and exploratory behaviour in adult life without interfering with anxiety levels or learning and memory in rats.

Palavras-Chave: Nicotine, lactation, programming, Learning/memory, anxiety, novelty-seeking, behaviour

Apoio Financeiro: Financial support: FAPERJ, CNPq, CAPES, SR2-UERJ.

Antidepressant-like effect of scopoletin, a coumarine isolated from *Polygala sabulosa* in models predictive of antidepressant activity

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Objectives: Scopoletin is a coumarine isolated from *Polygala sabulosa* A. W. Bennett, native to Santa Catarina (Brazil) that has been used in folk medicine as a topical anesthetic. Antioxidant properties have also been reported to scopoletin (1). In this study, the antidepressant-like effect of scopoletin was investigated in the tail suspension test (TST) and forced swimming test (FST), tests predictive of antidepressant activity. **Methods:** Swiss mice maintained at standard laboratory conditions were used. The immobility time in a 6-min session in the FST and TST was registered. Scopoletin or vehicle was administered by oral route (p.o.) (0.1-1-10-100 mg/kg) 60 min before FST or TST. Alternatively, scopoletin or vehicle was administered by intraperitoneal (i.p.) route (0.1-1-10 mg/kg) 30 min before FST and (0.001-0.01-0.1-1 mg/kg) TST. In order to rule out non-specific locomotor activity, mice were submitted to the open-field test. **Results:** Scopoletin given by p.o. route significantly decreased the immobility time in the TST at doses of 10 and 100 mg/kg ($67.3 \pm 4.7\%$ and $73.8 \pm 7.3\%$, as compared to the control group=100%, respectively). Scopoletin given by i.p. route at 1 and 10 mg/kg produced an antidepressant-like effect both in FST ($67.5 \pm 5.6\%$ and $80.6 \pm 2.6\%$, as compared to the control group=100%, respectively) and TST at doses of 0.01 and 0.1 mg/kg ($83.8 \pm 3.0\%$ and $69.7 \pm 2.3\%$, respectively). Scopoletin administered by p.o. route (10-100 mg/kg) and i.p. route (0.01-10 mg/kg) did not alter locomotor activity in the open-field test. **Conclusion:** Scopoletin produced a significant antidepressant-like response by oral route in TST but not in FST. Furthermore the administration of scopoletin by i.p. route was effective both in the TST and FST. The antidepressant-like effect elicited by scopoletin cannot be attributed to a psychostimulant action of this compound. **References:** 1–Biochem Pharmacol. 75:1416, 2008. **Palavras-Chave:** scopoletin; *Polygala sabulosa*; depression; tail suspension test; forced swimming test

Apoio Financeiro: FAPESC-SC, CAPES, CNPq, UFSC, FINEP-IBN-Net (01.06.0842-00)

Avaliação do potencial antidepressivo da *Polygala laureola* A.St.-Hil. & Moq (Polygalaceae) em camundongos: envolvimento do sistema monoaminérgico

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OBJETIVOS: *Polygala laureola* é nativa da Mata Atlântica brasileira, estando presente principalmente na região litorânea de Santa Catarina. Este estudo investigou o efeito antidepressivo do extrato aquoso (EAPL) e resinoso (ERPL) das partes aéreas desta planta no teste de suspensão da cauda (TSC) e teste do nado forçado (TNF) em camundongos, e o envolvimento do sistema monoaminérgico nesta ação.

MÉTODOS: O tempo de imobilidade foi registrado no TNF e TSC, bem como a ambulação no teste do campo aberto (TCA) em camundongos Swiss (n=6-8). EAPL ou veículo foram administrados por via oral (p.o.) 60 min antes do TNF e TSC (1-100 mg/kg e 0,0001-100 mg/kg, respectivamente). Adicionalmente, ERPL ou veículo foram administrados p.o. 60 min antes do TNF e TSC (0,1-100 mg/kg e 1-100 mg/kg, respectivamente). Para investigar os possíveis mecanismos de ação, os animais foram pré-tratados com veículo, WAY100635 (0,1 mg/kg, s.c., antagonista de receptores 5-HT_{1A}), cetanserina (5 mg/kg, i.p., antagonista de receptores 5-HT_{2A/2C}), prazosina (1 mg/kg, i.p., antagonista α 1-adrenérgico), ioimbina (1 mg/kg, i.p., antagonista α 2-adrenérgico), SCH23390 (0,05 mg/kg, s.c., antagonista de receptores dopaminérgicos D₁) ou sulpirida (50 mg/kg, i.p., antagonista de receptores dopaminérgicos D₂).

RESULTADOS: O tratamento com EAPL reduziu o tempo de imobilidade na dose de 0,001mg/kg no TSC ($70,1 \pm 10\%$; $p < 0,05$) quando comparado ao grupo controle = 100%. A administração de ERPL apresentou efeito tipo-antidepressivo no TNF e TSC, na dose de 1mg/kg ($88,2 \pm 1,5\%$; $p < 0,01$ e $84,4 \pm 3,6\%$; $p < 0,05$, respectivamente). Ambos extratos não alteraram significativamente a ambulação. O efeito do EAPL no TSC foi revertido pelo pré-tratamento dos animais com WAY100635 ($95,6 \pm 3,1\%$), cetanserina ($84,2 \pm 6,9\%$), prazosina ($87,3 \pm 4,9\%$), SCH23390 ($105,5 \pm 4,5\%$) e sulpirida ($101,1 \pm 3,3\%$), mas não com ioimbina ($81,4 \pm 6,1\%$), em relação ao grupo controle = 100%. **CONCLUSÕES:** O EAPL e o ERPL produzem efeito antidepressivo específicos. Os resultados sugerem que este efeito é mediado por uma interação com receptores 5-HT_{1A}, 5-HT₂, α 1-adrenérgicos e dopaminérgicos D₁ e D₂. **Palavras-Chave:** *Polygala laureola*, depressão, teste de suspensão da cauda, teste do nado forçado, serotonina, noradrenalina, dopamina

Apoio Financeiro: FAPESC-SC, CAPES, CNPq, UFSC, FINEP-IBN-Net (01.06.0842-00)

Reversão pela oxima K027 do comportamento tipo-depressivo induzido por malation em camundongos

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Objetivos: O organofosforado (OF) malation é utilizado como inseticida e sua toxicidade é atribuída à inibição da enzima acetilcolinesterase (AChE). Dados da literatura relatam que este OF induz a um comportamento tipo-depressivo no teste do nado forçado (TNF) em ratos, o qual é um modelo preditivo de atividade antidepressiva¹. Este trabalho investigou o efeito do tratamento repetido (3 dias) com malation e/ou com a oxima K027 no TNF e no teste do campo aberto (TCA) em camundongos Swiss.

Métodos: Decorridos 24 h após o último tratamento com o malation e/ou oxima K027, os camundongos foram submetidos aos testes comportamentais. A atividade da AChE foi mensurada no hipocampo. O tratamento repetido (3 dias) com malation (30, 100, 300 mg/kg, i.p.) causou um comportamento tipo-depressivo nos animais, pois aumentou o tempo de imobilidade no TNF ($121,77 \pm 2,02\%$, $125,21 \pm 4,56\%$, $124,80 \pm 5,64\%$; $p < 0,01$ respectivamente) em relação ao grupo controle = 100%, respectivamente. A fim de investigar um possível efeito protetor da oxima K027, os camundongos foram co-administrados por 3 dias consecutivos com malation (300 mg/kg, i.p.) e/ou com a oxima K027 (150 mg/kg, i.p.), e seus respectivos veículos.

Resultados: O OF aumentou o tempo de imobilidade no TNF ($113,46 \pm 2,06\%$; $p < 0,01$) e este efeito tipo-depressivo foi revertido pela co-administração da oxima K027 ($99,30 \pm 3,60\%$; $p < 0,01$). Porém a locomoção e o comportamento exploratório no TCA não foram alterados pelos tratamentos. A atividade da enzima AChE foi inibida no hipocampo dos animais tratados pelo malation ($55,8 \pm 5,43\%$; $p < 0,05$), este efeito não foi revertido pela oxima K027 ($72,27 \pm 5,43\%$) em relação ao controle 100%. Os resultados mostram que a oxima K027 foi capaz de proteger os animais contra o efeito tipo-depressivo induzido pelo malation, mas não da inibição da AChE hipocampal.

Conclusões: Este efeito da oxima K027 sobre o comportamento tipo depressivo induzido pelo malation parece não ser mediado pela reativação da enzima AChE hipocampal, o que sugere que outro mecanismo esteja envolvido.

Referências: 1- Toxicol. Sci. 97:140, 2007.

Palavras-Chave: acetilcolinesterase, depressão, malation, oxima K027, teste do nado forçado.

Apoio Financeiro: FAPESC-SC, CAPES, CNPq, UFSC, FINEP (IBN-Net - 01.06.0842-00).

Cognitive deficits induced by maternal deprivation in an animal model of mania

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Objectives: Extensive literature has indicated that environmental rearing conditions are critical for the establishment of neurobiological factors controlling cognition and behavior. The aim of the present study was to evaluate the effects of early maternal deprivation (a model of neonatal stress) in adult rats treated with D-amphetamine (D-AMPH) (a model of mania) on cognition. **Methods and Results:** Male litters from pregnant Wistar rats were exposed to one of the following rearing conditions from postnatal days 1-14: **(1)** handled (H) animals were exposed to a daily 15min period in which the dam was removed and the litter was weighed or **(2)** maternal deprived (MD) animals were exposed to the same conditions for a 180min period of separation. When they reached 3 months old, both groups received daily injections of saline or D-AMPH (2mg/Kg ip) for 7 days. We assessed recognition memory 24h [Experiment I] and 8 days [Experiment II] after the last injection. A recognition index, expressed by the ratio $T_N/(T_F+T_N)$ (T_F =time spent exploring the familiar object; T_N =time spent exploring the novel object), was calculated for each animal. In Experiment I, H rats that received D-AMPH showed normal short-term memory (STM) retention, while long-term memory (LTM) retention was impaired when compared to control group (median [interquartile ranges] = 0.73 [0.73/ 0.74] and 0.56 [0.49/0.58]; n=7-8). MD animals that received saline showed normal STM, but they also showed LTM impairments (0.42 [0.29/0.68]; n=7). Interestingly, MD rats that received D-AMPH showed impairments in both STM and LTM retention tests (0.57 [0.56/0.62]) and (0.49 [0.41/0.57]); n=7. In Experiment II, results indicated that the detrimental effects of D-AMPH on recognition memory persisted even 1 week after the last injection in both H and MD groups (0.49 [0.41/0.57]; n=8 and 0.50 [0.30/0.56]; n=7). **Conclusion:** Our findings suggest that individuals exposed to stressful environmental rearing conditions may be more susceptible to the detrimental effects of challenging events latter in life and this may have implications for the understanding of the neurobiological basis of some psychiatric disorders. **Palavras-Chave:** maternal deprivation, stress, D-amphetamine, recognition memory, rats

Apoio Financeiro: CNPq

Diphenyl diselenide exerts anxiolytic-like effect in Wistar rats: putative roles of GABA_A and 5HT receptors.

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Diphenyl diselenide [(PhSe)₂] is an organoselenium that presents pharmacological properties. Here we investigate the anxiolytic effect of (PhSe)₂ in rats, employing the elevated plus maze task. The involvement of 5HT and GABA receptors in the anxiolytic-like effect was also evaluated. (PhSe)₂ (5, 25 and 50 µmol/ kg, i.p.) did not affect locomotor activity as evaluated in the open field test, and learning and memory when assessed in the inhibitory foot-shock avoidance task. However, (PhSe)₂ at the 50 µmol/ kg dose produced an anxiolytic action with a decreased number of fecal boli in the open field test (F (3, 40) = 5.3, *P* < 0.01) and an increased time spent in (F (4, 69) = 5.7, *P* < 0.001) as well as an increased number of entries (F (4, 70) = 4.9, *P* < 0.001) to the open arms of the elevated plus maze test. All antagonists tested were injected 30 min before the administration of a single dose of 50 µmol/ kg (PhSe)₂ and evaluated by the two-way ANOVA. Pre-treatment with bicuculline (0.75 mg/ kg, i.p.) revealed a main effect of the pre-treatment x treatment interactions (F (1, 27) = 7.5, *P* < 0.01) to the time spent and a main effect of the pre-treatment x treatment interactions (F (1, 26) = 9.7, *P* < 0.01) to the number of entries in the open arms. The pre-treatment of animals with ketanserin (1 mg/ kg i.p.) revealed a main effect of the pre-treatment x treatment interactions (F (1, 35) = 4.55, *P* < 0.05) for the time spent and a main effect of the pre-treatment x treatment interactions (F (1, 33) = 4.7, *P* < 0.05) in the number of entries in the open arms. The pre-treatment with WAY100635 (0.1 mg/ kg s.c.) revealed a main effect of the pre-treatment x treatment interactions (F (1, 32) = 7.5, *P* < 0.01) on time spent in the open arms but not a main effect of pre-treatment x treatment interactions (F (1, 33) = 2.3, *P* = 0.135) in the number of entries to the open arms. Our results show that all antagonists used were able to abolish the anxiolytic-like effect of (PhSe)₂ suggesting that GABA_A, 5HT_{2A} and 5HT_{1A} receptors may be involved in the pharmacological properties of this selenocompound in the central nervous system. **Palavras-Chave:** Elevated plus maze; anxiety; diphenyl diselenide; GABA_A receptor; 5HT receptors

Apoio Financeiro: Capes, CNPq and PRONEX

Avaliação psicométrica de reações de imobilidade tônica, dissociação e pânico em pacientes com transtorno de estresse pós-traumático durante evocação de sintomas

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A imobilidade tônica é uma resposta defensiva muito estudada em diversas espécies animais, desencadeada em contexto de ameaça inescapável e caracterizada por inibição motora temporária e analgesia (1). Recentemente encontrou-se que a ocorrência de imobilidade tônica durante o trauma está associada à pior resposta a tratamento farmacológico em pacientes com transtorno de estresse pós-traumático (TEPT) (2).

Objetivo: Avaliar se há correlação entre a imobilidade tônica no contexto experimental de evocação de sintomas por roteiro dirigido e a gravidade e/ou o prognóstico do TEPT. Avaliar se outras respostas (dissociativas e de pânico) se correlacionam com a gravidade e/ou prognóstico do TEPT.

Método: 21 Pacientes com TEPT (SCID-I/DSM-IV) tratados naturalisticamente em ambulatório do IPUB/UFRJ submeteram-se à avaliação mensal de sintomas de TEPT (PCL-C) e de gravidade (CGI). Reações de imobilidade tônica, de dissociação, e pânico presentes durante o trauma foram avaliadas retrospectivamente através das escalas TIS, PDEQ e PRS. As mesmas reações foram avaliadas após a escuta de roteiro dirigido elaborado com base no relato do trauma feito pelo paciente.

Resultados: As respostas de imobilidade tônica correlacionaram-se com os sintomas de TEPT (TIS vs PCL $r=0,52$ $p<.05$) do mês do experimento e com o delta CGI ($r=0,44$ $p<.05$). As reações físicas de pânico correlacionaram-se tanto com os sintomas de TEPT (PRS vs PCL, $r=0,70$ $p<.05$) no mês do experimento quanto com a gravidade (PRS vs CGI, $r=0,69$ $p<.05$) do quadro clínico no mês do experimento e com os valores de delta PCL ($r=0,47$ $p<.05$) e delta CGI ($r=0,76$ $p<.05$). Os sintomas dissociativos correlacionaram-se somente com os sintomas de TEPT (PDEQ vs PCL, $r=0,67$ $p<.05$) do mês do experimento.

Conclusão: Os achados de associação significativa entre imobilidade tônica e pânico peritraumáticos e má resposta ao tratamento de TEPT bem como maior gravidade dos sintomas de TEPT no mês do experimento indicam que a reprodução desses fenômenos peritraumáticos através da escuta de roteiro dirigido podem ter valor prognóstico para este transtorno.

Referencias: (1) Psychol Bull 836–853,1974. (2) J Affect Disord 193-197, 2008.

Palavras-Chave: TEPT, Imobilidade tônica, dissociação, pânico

Apoio Financeiro: Instituto Milênio - CNPq

Efeito de estresse crônico sobre o comportamento de ratos machos e fêmeas no labirinto em cruz elevado

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Objetivos: O labirinto em cruz elevado é um modelo animal que tem sido amplamente utilizado na investigação farmacológica e na elucidação dos mecanismos envolvidos na ansiedade. No entanto, em tal modelo ainda não houve replicação das diferenças de gênero observadas em pacientes com transtornos de ansiedade. No presente estudo, foram investigados os efeitos da exposição crônica à natação forçada sobre o comportamento de machos e fêmeas testados no modelo.

Método: Ratos machos (n = 18) e fêmeas (n = 18) foram igualmente distribuídos em dois grupos: Nado Crônico (NC), submetido ao procedimento de nado forçado (15 min) diariamente por 14 dias; Controle-Manuseio (CM), submetido ao mesmo procedimento que o grupo anterior, porém, com o tanque sem água; e, Controle (CO), permaneceu no biotério durante o período. No 15º dia os animais foram testados no labirinto por 5 min.

Resultados: De acordo com uma ANOVA de duas vias, os fatores sexo e grupo tiveram efeitos sobre a exploração dos braços abertos: tempo gasto (sexo: $F[1,30] = 4,63$; $p < 0,05$ e nado: $F[2,30] = 12,38$; $p < 0,01$) e porcentagem de entradas (sexo: $F[1,30] = 4,45$; $p < 0,05$ e nado: $F[2,30] = 7,41$; $p < 0,01$). O teste post hoc Fisher LSD mostrou que os grupos NC de ambos os sexos apresentaram valores menores de tempo nos braços abertos (machos - CM: $127,6 \pm 7,4$; NC: $66,1 \pm 7,8$ e fêmeas - CM: $112,3 \pm 16,3$; NC: $52,4 \pm 11,8$) e de porcentagem de entradas nos mesmos (machos - CM: $54,0 \pm 1,5$; NC: $36,1 \pm 2,3$ e fêmeas - CM: $43,7 \pm 3,7$; NC: $31,7 \pm 6,9$). A ANOVA mostrou ainda uma interação entre os fatores no número de entradas nos braços fechados ($F[2,30] = 3,68$; $p < 0,05$), indicando que o efeito das variáveis manuseio e natação crônica pode depender do gênero. Porém o teste post hoc não detectou diferenças entre os grupos nesta medida.

Conclusões: A exposição crônica à natação forçada levou a uma equivalente redução na exploração dos braços abertos exibida por machos e fêmeas. Curiosamente, o número de entradas nos braços fechados apresentou uma interação que sugere que o efeito do estressor sobre a atividade locomotora dependeu do gênero. Tal dependência precisa ser mais extensamente investigada em novos estudos.

Palavras-Chave: diferenças de gênero, labirinto em cruz elevado, estresse crônico, natação forçada

CAN MINDFULNESS PRACTICE IMPROVE CARDIORESPIRATORY VARIABLES IN PEOPLE WITH EXCESSIVE WORRY?

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Introduction: Mindfulness has been described as a process to bring certain quality of attention to moment-by-moment experience. The origin of Mindfulness is found in Budist meditation. It has been applied to treatment of chronic pain, stress, anxiety, and depression. On the other hand, worry has been associated with reduced heart rate variability, higher heart rate, and higher number of health complaints. **Objectives:** We present a study aimed at examining the effect of training in Mindfulness on reduction of chronic worry and its associated psychophysiological indices. **Methods:** A group of 31 women with high scores in a trait worry questionnaire (PSWQ) participated in the study. Participants were split into two groups: one group received progressive relaxation plus the instruction to delay worry and the other group received training in mindfulness skills. Pre and post treatment measures included subjective indices of health complaints, worry, anxiety, depression, and emotional intelligence. In addition, participants completed a daily worry registration form and a psychophysiological test consisting of a baseline period followed by a cardiac defense trial, several startle trials, a period of Mindfulness or Relaxation (depending on the group), and a final period of worry. Physiological measures included electromiography of orbicularis oculi, heart rate, skin conductance and respiration. **Results and Conclusions:** Both intervention programs were successful in reducing worry as indexed by both subjective and psychophysiological measures. On top of that, the Mindfulness group showed more adaptive cardiac and respiratory indices as well as higher indices of emotional understanding.

Palavras-Chave: Emotion, Mindfulness, Worry, Psychophysiology measures

Expressão da subunidade γ_2 do receptor GABA_A no estriado de ratos machos e fêmeas nas diferentes fases do ciclo estral

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Objetivos: A depressão é um transtorno afetivo multifatorial que apresenta diferenças quanto ao sexo e à resposta aos hormônios gonadais. Um mecanismo proposto para estas diferenças é a ação dos neuroesteróides derivados da progesterona sobre a expressão das subunidades do receptor GABA_A. O objetivo deste trabalho foi verificar a influência do sexo e das diferentes fases do ciclo estral na expressão da subunidade γ_2 do receptor GABA_A no estriado de ratos Wistar.

Métodos: Foram utilizados ratos Wistar adultos, machos (M) e fêmeas nas diferentes fases do ciclo estral: Proestro (P), Estro (E), Diestro I (DI) e Diestro II (DII) (n=3-5/grupo). O ciclo estral das fêmeas foi acompanhado mediante esfregaço vaginal diário e foram selecionados os animais que realizaram dois ciclos estrais completos e regulares, confirmados por análise citológica vaginal mediante microscopia óptica. Os estriados foram dissecados e congelados para posterior extração do RNA e análise da expressão do mRNA da subunidade γ_2 do receptor GABA_A por RT-PCR. A extração do RNA total foi realizada pelo método do trizol, seguida de síntese de cDNA. A PCR foi feita utilizando-se oligonucleotídeos específicos para a subunidade γ_2 do receptor GABA_A e β -actina (normalizador). A análise densitométrica das bandas foi medida por um sistema de processamento de imagem e os resultados foram apresentados como média $\pm$ desvio padrão da relação subunidade γ_2 / β -actina.

Resultados: As médias dos grupos foram: M= 0,49 $\pm$ 0,170; P= 0,40 $\pm$ 0,168; E= 0,55 $\pm$ 0,040; DI= 0,73 $\pm$ 0,173 e DII= 0,64 $\pm$ 0,179. Não houve diferença significativa entre os grupos (ANOVA $F_{(4,16)} = 2.785$ $P = 0.06$)

Conclusão: A expressão do mRNA da subunidade gama 2 do receptor GABA_A não é diferente no estriado de ratos machos e fêmeas nas diversas fases do ciclo. No entanto, estes resultados são preliminares, sendo necessário aumentar o número de animais por grupo para análise mais conclusiva.

Palavras-Chave: depressão, GABAA, gênero

Apoio Financeiro: CNPq

COMPARISON OF BEHAVIOR IN FOUR NON DEPRESSIVE RAT STRAINS TO FORCED SWIMMING TEST.

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Introduction: It is well known that rats forced to swim in a narrow space from which there is no escape will, after an initial period of vigorous activity, cease to move altogether making only those movements necessary to keep its head above water. It was hypothesized that immobility reflected the animals' have learned that escape was impossible and they have given up hope. Immobility was subsequently found to be reduced by a wide range of clinically active antidepressant drugs, making it a good test to study depressive behavior in rats. Objective: To compare the behavior to the forced swimming test in four non depressive rat strains. Methods: 2 inbred strains (Fisher 344 N=7 and Lewis N=7) and 2 outbred strains (Sprague-Dawley N=9 and Wistar N=5) were placed in a container (60 cm in height × 30 cm in diameter) filled with water to a height of 50 cm, for 15 minutes. Water temperature was approximately 20°C. The parameters observed were immobility and swimming and climbing. One-way ANOVA followed, by post-hoc Tukey test was used to identify possible differences among strains. Results: We have found that the Wistar rats spent more time swimming than all the other strains (and less time in immobility) and the other strains did not differ among them. When comparing climbing, Wistar have climbed more than Sprague-Dawley and Fisher, thus, Lewis have climbed more than Sprague-Dawley. Conclusion: Our results show that the Wistar rats have the less depressive behavior in the forced swimming test when compared with the outbred strain Sprague-Dawley, and with the inbred strains Fisher 344 and Lewis.

Palavras-Chave: FORCED SWIMMING, RATS, DEPRESSION.

Apoio Financeiro: AFIP, CNPq and FAPESP.

Efeitos da progesterona na expressão da subunidade γ_2 do receptor GABA_A no estriado de ratos Wistar submetidos ao teste do nado forçado

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Objetivos: Os receptores GABA_A medeiam a maior parte da neurotransmissão inibitória no SNC e alterações em sua função estão envolvidas na fisiopatologia de transtornos afetivos, como a depressão maior. A expressão gênica das subunidades do receptor GABA_A é afetada por esteróides neuroativos como a progesterona e seu metabólito ativo alopregnanolona, que atuam como moduladores positivos dos receptores GABA_A. O objetivo foi investigar os efeitos da progesterona na expressão da subunidade γ_2 do receptor GABA_A no estriado de ratos submetidos ao teste do nado forçado, um modelo animal para comportamento tipo-depressivo.

Métodos: Foram utilizados ratos Wistar adultos, machos (n=3/grupo). No primeiro dia os animais foram treinados por 15 minutos em aquários contendo água a 25 ± 1° C e 27 cm de profundidade. Imediatamente após o nado, os animais receberam injeção de progesterona (P), 0,4 mg/kg ou controle (C), repetida a cada 24 horas por 8 a 10 dias. No dia da última injeção, os animais foram submetidos ao teste, por 5 minutos. Trinta minutos após o teste, os animais foram eutanasiados por decapitação e os estriados foram dissecados e congelados para posterior extração do RNA e análise da expressão do RNA da subunidade γ_2 do receptor GABA_A por RT-PCR. A extração do RNA total foi realizada pelo método do trizol, seguida de síntese de cDNA. A PCR foi feita utilizando-se oligonucleotídeos específicos para a subunidade γ_2 do receptor GABA_A e β -actina (normalizador). A análise densitométrica das bandas foi medida por um sistema de processamento de imagem e os resultados foram apresentados como média ± desvio padrão da relação subunidade γ_2/β -actina.

Resultados: Embora a relação γ_2/β -actina no estriado dos animais tratados esteja 3,5 vezes maior em relação aos controles (P = 0,678 ± 0,357; C = 0,222 ± 0,053), não houve diferença significativa entre os grupos (ANOVA F_(2,6) = 4,854 P = 0,05).

Conclusão: Apesar destes resultados serem preliminares, sendo necessário aumentar o número de animais por grupo, pode-se concluir que o tratamento com progesterona não altera significativamente a expressão do mRNA da subunidade γ_2 do receptor GABA_A no estriado de ratos submetidos ao teste do nado forçado. **Palavras-Chave:** Receptor GABA-A, progesterona, depressão, nado forçado

Apoio Financeiro: CNPq

Depressão e suicidalidade em gestantes, Pelotas, R. S.

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Objetivos: Os objetivos deste trabalho são identificar a proporção de potencial suicida e de depressão em gestantes, verificando fatores associados a esse potencial. **Método:** Este estudo tem como população-alvo gestantes atendidas no Hospital Universitário São Francisco de Paula/UCPel, no Campus da Saúde/UCPel, no Hospital Escola UFPel /FAU, no Centro de Especialidades, em Pelotas (Rio Grande do Sul) no período de agosto de 2005 a dezembro de 2006. Estas gestantes eram usuárias do sistema público de saúde nos locais referidos, residentes na zona urbana da cidade com capacidade de compreender e responder o questionário aplicado. Este questionário era composto por perguntas sobre dados socioeconômicos, informações sobre a saúde da mulher e escala HAD (*Hospital Anxiety and Depression Scale*). Potencial suicida foi avaliado segundo a questão 10 da escala EPDS (*Edinburgh Post-natal Depression Scale*). **Resultados:** Em uma amostra composta por 868 gestantes na faixa etária de 12 a 45 anos, 26,0% das gestantes manifestaram um quadro depressivo e 15,8% apresentaram presença de potencial suicida, sendo assim distribuído: 7,1% pensaram em fazer algo contra si pouquíssimas vezes; 5,2% algumas vezes; e, 3,5% muitas vezes. Na análise multivariada, a presença de potencial suicida associou-se com baixa escolaridade ($p=0,017$), classificação socioeconômica “D” ou “E” ($p=0,041$), o fato de ter pensado em aborto ($p=0,000$) e depressão ($p=0,016$). **Conclusões:** A alta prevalência de depressão e de potencial suicida em gestantes deve servir de alerta aos profissionais de saúde. A implantação de um serviço de diagnóstico e tratamento de depressão durante avaliação pré-natal pode ser uma alternativa para diminuição destes índices na gestação e pós-parto, evitando-se prejuízos no vínculo entre a mãe e o bebê bem como altos índices de insegurança vincular e atrasos no desenvolvimento cognitivo e emocional da prole. **Palavras-Chave:** gestação, depressão, potencial suicida

Apoio Financeiro: PROSUP/CAPES

O modelo de Bulbectomia olfatória induz estresse oxidativo no córtex cerebral de camundongos.

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Objetivos: A ablação bilateral dos bulbos olfatórios resulta em alterações comportamentais compatíveis ao que é observado em pacientes com depressão. Desta forma, a bulbectomia olfatória (B.O.) em roedores tem sido proposta como um modelo animal de depressão¹. Dados na literatura remontam da correlação existente entre a depressão e o estresse oxidativo². Neste contexto, o presente estudo investigou o efeito da bulbectomia olfatória bilateral em parâmetros comportamentais e de estresse oxidativo em camundongos. **Métodos:** Camundongos Swiss de ambos os sexos (40-50g) foram divididos em 2 grupos experimentais: SHAM (controle) e B.O. (bulbectomizados, com a ablação dos bulbos olfatórios). Decorridos 14 dias pós-operatório os animais foram avaliados quanto a atividade locomotora no teste do campo aberto (TCA), posteriormente, o córtex cerebral foi removido para a determinação da lipoperoxidação (TBARS), níveis de GSH, atividade das enzimas antioxidantes glutathione peroxidase (GPx) e glutathione redutase (GR) e atividade dos complexos I e II da cadeia respiratória. **Resultados:** Os resultados indicaram que a B.O. aumentou significativamente a atividade locomotora ($239,4 \pm 15,5$ %; $p < 0,01$), comprovando o efeito tipo depressivo característico deste modelo. Em relação aos parâmetros de estresse oxidativo, a B.O. aumentou significativamente a lipoperoxidação no córtex cerebral ($163,0 \pm 6,6$ %, $p < 0,01$), todavia os níveis de GSH não foram modificados. A atividade da GPx ($71,23 \pm 4,5$ %; $p < 0,05$) e GR ($71,85 \pm 4,34$ %; $p < 0,05$) diminuíram significativamente com a B.O., % em relação aos grupos controle SHAM=100%. Todavia, a B.O. não interferiu na função mitocondrial no córtex cerebral dos animais bulbectomizados. **Conclusão:** Os dados do presente trabalho demonstram o envolvimento do estresse oxidativo na depressão induzida pela Bulbectomia olfatória, sendo que este efeito pode ser relacionado com a patogênese deste transtorno de humor.

Palavras-Chave: depressão, bulbectomia olfatória, estresse oxidativo.

Apoio Financeiro: FAPESC-SC, CAPES, CNPq, UFSC, IBN-Net (01.06.0842-00).

Parallel memory processing by the CA1 region of the dorsal hippocampus and the basolateral amygdala

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There is abundant literature on the role of the basolateral amygdala (BLA) and the CA1 region of the hippocampus in memory formation of inhibitory avoidance (IA) and other behaviorally arousing tasks. Here we investigate molecular correlates of IA consolidation in the two structures, and their relation to N-methyl-D-aspartate glutamatergic receptors (NMDAr) and β noradrenergic receptors β -ADr. The separate posttraining administration of antagonists of NMDAr and β -ADr to BLA and CA1 is amnesic. IA training is followed by an increase of the phosphorylation of calcium and calmodulin-dependent protein kinase II (CaMKII) and extracellular signal regulated protein kinase 2 (ERK2) in CA1 but only of that of ERK2 in BLA. The changes are blocked by NMDAr antagonists but not β -ADr antagonists in CA1, and by β -ADr but not NMDAr antagonists in BLA. In addition, the changes are accompanied by increased phosphorylation of tyrosine hydroxylase (TH) in BLA but not in CA1, suggesting that β noradrenergic modulation results from local catecholamine synthesis in the former but not in the latter structure. NMDAr blockers in CA1 do not alter the learning-induced neurochemical changes in BLA and β -ADr blockade in BLA do not hinder those in CA1. When put together with other data from the literature, the present findings suggest that CA1 and BLA play a role in consolidation, but they operate to an extent in parallel, suggesting that each is probably involved with different aspects of the task studied.

Palavras-Chave: Memory; Basolateral amygdala; CA1 and NMDA

Apoio Financeiro: CNPq, CAPES

Paralisia do medo: imobilidade tônica no transtorno do estresse pós-traumático

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Objetivos: A imobilidade tônica constitui uma reação extrema à percepção de ameaça inescapável, quando outros recursos como fugir e lutar são ineficazes. Estudos têm descrito a presença de imobilidade tônica durante eventos intensamente traumáticos em humanos. Neste trabalho procuramos substratos somato-motores que pudessem estar associados ao relato de ocorrência de paralisia e congelamento peritraumáticos em pacientes com transtorno do estresse pós-traumático (TEPT).

Métodos: A amplitude (área) das oscilações posturais durante a escuta do relato do evento traumático foi mensurada em 23 vítimas de violência urbana (18 pacientes com TEPT e 15 controles) através de uma plataforma de força que registrava o deslocamento do centro de pressão. A presença de imobilidade tônica foi estimada através de questionário, cujo escore médio foi utilizado para classificar os pacientes em um sub-grupo “com” e outro “sem” imobilidade tônica. O relato subjetivo da intensidade do medo foi coletado. O experimento foi aprovado pelo Comitê de Ética e todos participantes assinaram o termo de consentimento.

Resultados: Encontramos um efeito principal para medo (ANOVA: $F(2,30) = 17,77$; $p < 0.001$). Pacientes com imobilidade tônica apresentaram os escores de medo significativamente mais altos em relação aos pacientes sem imobilidade tônica ($p \leq 0,006$) e aos controles ($p < 0.001$). Encontramos efeito principal para a área de oscilação (ANOVA: $F(2,30) = 7,24$; $p = 0.003$). Pacientes sem imobilidade tônica apresentaram área de oscilação significativamente maior que controles ($p = 0,002$), enquanto que pacientes com imobilidade tônica não diferiram dos controles ($p = 0,39$).

Conclusões: Mostramos que os relatos de imobilidade tônica estavam associados ao medo intenso. A área de oscilação destes pacientes apresentou valores próximos ao dos controles sugerindo que este achado possa refletir o aumento, da rigidez muscular e inibição motora que caracterizam a imobilidade tônica.

Referências: 1- Beh Res & Therapy 43:1157,2005.; 2- Psychophysiology 42:255,2005; 3- Neuroscience Letters 410:52,2006. **Palavras-Chave:** imobilidade tônica; estabilometria; TEPT

Apoio Financeiro: MCT/CNPq, FAPERJ, FINEP

Efeitos das condições de disponibilidade e abstinência de sacarose sobre a atividade da acetilcolinesterase central e desempenho de ratos em tarefas de navegação espacial

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Tem-se estudado o mecanismo molecular subjacente à relação entre transtornos alimentares e psiquiátricos. Ratos tratados com solução de sacarose em esquema intermitente apresentam, durante abstinência, sinais de dependência e sensibilização cruzada com drogas de abuso, além do aumento de acetilcolina no núcleo acumbente. Os objetivos do presente trabalho foram avaliar os efeitos da privação de sacarose sobre: i) aspectos da aprendizagem e memória espacial no Labirinto Aquático de Morris (LAM) e ii) atividade da acetilcolinesterase (AChE) no neocórtex e hipocampo. Foram utilizados 15 ratos Wistar, tendo livre acesso à ração, divididos em três grupos quanto à disponibilidade de sacarose 10% p/v: 1. Sacarose Livre (SL): acesso livre à solução de sacarose; 2. Sacarose Intermitente (SI): 12h de acesso/ 12h de privação; 3. Controle (C): sem acesso à sacarose. Para controle do estado nutricional dos animais dos grupos SL e SI, foram incluídos mais dois grupos (n=5 cada) pareados quanto ao consumo de ração. Todos os animais tiveram acesso livre à água. O tratamento durou 21 dias e após 36 horas de abstinência os animais foram treinados e testados no LAM. O ensaio da atividade da AChE foi realizado por espectrofotometria. A ANOVA com medidas repetidas mostrou que a latência de escape sofreu efeito significativo ($p<0,01$) do decorrer das sessões, indicando aprendizado da tarefa por todos os ratos. Houve também efeito ($p<0,01$) da disponibilidade da sacarose. Teste *post hoc* mostrou que a latência dos ratos do grupo SI foi menor ($p<0,02$) que a dos ratos dos grupos SL e C, que não diferiram entre si. Os dados comportamentais obtidos com os grupos controles pareados mostraram que a diferença não se deve ao estado nutricional dos animais. Não houve diferença significativa entre os diferentes grupos quanto à memória espacial e atividade da AChE neocortical e hipocampal. A diferença no desempenho entre os animais dos grupos SI e SL pode ser atribuída ao limite à disponibilidade de sacarose imposto aos animais do grupo SI, o qual pode resultar em um estado de desamparo. Essa hipótese é corroborada por dados da literatura sobre efeitos da limitação de outros tipos de reforços.

Palavras-Chave: Sacarose, Acetilcolinesterase, Aprendizado e Memória Espacial, Labirinto Aquático de Morris, Rato

Apoio Financeiro: CNPq e FAPEMIG

Decreased cognition in apolipoprotein E epsilon4 allele carriers with major depressive disorder: preliminary results

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Introduction: The epsilon4 allele of apolipoprotein E (ApoE4) is a well-established risk factor for late onset Alzheimer's disease (AD). This knowledge has generated interest in the role of ApoE variants in normal cognition. Varying degrees of cognitive dysfunction have been described in non-demented individuals with one or two epsilon4 alleles leading to suggestions that the gene could play a role in normal cognition or helps calibrate the aging process. Thus we have performed a study in order to investigate the role of the ApoE4 allele in cognition of patients with major depressive disorder according to the DSM-IV criteria. **Methods:** Thirteen elderly patients older than 60 years, with a diagnosis of major depressive disorder according to DSM-IV criteria were included in the study and blood samples were collected of all these subjects for the genetic investigation. The patients were divided into two groups according to the presence of the epsilon4 allele of the ApoE4 (ApoE3/ApoE3 or ApoE3/ApoE4). In order to evaluate cognition impairment the patients have received a detailed neuropsychological evaluation, which included the Cambridge Assessment of Mental Disorders in the Elderly (CAMCOG), Mini-Mental State Examination (MMSE), Fuld Object Memory Evaluation (FOME) and IQ Test. **Results:** There was an association between the presence of ApoE4 allele and cognitive decline measured by CAMCOG ($p=0.026$) (ApoE3/ApoE3: Mean=90.2 and SD=11.5; ApoE3/ApoE4: Mean=68.9 and SD=18.4) and FOME ($p=0.020$). There was a trend of association between cognitive decline obtained by MMSE and the investigated allele ($p=0.055$). Regarding the IQ test results, there was no association between cognitive decline and ApoE4 allele. **Discussion:** In older patients with major depressive disorder, the presence of an APOE epsilon4 allele was associated with greater cognitive impairment. These preliminary results may suggest the role of such allele not only in cognitive decline related to Alzheimer disease.

Palavras-Chave: apolipoprotein E, cognitive impairment, elderly depression

EFEITOS COMPORTAMENTAIS E GABAÉRGICOS DA COCAÍNA EM RATOS HIPERATIVOS.

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Introdução: Evidências clínicas têm demonstrado a relação entre o Transtorno do Déficit de Atenção e Hiperatividade (TDAH) e uso/abuso de cocaína. Em ratos, a administração intracisterna de 6-hidróxidopamina (6-OHDA) constitui-se o modelo animal de TDAH. Estudos sugerem que o TDAH esteja associado a uma disfunção dopaminérgica no córtex pré-frontal (CPF) e em estruturas subcorticais, como hipocampo e estriato. Essa hipótese hipodopaminérgica poderia modular a transmissão GABAérgica e de alterar a sua síntese, pelas enzimas GAD₆₅ e GAD₆₇. **Métodos e Resultados:** Utilizou-se 40 ratos machos distribuídos em 4 grupos ($n=10$ animais/grupo). Dividiram-se os grupos pelo comportamento (6-OHDA, *naive* e *sham*) e auto-administração oral (solução de cocaína ou água). Os grupos 6-OHDA (cocaína ou água) sofreram lesão dopaminérgica aos 3 dias de vida, enquanto que o grupo *sham*-água sofreu apenas intervenção com veículo e o grupo *naive*-água foi intercedido. Em 14 semanas, foi iniciado o protocolo de auto-administração oral de cocaína a 0,1mg/ml, pela Técnica de Escolha de Dois Bebedouros, por 23 dias. Nesse ínterim, verificou-se a atividade locomotora dos animais em Campo Aberto. Decapitou-se os animais e retirou-se o córtex pré-frontal, hipocampo e estriato para análise de mRNA, por RT-PCR (Invitrogen®). Na amplificação, foram utilizados primers para GAD₆₅ e GAD₆₇. Os amplicons foram analisados em gel de agarose e os resultados foram expressos em razão de densitometria entre os genes em estudo e a b-actina. As análises estatísticas da atividade locomotora e de mRNA foram realizadas por ANOVA de duas vias, e o teste de Snk foi utilizado para comparações *post-hoc*. Os dados foram demonstrados em médias $\pm$ erro padrão, e valores de $p<0,05$ foram considerados significativos. Os ratos 6-OHDA consumiram solução de cocaína na dose de $5,9 \pm 0,40$ mg/kg/dia. Ainda, foi verificado que a cocaína diminuiu a expressão de mRNA de GAD₆₅ e GAD₆₇ no hipocampo e estriato dos ratos 6-OHDA em comparação com os grupos-controle ($p<0,001$; $SNK<0,05$). No CPF, a cocaína diminuiu a expressão de mRNA de GAD₆₅ e aumentou a expressão de GAD₆₇ dos ratos 6-OHDA em comparação com os grupos-controle ($p<0,001$; $SNK<0,05$). A lesão por 6-OHDA resultou em progressivo aumento da locomoção total no campo aberto. **Conclusões:** Os resultados sugerem que a cocaína altera o sistema GABAérgico em ratos em modelo de TDAH, regulando a expressão de mRNA de GAD₆₅ e GAD₆₇ no CPF, hipocampo e estriato, que podem estar relacionadas com as anormalidades comportamentais induzidas pela cocaína.

Palavras-Chave: Cocaína, GABA, TDAH

Apoio Financeiro: CNPq

Cellular Prion Protein (PrP^c) modulates rewarding properties, rapid tolerance and ethanol consumption in mice.

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Objective: Ethanol addiction has enormous individual and social importance and the responses related to this phenomenon mainly involve plasticity processes at the mesocorticolimbic system. In the light of the direct modulation of prion protein on synaptic plasticity, here we investigated the role of this non-infectious prion isoform in ethanol addiction related tasks in mice.

Methods: Wild-type (*Prnp*^{+/+}) and PrP^c knockout (*Prnp*^{0/0}) female mice were tested in the following behavioral paradigms (n=6-10 per group): Conditioned Place Preference (CPP), Rapid tolerance Task (RTT) and the Two-bottle choice oral self-administration. Moreover, after the CPP and RTT the blood samples were collected by tail puncture and the blood ethanol concentration was evaluated enzymatically.

Results: Overall, the lowest ethanol dose tested (0.5 g/kg, i.p.) produced CPP only in the *Prnp*^{0/0} mice [pre vs. postconditioning (s): 38.33 ± 17.25 vs. 122.00 ± 61.09], while the highest ethanol dose (2 g/kg) induced rewarding effects in the *Prnp*^{+/+} mice [26.00±17.28 vs. 86.20±22.44]. Twenty-four hours after CPP each mouse received i.p injection of saline or ethanol (0.5 or 2 g/kg) and 30 min later differences were also noted in the blood ethanol assay [0.5 g/kg: concentration (mg/dL); *Prnp*^{+/+}: 0.0±0.0; *Prnp*^{0/0}: 27.98±6.67] and [2 g/kg: *Prnp*^{+/+}: 118.90±10.39; *Prnp*^{0/0}: 226.75±14.11]. The absence of PrP^c was able to disrupt the acquisition of rapid tolerance to ethanol incoordination evaluated in the rota-rod [latency to fall (s) Day 2: *Prnp*^{+/+}: 7.50±1.25; *Prnp*^{0/0}: 2.50±0.34]. Blood ethanol assay performed after RTT indicated no significant inter-strain differences. In the two-bottle choice, *Prnp*^{0/0} mice showed decreased ethanol consumption in 10 and 20% solutions [Ethanol 10% g/kg/day; *Prnp*^{+/+} 12.27±3.72; *Prnp*^{0/0} 5.96±0.76] and [Ethanol 20% g/kg/day; *Prnp*^{+/+} 15.94±1.38; *Prnp*^{0/0} 11.85±1.07].

Conclusion: This is the first study indicating the participation of PrP^c in rewarding properties, acquisition of rapid tolerance and ethanol consumption in mice. Interestingly PrP^c could also be involved in ethanol metabolism. These findings suggest PrP^c as a genetic predictor target for ethanol addiction. **Palavras-Chave:** Cellular Prion Protein, Ethanol Addiction.

Apoio Financeiro: CAPES, CNPq e FAPESC

C.08.003

Estudo das atividades epileptiformes sustentadas por conexões não sinápticas no hipocampo de ratos submetidos à doses variadas de álcool.

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Objetivo: Sabe-se que o álcool promove aumento da excitabilidade das células nervosas, principalmente por distúrbios do fluxo de íons através da membrana celular e acredita-se que o mesmo pode modificar as características eletrofisiológicas e morfológicas dos neurônios, principalmente nas regiões hipocâmpais, o que pode levar à morte celular e ao *status epilepticus*. Sendo assim os objetivos desse trabalho é estudar o efeito do alcoolismo crônico em ratos visando: a) analisar a eletrofisiologia de atividades epileptiformes na camada granular do giro denteado. b) caracterizar a morfologia da camada granular do ápice do giro denteado através do processo histoquímico.

Metodologia: Ratos Wistar machos de 6 semanas foram separados em grupos A1, A2 e controle, sendo submetidos, respectivamente, à doses de 1,50g/kg e 3,00g/kg de solução de etanol à 20% v/v e volumes equivalentes às doses anteriores em solução salina. Após 4 semanas de tratamento, os ratos foram sacrificados e os hipocâmpos isolados para os procedimentos de eletrofisiologia e histologia.

Resultados: As análises dos registros eletrofisiológicos obtidos demonstram que o álcool altera as conexões não sinápticas, sendo essas alterações específicas em cada uma das doses aplicadas. Houve aumento da excitabilidade nos animais dos grupos A1 e A2, sendo essa mais significativa em A1. A característica mais evidenciada nos animais do grupo A2 em relação aos outros grupos foi a resistência ao início das atividades epileptiformes quando sustentadas por conexões não sinápticas, porém ao se iniciarem tendem a ser mais frequentes que nos demais grupos. A análise quantitativa histológica mostrou diminuição da densidade celular no grupo A1 de $0,0016 \pm 0,0001$ (redução de 20%) e no grupo A2 de $0,0011 \pm 0,0001$ (redução de 45%) quando comparados com o grupo controle de $0,0020 \pm 0,0001$. O *p-value* encontrado na comparação desses grupos é igual a 0,00075955.

Conclusões: Os resultados obtidos demonstraram que o álcool quando ministrado em doses variadas promove alterações específicas nas atividades epileptiformes sustentadas por conexões não sinápticas associadas à morte e à excitabilidade neuronal, dose dependente.

Palavras-Chave: Álcool, atividades não-sinápticas, eletrofisiologia, giro denteado, epilepsia.

Apoio Financeiro: FAPEMIG/CNPq

C.08.004

Damage to the tuberomammillary nucleus disrupts drug-seeking behavior.

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Addiction profoundly alters motivational circuits so that drugs become powerful reinforcers of behavior. Histamine neurons of the tuberomammillary nucleus are essential for the food and water searching behavior. We tested the idea that the histamine neurons are critical for drug-seeking behavior in addicted rats. Male rats group was conditioned using the place preference paradigm, where one of two chambers was repeatedly paired with the administration of amphetamine (1.5 mg/Kg i.p.). Conditioned rats showed a significant preference for amphetamine-paired room and increase in the number of Fos-ir in the tuberomammillary nucleus, compared to the non-conditioned rats or saline controls. The lesion of the histamine neurons of the tuberomammillary nucleus using saporin conjugated to the hypocretin 2, suppressed the preference for amphetamine-paired room, while producing no effects on general ambulation. Our results indicate a role for the histamine neurons of the tuberomammillary nucleus in drug-seeking behavior.

Palavras-Chave: tuberomammillary nucleus, histamine, drug-seeking behavior, addiction

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Cocaine modulates A2a and D2 receptors interactions in embryonic mouse brain.

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Dopamine and adenosine receptors appear in the developing brain. However, the neurochemical interactions between these receptors in the embryonic brain are not fully understood. We examined the activity and interactions between D2 and A2a receptors in the embryonic mouse brain and the effects of cocaine on these interactions. CD1 mice were exposed to cocaine (40mg/Kg/day; s.c.) or saline from embryonic day 8 (E8) to E15. The striatum (STR) and cerebral cortex (CTX) were dissected on E15 and incubated with 0.5mM Rolipram 0.5mM; 10min. Then, the samples were exposed to dopamine (DA); forskolin (10μM); SKF 81297 (10μM; D1 receptor agonist); SCH23390; (10μM; D1 receptor antagonist); TNPA (10μM, D2 receptor) CGS 21680; (1μM; A2a receptor agonist) or SCH58261 (1μM; A2a receptor antagonist) for 30 minutes. The reaction was stopped with TCA (10%) and cAMP accumulation was assayed. Immunoblot, immunohistochemistry assays and ³H-DA uptake were done to investigate pre and pos-synaptic dopaminergic components. We found that, SKF81297 induced cAMP accumulation, was more pronounced in STR than in CTX (105±5 versus 89±4 pmol cAMP/mg protein). The basal levels of cAMP, in saline group, were comparable in both regions (22±3 pmol/mg protein) but in cocaine group, the basal levels increased to 80±6 pmol/mg protein. The cAMP levels induced by SKF81297, in cocaine group, were reduced to 52±5pmol/mg in CTX and 23 ±2 pmol/mg in STR as well as, D1 receptors expression and DA uptake. In an opposite way, D2 receptor expression and AC5 expression was increased by cocaine exposure in CTX and STR. TNPA was able to block the cAMP increases induced by forskolin, in cocaine group, but not in saline group. CGS 21680 induced cAMP accumulation (STR 110±5 pmol/mg and CTX 92±8pmol/mg) and this effect was completely blocked by TNPA and by SCH58261. These results indicate that, D2 receptors is up-regulated by cocaine exposure and may modulate purinergic responses in embryonic brain.

Palavras-Chave: cocaine, striatum, cerebral cortex, A2a receptors ,

Apoio Financeiro: CNPq, NIH grant NS43246

Efeitos da administração intra-estriatal dos agonistas D1, SKF 38393 e D2, quimpirola sobre a locomoção e auto-limpeza incondicionada e condicionada.

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Investigou-se os efeitos da administração intra-estriatal de SKF 38393 (SKF) e do quimpirola (QUI) sobre o condicionamento induzido por drogas. Ratos Wistar foram divididos em dois experimentos. No primeiro, administrou-se QUI (QUI-0,1, 3,0, 10,0 mg/ml) em duas situações: associada (QUI-0,1-A; n=6; QUI-3-A; n=7; QUI-10-A; n=6) e não-associada (QUI-0,1-NA; n=6; QUI-3-NA; n=7; QUI-10-NA; n=6) ao ambiente experimental durante cinco dias na fase de condicionamento (FC), sendo o número de cruzamentos e o tempo de auto-limpeza registrados por 15 minutos. O grupo controle recebeu veículo (QUI-VEI; n=11). Seguindo um período de retirada de droga de 2 dias, administrou-se salina (teste de condicionamento; TC). No segundo experimento, os ratos foram divididos, obedecendo ao mesmo protocolo, em grupos que receberam SKF (SKF-3-A; n=6; SKF-10-A; n=6; SKF-3-NA; n=6; SKF-10-NA; n=6). O grupo controle recebeu veículo (SKF-VEI; n=8). No experimento 1, no TF, o grupo QUI-10-A ($117,36 \pm 59,41$) apresentou nº de cruzamentos maior que os demais. O QUI-3-A ($95,97 \pm 12,85$) apresentou nº cruzamentos maior do que todos os demais grupos exceto do QUI-10-A. O QUI-0,1-A ($9,57 \pm 6,71$) apresentou menor nº cruzamentos e maior auto-limpeza ($61,33 \pm 52,35$). No TC, os QUI-10-A ($107,06 \pm 1,63$) e QUI-3-A ($95,57 \pm 2,01$) apresentaram nº de cruzamentos maior que os demais. Não houve diferença em relação à auto-limpeza no TC. No experimento 2, no TF, o SKF-10-A ($103,23 \pm 48,07$) apresentou auto-limpeza maior. O SKF-3-A ($70,96 \pm 12,75$) apresentou auto-limpeza maior do que os demais grupos exceto do SKF-10-A. No TC, os SKF-10-A ($60,33 \pm 12,61$) e SKF-3-A ($47,60 \pm 3,65$) apresentaram uma auto-limpeza maior do que os demais. Não houve diferença em relação ao nº cruzamentos na FC e TC (ANOVA seguida de Duncan, $p < 0,05$). Os receptores D2 estão relacionados à locomoção incondicionada e condicionada enquanto os receptores D1 com a auto-limpeza incondicionada e condicionada.

Palavras-Chave: Estriado dorsal, locomoção, auto-limpeza, condicionamento induzido por drogas

Apoio Financeiro: TECNORTE

Role of gabaergic and opioid mechanisms of the central nucleus of amygdala in the modulation of the morphine withdrawal-potentiated startle

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Objectives: Anxiety is one of the main affective symptoms of opiate withdrawal. In a paradigm of acute morphine dependence, opioid antagonist-precipitated withdrawal increases the acoustic startle response, which has been considered an index of elevated anxiety. Since the amygdala is a key structure in the production of this class of emotional response, this study aimed to examine the influence of gabaergic and opioid mechanisms of the central nucleus (CeA) of this structure on the modulation of the morphine withdrawal-potentiated startle.

Methods: The amplitude of startle to a loud noise was accessed in rats before and after they were submitted to an acute morphine dependence procedure. For this, rats implanted with a guide-cannula in the CeA received a morphine injection (10 mg/kg; s.c.) followed, 2h later, by a challenge injection of the opioid antagonist naltrexone (0,1 mg/kg; s.c.). The effects of microinjections of muscimol, a GABA_A receptor agonist, or DAMGO, a μ -opioid receptor agonist, into the CeA on the aversive effects of morphine withdrawal were also studied.

Results: The results showed that the morphine withdrawal significantly increased the startle amplitude (control: 313,1 $\pm$ 52,2; morphine: 522,5 $\pm$ 67,3). Microinjections of muscimol (control: 311 $\pm$ 50,3; morphine: 362,9 $\pm$ 63,2) and DAMGO (control: 289,2 $\pm$ 71,9; morphine: 371,5 $\pm$ 38,8) into the CeA counteracted the aversive effects of morphine withdrawal.

Conclusions: These results suggest that mechanisms mediated by GABA_A and μ -receptors in the CeA exert an inhibitory control on the anxiety induced by withdrawal from acute morphine, as accessed by the morphine withdrawal-potentiated startle model used here. **Palavras-Chave:** Morphine withdrawal, amygdala, GABA, opioids

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The unconditioned fear produced by morphine withdrawal is differentially regulated by μ - and κ -opioid receptors in the midbrain tectum

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Objectives: The dorsal periaqueductal gray (dPAG) and the inferior colliculus (IC) are involved in the organization of fear and anxiety states. Since opiate withdrawal is associated with increased anxiety levels, this study aimed to investigate whether these midbrain tectum (MT) structures are sensitized during the morphine withdrawal and the influence of opioid mechanisms on this process.

Methods: Rats submitted to a chronic treatment with morphine (10 days; 10 mg/kg; s.c.) were implanted with an electrode glued to a guide-cannula into the dPAG or IC. Forty-eight hours after the last injection, they were submitted to the electrical stimulation of these structures to determine the freezing (FR) and escape (ESC) thresholds in two conditions – before and after local microinjections of DAMGO, a μ -receptor agonist, or nor-binaltorphimine (BNI), a κ -receptor antagonist (only IC group).

Results: The results showed that morphine withdrawal significantly reduced the aversive thresholds determined by the electrical stimulation of the dPAG (control: FR: $60 \pm 8,2$; ESC: $86,2 \pm 13,1$; morphine: FR: $30,4 \pm 3,6$; ESC: $50,4 \pm 5,1$) and the IC (control: FR: $35,9 \pm 3,9$; ESC: $91,8 \pm 9,6$; morphine: FR: $18,8 \pm 1,5$; ESC: $63,1 \pm 6,1$). Microinjections of DAMGO into the dPAG promoted antiaversive effects in the control group (FR: $87,8 \pm 9,3$; ESC: $112,1 \pm 12,1$) without changing the aversive effects of morphine withdrawal (FR: $30 \pm 4,2$; ESC: $57,1 \pm 4,8$). Microinjections of DAMGO (control: FR: $62,5 \pm 13,9$; ESC: $167,5 \pm 7,9$; morphine: FR: $33,8 \pm 4,7$; ESC: $115,6 \pm 24,6$) or BNI (control: FR: $50 \pm 6,9$; ESC: $153,6 \pm 18,6$; morphine: FR: $37,5 \pm 3,6$; ESC: $96,2 \pm 11,1$) into the IC increased the aversive thresholds in both groups.

Conclusions: These results suggest that morphine withdrawal leads to fear states engendered by the activation of the dPAG and IC. The activation or the blockade of μ - and κ -receptors, respectively, inhibit the sensitization produced by morphine withdrawal on the neural substrates of fear in the IC, but not in the dPAG. **Palavras-Chave:** Morphine withdrawal, dPAG, inferior colliculus, opioid receptors

Apoio Financeiro: FAPESP (04/02859-0)

ANTAGONISTAS NMDA (MEMANTINA E MK-801) NO CÓRTEX PRÉ-FRONTAL MEDIAL REDUZEM OS PREJUÍZOS DA MEMÓRIA OPERACIONAL ESPACIAL PRODUZIDO PELO ETANOL EM RATOS

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Objetivos: Este estudo investigou os efeitos de bloqueadores de canal aberto, memantina e MK-801, administrados no corte pré-frontal medial (CPFm) sobre os prejuízos da memória operacional produzidos pelo etanol (EtOH). **Métodos:** Ratos Wistar machos (n = 24, 250-300 g), treinados no labirinto radial de 8 braços e com cânulas bilaterais implantadas no CPFm (B: 2,5 mm A, +/-1 mm L, 2,7 mm V), receberam administrações intracorticais (IC) de MK-801 (0,32, 1,0 e 3,2) ou salina (SAL) e memantina (1,0, 3,2 e 10,0 µg) ou salina (SAL) 10 minutos antes da administração IC de SAL ou EtOH 100 µg, e submetidos ao teste com 1 h de retardo no labirinto 5 minutos depois. No desempenho após o retardo de 1 h foram considerados “erro tipo 1”, a repetição dos braços visitados no pré-retardo, e, “erro tipo 2”, a repetição de braços visitados no pós-retardo. **Resultados:** Em ambas as curvas dose-efeito, o EtOH 100 µg IC produziu significativamente ($p < 0,01$) maior número de erros tipo 1 ($1,4 \pm 0,4$ e $1,3 \pm 0,3$) comparado aos controles ($0,3 \pm 0,1$ e $0,2 \pm 0,1$). A administração prévia de MK-801 0,32 µg reduziu os erros tipo 1 ($0,5 \pm 0,3$) produzidos pelo EtOH 100 µg, o que não ocorreu com as doses maiores. A memantina 3,2 µg por si só produziu maior número de erros tipo 1 ($1,5 \pm 0,3$) ($p < 0,01$) comparado ao controle ($0,2 \pm 0,1$). Entretanto, quando administrada antes do EtOH 100 µg, notadamente na maior dose (10,0 µg), a memantina reduziu significativamente ($p < 0,05$) o número de erros tipo 1 ($0,3 \pm 0,2$) comparado à SAL e EtOH (100 µg) ($1,3 \pm 0,3$). Adicionalmente, foi observado que em todas as análises o EtOH produziu significativamente ($p < 0,01$) maior número de erros tipo 1 ($1,4 \pm 0,4$ e $1,3 \pm 0,3$) comparados ao tipo 2 ($0,1 \pm 0,1$ e $0,0 \pm 0,0$). **Conclusões:** Antagonistas NMDA administrados no CPFm reduzem os prejuízos da MO de duração prolongada (retardos de 1 h) produzida pelo EtOH IC, corroborando a participação do sistema glutamatérgico, na ação do EtOH no CPFm. A prevalência do “erro tipo 1” sugere que o EtOH poderia interferir na retenção e/ou evocação da informação, componentes essenciais da memória operacional, e este prejuízo foi revertido pelos antagonistas NMDA.

Palavras-Chave: etanol, MK-801, memantina, memória operacional espacial, córtex pré-frontal medial, ratos

AValiação da função frontal em condutores de veículos nas noites de Vitória/ES

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Objetivos: O consumo do álcool tem sido associado a vários tipos de violência, destacando-se os acidentes de trânsito. Este estudo investigou os efeitos da ingestão de álcool sobre as funções executivas empregando um teste de rastreio (Bateria de Avaliação Frontal – FAB) em condutores de veículos na noite de Vitória/ES. **Métodos:** 592 condutores selecionados aleatoriamente, entre 22:00 e 04:00 h, em locais de tráfego intenso, foram convidados a responder um questionário com dados sobre idade, escolaridade, renda familiar, participação em acidentes de trânsito e consumo alcoólico. Em seguida, caso aceitassem, foram submetidos à leitura da alcoolemia por um bafômetro ativo e posteriormente à aplicação do FAB. **Resultados:** 490 (82,8%) condutores aceitaram participar do estudo, sendo 401 (82%) do sexo masculino e 88 (18%) do sexo feminino, 342 (69,8%) tinham idade entre 18 e 40 anos, 326 (66,7%) com grau superior completo ou incompleto. 108 (24,4%) apresentaram registro de consumo alcoólico, sendo que em 62 (14%) foi acima de 0,06 g/l, limite permitido pelo Código Nacional de Trânsito. 389 (79,4%) destes indivíduos se submeteram ao teste de avaliação frontal (FAB), sendo a mediana da FAB de 293 indivíduos com alcoolemia zero de 15 (13-16), de 40 com alcoolemia acima de zero e abaixo de 0,06 g/l de 14 (11-15,5), e de 56 com alcoolemia acima de 0,06 g/l de 13 (11-15). A pontuação da FAB em condutores com alcoolemia acima de 0,06 g/l foi significativamente menor ($p < 0,05$) comparado aos que não apresentavam registro de álcool. Análises mais pormenorizadas destes dados demonstraram que esta redução da FAB sob o efeito do álcool foi significativa na população masculina jovem (20-30 anos de idade). **Conclusões:** O consumo de álcool em condutores na cidade de Vitória/ES, durante as noites, foi mais prevalente em jovens do sexo masculino, nos quais o desempenho da FAB apresentou-se significativamente reduzido. Estes dados refletem alterações de funções cognitivas e do comportamento motor, como flexibilidade mental, tomada de decisões, programação motora e controle inibitório, funções essenciais para a condução adequada de um veículo automotivo **Palavras-Chave:** álcool, direção automotiva, funções frontais, funções executivas, FAB

Apoio Financeiro: CAPES, FAPES

ALTERAÇÃO DAS SUBUNIDADES GluR1 E NR1 DOS RECEPTORES GLUTAMATÉRGICOS NO NÚCLEO ACUMBENS E CÓRTEX PRE-FRONTAL APÓS ADMINISTRAÇÃO REPETIDA DE COCAÍNA EM RATOS ADOLESCENTES

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Objetivos: Demonstramos previamente que a administração de cocaína em ratos adolescentes causa sensibilização comportamental que persiste até a idade adulta (30 dias após o tratamento). Estudos em ratos adultos, mostram que a administração repetida de cocaína pode alterar de forma duradoura a expressão de receptores glutamatérgicos em áreas dopaminérgicas associadas aos efeitos comportamentais dessa substância, como Núcleo Acumbens (NAc) e o Córtex Préfrontal Medial (CPFm). O objetivo do presente trabalho foi avaliar se a sensibilização comportamental à cocaína em ratos adolescentes está relacionada a alterações da quantidade das subunidades GluR1 e NR1 dos receptores glutamatérgicos, AMPA e NMDA, respectivamente, no NAc e CPFm.

Métodos: Ratos Wistar machos adolescentes receberam injeções intraperitoneais de salina (SAL) (1 mL/kg) ou cocaína (COC) (10 mg/kg), 2 vezes ao dia entre os dias pós-natais (DPN) 30-34. Três, 30 ou 60 dias após a última injeção os animais foram sacrificados para dissecação do NAc e CPFm. As proteínas GluR1 e NR1 foram quantificadas por Western Blotting e a análise estatística consistiu do teste *t*-Student.

Resultados: Os dados representam a média $\pm$ EPM (N=7-8 por grupo). Os ratos tratados com cocaína apresentaram aumento significativo ($p < 0,05$) da quantidade da proteína GluR1 no CPFm 3 dias após o fim tratamento (SAL: $100,0 \pm 3,8$; COC: $124,5 \pm 8,0$). Essa alteração não foi significativa para o NAc (SAL: $100,0 \pm 5,6$; COC: $110,9 \pm 7,8$). Trinta ou 60 dias após o tratamento (idade adulta) não foi observada alteração significativa da proteína GluR1, como também não houve alteração da proteína NR1 em nenhum dos períodos avaliados.

Conclusão: A administração repetida de cocaína em ratos adolescentes pode ter seus efeitos relacionados ao aumento transitório da quantidade da subunidade GluR1 dos receptores AMPA no CPFm. No entanto, essa alteração não permanece até a idade adulta e parece dissociada da expressão da sensibilização comportamental observada nessa idade.

Palavras-Chave: cocaína, receptores glutamatérgicos, adolescentes, sensibilização comportamental

Apoio Financeiro: FAPESP, CAPES

O enriquecimento ambiental na adolescência diminui respostas emocionais e o consumo de etanol em ratos espontaneamente hipertensos adultos

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Objetivos: A adolescência é um período crítico para o desenvolvimento biológico e também para o início de vários distúrbios neuropsiquiátricos, como o transtorno de déficit de atenção e hiperatividade (TDAH) e a dependência às drogas. Os ratos espontaneamente hipertensos (SHR) têm sido utilizados como modelo para o estudo do TDAH. No presente estudo, investigamos se a exposição ao ambiente enriquecido (AE) durante a adolescência seria capaz de alterar as respostas emocionais e o consumo de álcool na idade adulta em ratos SHR.

Métodos: Ratos SHR foram mantidos em AE ou em ambiente padrão (AP) desde o período de desmame (24º dia pós-natal) até atingirem a idade adulta, quando foram testadas. O AE era composto por duas gaiolas interconectadas por um tubo, com uma roda de correr, um túnel, brinquedos e escadas. As respostas emocionais e locomotoras foram avaliadas no teste do campo aberto (CA) durante 5 min. Um dia após o CA, os animais foram separados em gaiolas individuais e submetidos ao protocolo de auto-administração utilizando duas garrafas (livre escolha sacarina (7,5mM) - água; consumo forçado de etanol 10% (v/v) e livre escolha etanol 10%(v/v) - água).

Resultados: Os animais mantidos no AE apresentaram uma diminuição significativa na atividade locomotora horizontal (AE: 80,2±8,0 vs AP: 146,8±9,4), vertical (AE: 28,2±5,0 vs AP: 55,1±2,6) e na emocionalidade (% Locomoção central: AE: 35,5±0,9 vs AP: 28,5±1,5; Tempo no centro(s): AE: 109,2±13,7 vs AP: 67,4±3,9) quando comparados aos animais mantidos no AP. Além disso, o AE diminuiu significativamente a preferência à sacarina (AE: 83,6±0,1 vs AP: 86,7±0,5), o consumo forçado de etanol (AE: 14,1±1,6 vs AP: 23,2±3,0 g/kg/dia) e a preferência ao etanol (AE: 16,0±2,5 vs AP: 6,4±1,0) quando comparados aos animais do AP.

Conclusões: Esses resultados demonstram, de maneira inédita, que o enriquecimento ambiental durante o período crítico do desenvolvimento diminui as respostas locomotoras, emocionais e reforçadoras do etanol em ratos SHR. linhagem utilizada como modelo do TDAH.

Palavras-Chave: ambiente enriquecido, ratos SHR, etanol

Apoio Financeiro: CNPq, CAPES

Diferenças individuais espontâneas e a sua relação com o processo de sensibilização comportamental induzido por apomorfina em ratos.

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O objetivo deste trabalho foi verificar a manutenção das diferenças individuais na emissão do comportamento orofacial e locomoção em ratos após o desenvolvimento da sensibilização produzida por apomorfina (APO). Ratos machos Wistar foram colocados em uma arena-teste por 60 min (teste à novidade - TN) e separados em respondedores altos (RA) e baixos (RB), de acordo com a média do tempo de emissão do comportamento orofacial. Sete dias após o TN, metade dos animais de cada grupo recebeu APO (2,0 mg/kg) e a outra metade recebeu veículo [RB-VEIC (n=8), RB-APO (n=8), RA-VEIC (n=8) e RA-APO (n=8)]. Os tratamentos foram administrados por 14 dias (tratamento farmacológico – TF) nas caixas-viveiro, sendo que nos dias 1 (teste agudo com apomorfina), 7 e 14, os animais foram colocados na arena-teste imediatamente após receberem seus tratamentos e os comportamentos orofacial e locomoção foram registrados. Após 5 dias de retirada de droga, houve o teste de sensibilização (TS), onde os grupos APO receberam APO (2,0 mg/kg), a metade dos grupos veículo recebeu APO [RB-VEIC-APO (n=4) e RA-VEIC-APO (n=4)] e a outra metade recebeu veículo [RB-VEIC-VEIC (n=4) e RA-VEIC-VEIC (n=4)]. Os resultados mostraram que no TN, o grupo RA apresentou maior nível de comportamento orofacial e locomoção ($87,1 \pm 46,7$; $13,2 \pm 11,4$) que o grupo RB ($54,9 \pm 53,8$; $9,6 \pm 11,6$). No dia 1 do TF, a locomoção do grupo RA-APO ($18,0 \pm 20,2$) foi maior que o RB-APO ($8,1 \pm 8,7$) e esse aumento foi progressivo, sugerindo o desenvolvimento da sensibilização. O grupo RB-APO também apresentou sensibilização, mas esta só foi iniciada a partir do dia 7 ($34,8 \pm 34,0$) e foi menor que a do grupo RA-APO ($54,4 \pm 46,5$). No dia 14 e no TS não houve diferenças entre os grupos RA-APO ($63,3 \pm 50,0$ e $58,3 \pm 44,0$) e RB-APO ($61,7 \pm 54,2$ e $54,7 \pm 46,6$). Também não houve diferenças entre os grupos RB-APO e RA-APO quanto ao comportamento orofacial. Os resultados sugerem que o desenvolvimento da sensibilização diluiu as diferenças individuais obtidas no teste à novidade e no teste com apomorfina.

Palavras-Chave: Diferenças individuais, apomorfina, sensibilização comportamental

Apoio Financeiro: PIBIC/UENF

COMPARAÇÃO ENTRE RATOS ADOLESCENTES E ADULTOS DA NEUROTRANSMISSÃO DOPAMINÉRGICA EM RESPOSTA À NICOTINA

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Objetivo: A nicotina é uma substância psicoativa que causa farmacodependência e pode alterar a neurotransmissão dopaminérgica no núcleo acumbens (NAc). Ratos adolescentes apresentam menor sensibilidade comportamental à estimulação psicomotora induzida pela nicotina. Assim, comparamos ratos adolescentes e adultos quanto à ação da nicotina na neurotransmissão dopaminérgica pela medida do neurotransmissor e seus metabólitos em homogenato tecidual do NAc.

Métodos: Foram utilizados ratos Wistar machos adolescentes e adultos, dia pós-natal 30 e 70, respectivamente. Os animais receberam injeção subcutânea de nicotina (0,4 mg/kg) ou salina (1 mL/kg) e após trinta minutos foram decapitados e seus encéfalos removidos para dissecação do NAc. As concentrações de dopamina (DA) e seus metabólitos [ácido diidroxifenil acético (DOPAC) e ácido homovanílico (HVA)] foram quantificadas no homogenato do NAc por cromatografia líquida de alta performance. Os resultados são expressos com ng da substância por mg de tecido neural e foram analisados por ANOVA seguida do teste Newman-Keuls (N = 6 por grupo).

Resultados: A administração de nicotina aumentou significativamente ($p < 0,05$) a concentração tecidual de HVA em animais adolescentes (salina: $0,334 \pm 0,036$; nicotina: $0,459 \pm 0,024$) e adultos (salina: $0,333 \pm 0,022$; nicotina: $0,414 \pm 0,024$). A razão HVA/DA também aumentou tanto nos ratos adolescentes (salina: $0,078 \pm 0,006$; nicotina: $0,104 \pm 0,006$) quanto nos adultos (salina: $0,079 \pm 0,006$; nicotina: $0,098 \pm 0,005$) após a injeção de nicotina. Nenhuma alteração significativa da concentração de DA ou DOPAC foi encontrada.

Conclusões: Ratos adolescentes e adultos apresentaram ativação semelhante na neurotransmissão dopaminérgica no NAc em resposta à nicotina.

Palavras-Chave: nicotina, dopamina, DOPAC, HVA, adolescentes

Apoio Financeiro: FAPESP, CAPES e PADCF/UNESP

MORPHINE ATTENUATES THE EXPRESSION OF SENSITIZATION TO ETHANOL, BUT OPIOID ANTAGONISTS DO NOT.

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Background: Behavioral sensitization to ethanol is characterized by increased locomotion after repeated exposure to the drug. A great variability exists among species and strains in the development of sensitization. The opioid system is involved in alcoholism, and therefore it is possible that this system also modulates the ethanol sensitization. **Objective:** We evaluated the role of the opioid system in determining the variability of the sensitized response to ethanol. **Methods and Results:** Mice received repeated administrations of ethanol (2.2 g/kg) or saline. According to their locomotor response on the last day of treatment, ethanol-treated animals were classified into two groups: sensitized or non-sensitized mice. After the treatment, in experiments 1 and 2, mice were challenged, respectively, with ip opioid antagonists (naloxone or naltrexone) or an ip opioid agonist (morphine) followed by ethanol (ip). In experiment 3, animals received morphine (ICV), followed by ethanol (ip). Opioid antagonists did not block the expression of ethanol sensitization. However, morphine attenuated the increased locomotor activity after ethanol administration in sensitized mice (sensitized group of mice in morphine/ethanol challenge: 1285 ± 217 (mean $\pm$ SEM) Exp2, 986 ± 344 in Exp3; and in saline/ethanol challenge: 2278 ± 245 in Exp2, 2919 ± 294 in Exp3). For experiment 4, after chronic treatment, mice brains were processed and brain mu opioid binding was assessed by [³H]DAMGO ligand autoradiography. No differences were detected between any of the groups of mice (Example: *core* NAcc – binding means $\pm$ S.E.M. in μ Ci/g tissue – saline: 0.57 ± 0.05 ; non-sensitized: 0.62 ± 0.06 ; sensitized: 0.63 ± 0.03). **Conclusions:** Although opioid antagonists did not alter the ethanol sensitization, previous stimulation of the opioidergic system by morphine prevented its expression. Considering that the mu opioid receptor binding was similar among the groups, the agonist effect is not likely to be mediated by mu receptors alterations.

Palavras-Chave: ethanol, opioidergic system, locomotor activity, mice

Apoio Financeiro: FAPESP, AFIP, CNPq

Efeitos da nicotina no teste da natação forçada: envolvimento da transmissão serotoninérgica e do eixo hipófise-hipotálamo-adrenal.

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Estudos mostram grande prevalência de fumantes entre pacientes com depressão. Sendo o teste da natação forçada (TNF) amplamente empregado no "screening" de fármacos antidepressivos, o trabalho teve por objetivo avaliar as alterações neuroendócrinas subjacentes ao efeito do tratamento com nicotina no TNF. Ratos Wistar machos (200-250g) foram submetidos ao TNF (30 cm de água, 24 ± 1°C) por 15 min e tratados (1, 19 e 23h) com nicotina (NIC: 0,5 mg/kg, sc), imipramina (IMI: 15 mg/kg, ip, antidepressivo referência) ou salina (SAL). Uma hora após a última injeção, o animais foram reexpostos (5 min) à mesma cuba e os comportamentos de imobilidade, natação ou escalada analisados. Imediatamente após o teste, o animais foram sacrificados e o hipocampo e sangue coletados. A 5-HT e o 5-HIAA foram quantificados por HPLC e a corticosterona, por radioimunoensaio. Os dados (média ± e.p.m.) foram analisados por ANOVA bifatorial (1-tratamento, 2-TNF) seguido do teste-F de comparações planejadas quando $p \leq 0,05$. A NIC e IMI reduziram em 39% e 50%, respectivamente, o tempo de imobilidade em relação ao grupo salina (SAL: 22,4±2,6; NIC: 13,6± 2,9; IMI: 11,1± 2,2 contagens/5min). Ambas as drogas aumentaram significativamente em 52% e 66%, respectivamente, o tempo de escalada (SAL: 25,30±2,7; NIC: 38,5± 4,4; IMI: 41,9± 3,3 contagens/5min), sem afetar o tempo de natação. O tratamento per se com IMI ou NIC não promoveu alterações na razão 5-HIAA/5-HT (turnover) da serotonina hipocampal. Entretanto, o aumento do "turnover" da serotonina induzido pela exposição ao TNF (2,44±0,21) foi bloqueado pelo tratamento com NIC (1,77±0,13) ou IMI (1,73±0,19). A NIC per se não promoveu alterações significativas do nível basal da corticosterona mas reduziu em 51,4% o aumento de 337% (34,71 ± 3,53 m g/100 mL) da corticosterona basal (7,95 ± 2,08 m g/100 mL) após exposição ao TNF. A IMI promoveu aumento de 206% da corticosterona basal, não reduzindo significativamente a hipercorticosterolemia induzida pelo TNF. Os resultados sugerem que o mecanismo subjacente ao efeito antidepressivo da NIC detectado pelo TNF parece envolver a regulação do eixo HPA via modulação da 5-HT hipocampal.

Palavras-Chave: nicotina, natação forçada, serotonina, corticosterona

Apoio Financeiro: FAPESP

A EXPOSIÇÃO REPETIDA À NICOTINA ALTERA A TAXA DE RECICLAGEM (TURNOVER) DE DOPAMINA NO NÚCLEO ACUMBENS E CÓRTEX PRÉ-FRONTAL EM RESPOSTA À ADMINISTRAÇÃO AGUDA DE ANFETAMINA, EM RATOS ADOLESCENTES.

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Introdução: Anfetamina e nicotina são substâncias de abuso que têm em comum a propriedade de aumentar a transmissão dopaminérgica e a atividade locomotora. Resultados anteriores do nosso laboratório mostram que ratos adolescentes tratados com nicotina durante 7 dias apresentaram aumento da atividade locomotora em resposta à administração aguda de anfetamina. O presente trabalho investigou se a exposição repetida à nicotina altera os efeitos agudos da anfetamina nas vias dopaminérgicas.

Materiais e Métodos: Ratos Wistar adolescentes (DPN=28) receberam injeções subcutâneas (sc) de nicotina (NIC) (0,4 mg/Kg) ou salina (SAL) (1 mL/Kg) durante 7 dias. No décimo dia os animais receberam uma injeção intra-peritoneal (ip) de anfetamina (ANF) (5,0 mg/Kg) ou SAL (1 mL/Kg). Trinta minutos após as injeções os animais foram decapitados, os encéfalos dissecados e as áreas de interesse, núcleo acumbens (NAc) e córtex pré-frontal (CPF), retiradas pela técnica de "minipunch". A dopamina e seus metabólitos, ácido diidroxifenil acético (DOPAC) e ácido homovanílico (HVA), foram quantificados por cromatografia líquida de alta performance. A análise estatística foi feita através de ANOVA unifatorial.

Resultados: Os dados representam a média $\pm$ EPM (n=5 animais por grupo). O pré-tratamento com NIC alterou significativamente ($p < 0,05$) o "*turnover*" no NAc, expresso como relação DOPAC/Dopamina (SAL-SAL: $0,78 \pm 0,06$; SAL-ANF: $0,41 \pm 0,02$; NIC-SAL: $0,57 \pm 0,04$; NIC-ANF: $0,27 \pm 0,05$) e HVA/Dopamina (SAL-SAL: $0,14 \pm 0,02$; SAL-ANF: $0,06 \pm 0,004$; NIC-SAL: $0,12 \pm 0,005$; NIC-ANF: $0,06 \pm 0,005$). No CPF, o tratamento crônico também alterou a relação DOPAC/Dopamina (SAL-SAL: $0,65 \pm 0,05$; SAL-ANF: $0,36 \pm 0,07$; NIC-SAL: $0,57 \pm 0,04$; NIC-ANF: $0,21 \pm 0,01$), mas não a relação HVA/Dopamina (SAL-SAL: $0,32 \pm 0,03$; SAL-ANF: $0,28 \pm 0,1$; NIC-SAL: $0,35 \pm 0,05$; NIC-ANF: $0,16 \pm 0,03$).

Conclusão: Os resultados indicam que o tratamento crônico com nicotina diminuiu o efeito agudo da anfetamina no "*turnover*" de dopamina em regiões encefálicas envolvidas nos efeitos reforçadores das substâncias psicoativas de abuso.

Palavras-Chave: Anfetamina, Nicotina, Dopamina

Apoio Financeiro: Capes

ESTUDOS MORFOLÓGICOS PARA AVALIAÇÃO DOS DANOS CEREBRAIS CAUSADOS PELO USO DE NICOTINA E ETANOL DURANTE A ADOLESCÊNCIA DE CAMUNDONGOS

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Objetivo: O objetivo desse trabalho é avaliar morfologicamente os danos cerebrais causados pela exposição à nicotina e/ou etanol durante a adolescência de camundongos C57BL/6.

Metodologia: De PN30 (30º dia pós-natal) até PN45, 84 animais foram expostos à nicotina *free base* (NIC) e/ou etanol (ETOH) em 4 grupos experimentais: exposição combinada à NIC [50µg/ml em sacarina a 2% para beber] +ETOH [25%, 2g/kg i.p. em dias alternados]; exposição à NIC; exposição ao ETOH; exposição ao veículo (VEH). No último dia de exposição (PN45, n=38) e cinco dias após seu término (PN50, n=46), os cérebros foram processados para quantificação de morte celular por apoptose pelo método do TUNEL (marcação do terminal 3'-OH do DNA pela desoxinucleotidil transferase) em áreas do hipocampo: Camada Granulosa do Giro Denteado (GrDG), Camada Molecular (Mol) e regiões CA1, CA2 e CA3.

Resultados: Em PN45, a exposição ao etanol provocou aumento no número de células em apoptose em todas as áreas do hipocampo quando comparado ao grupo veículo: GrGD (ETOH: M=28,5; EPM=1,0; VEH: M=11,7 EPM=3,1; P<0,0001), Mol (ETOH: M=16,4; EPM=0,5; VEH: M=5,4; EPM=2,0; P<0,0001); CA1 (ETOH: M=15,8; EPM=0,6; VEH: M=6,8; EPM=1,0; P<0,0001), CA2 (ETOH: M=7,1; EPM=0,2; VEH: M=3,2; EPM=0,7; P<0,0001) e CA3 (ETOH: M=28,3; EPM=0,8; VEH: M=11,3; EPM=1,9; P<0,0001). A nicotina provocou uma resposta menos severa e dependente da região: o aumento no número de células em apoptose foi significativo apenas nas camadas Mol (NIC: M=9,3; EPM=0,7; VEH: M=5,4; EPM=2,0; P<0,03) e na CA1 (NIC: M=10,1; EPM=0,6; VEH: M=6,8; EPM=1,0; P<0,002) quando comparado com o grupo veículo. Não houve diferenças significativas entre o grupo NIC+ETOH e o grupo VEH. Em PN50 não houve diferenças no número de células em apoptose entre os grupos experimentais.

Conclusão: Concluimos que, no hipocampo, a exposição ao etanol durante a adolescência provoca dano celular mais intenso que a nicotina. A exposição combinada a NIC+ETOH reverteu os efeitos neurotóxicos do etanol e da nicotina administrados separadamente. Esses efeitos não persistem até cinco dias após o término da exposição.

Palavras-Chave: Adolescência; Nicotina; Etanol; Morfologia

Apoio Financeiro: Philip Morris USA Inc. e Philip Morris International, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), SR-2 UERJ

Relationship between cerebellar cortex expression of CB1-R, CB2-R, GABA and GLAST-1 after chronic agonist cannabinoid exposure in adult male rat.

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There are two well characterized cannabinoid receptors (CB-Rs) that mediate the effects of endocannabinoids and cannabis use. CB1-Rs are mainly presynaptic and are G-protein coupled, while CB2-Rs are mainly postsynaptic and both are present in cerebellar cortex. WIN 55,212-2 is an agonist that activates both types of CB-Rs. The Purkinje cell is the only projection neuron of cerebellar cortex and is a GABAergic neuron with a rich dendritic tree in the molecular layer. In order to study the response of the cerebellar cortex CB1-R and CB2-R to a cannabinoid agonist exposure we used an animal model of rats treated with WIN55,212-2. Ten adult male Wistar rats were divided into 2 groups: one group of 5 animals (treated group = W) were injected twice daily subcutaneously with WIN55212-2, mesylate (3mg/kg) dissolved in DMSO for 14 days. The other 5 rats (control group = C) were injected with an equivalent volume of sterile DMSO. On the fifteenth day, rats were perfused intracardially with 4% paraformaldehyde in 0.1M phosphate buffer. 50 μ m thick sections were obtained using a vibratome. Sections were immunostained using primary antibody to CB1-R, CB2-R, GABA and GLAST-1 (a glial glutamate transporter). In the basket of Purkinje cells, there was decreased CB1-R immunoreactivity (ir), determined by measuring the relative area of ir (C: 2.8 ± 0.11 vs. W: 1.66 ± 0.10 [$P=0.02$]) and for GABA-ir (C: 0.15 ± 0.01 vs. W 0.06 ± 0.007 [$P<0.0001$]). In contrast the treatment with WIN55,212-2 increased the percentage of CB2-R-ir (C: 0.137 ± 0.02 vs. W: 0.38 ± 0.02 [$P<0.0001$]) and GLAST-1-ir (C: 0.10 ± 0.008 vs. W: 0.32 ± 0.01 [$P<0.0001$]) in the molecular layer. The present results showed that chronic treatment with cannabinoid agonist modified the expression CB-Rs, GABA, and GLAST-1 in the cerebellar cortex. We conclude, that chronic treatment with WIN55,212,2 produced downregulation of CB1-R expression and reduced the expression of GABA in Purkinje's basket cells. However, the increase in the expression of CB2-R may be associated with inflammatory response that may be related with an astrocytic reaction modulated by the increase in the GLAT-ir.

Palavras-Chave: drug abuse, cannabinoid agonist

Apoio Financeiro: UBACYT M-072, ANPCYT PICT 31964, CONICET PIP 5034

ESTRESSE CRÔNICO DE IMOBILIZAÇÃO CAUSA SENSIBILIZAÇÃO COMPORTAMENTAL À ANFETAMINA, MAS NÃO À CAFEÍNA

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Introdução: O estresse é um fator importante no abuso e desenvolvimento de dependência a psicostimulantes, como anfetamina. A exposição ao estresse crônico causa sensibilização comportamental cruzada à anfetamina, a qual está relacionada a mecanismos neuroadaptativos envolvidos no desenvolvimento da farmacodependência. A cafeína é um psicostimulante com efeitos comportamentais semelhantes à anfetamina, no entanto poucos estudos têm investigado a sensibilização cruzada entre estresse e cafeína.

Objetivo: Investigar se a exposição ao estresse crônico de imobilização é capaz de induzir sensibilização comportamental cruzada com a anfetamina ou cafeína em ratos.

Métodos: Foram utilizados ratos Wistar machos adultos. Os animais foram expostos a estresse por imobilização diariamente durante uma hora por 10 dias (grupo Estresse) ou não foram perturbados (grupo Controle). Setenta e duas horas após a última sessão de estresse os animais foram colocados em uma caixa de atividade para habituação por 20 minutos. Em seguida, cada animal recebeu injeção intraperitoneal de anfetamina (1 mg/kg), cafeína (10 mg/kg) ou salina (1 ml/kg) e sua atividade locomotora foi registrada durante 40 minutos. Os resultados foram analisados por ANOVA bifatorial seguida do teste Newman-Keuls (N = 6-7 por grupo).

Resultados: Os animais expostos ao estresse apresentam aumento significativo ($p < 0,05$) da atividade locomotora em resposta à anfetamina (Controle: 2063 ± 276 , Estresse: 4353 ± 654); enquanto a locomoção induzida pela cafeína (Controle: 2873 ± 257 , Estresse: 3810 ± 666) ou salina (Controle: 775 ± 197 , Estresse: 880 ± 126) não foi modificada significativamente pela exposição prévia ao estresse.

Conclusões: Nossos resultados indicam a presença de sensibilização cruzada entre estresse e anfetamina, enquanto nenhum efeito foi observado para a cafeína.

Palavras-Chave: Anfetamina, cafeína, estresse

Apoio Financeiro: FAPESP e CNPq.

EFEITOS ISOLADOS E COMBINADOS DA NOVIDADE E DA LUMINOSIDADE SOBRE OS EFEITOS COMPORTAMENTAIS DO ETANOL EM CAMUNDONGOS

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Objetivos: O comportamento de animais seja espontâneo ou induzido por drogas de abuso pode ser modificado pela exposição à novidade e diferentes condições de luminosidade. Nosso objetivo foi investigar os efeitos desses 2 fatores, conduzidos separadamente ou em conjunto, sobre o efeito estimulante motor do etanol e sua sensibilização, modelos animais de euforia e dependência, respectivamente. **Métodos:** 94 camundongos Swiss machos (3 meses) foram distribuídos em 8 grupos com 11-12 animais cada: HAB-LUZ-SAL (A), NOV-LUZ-SAL (B), HAB-PEN-SAL (C), NOV-PEN-SAL (D), HAB-LUZ-ETN (E), NOV-LUZ-ETN (F), HAB-PEN-ETN (G) e NOV-PEN-ETN (H). Os animais foram habituados (HAB) ou não (NOV) ao campo aberto, na presença de luz (200 lx) (HAB-LUZ) ou penumbra (9 lx) (HAB-PEN), por 20 minutos, durante 3 dias consecutivos. Os animais receberam, então, uma injeção de salina (SAL) ou etanol (ETN) e, imediatamente após, sua atividade geral foi quantificada em campo aberto na presença de luz (HAB-LUZ e NOV-LUZ) ou penumbra (HAB-PEN e NOV-PEN), durante 10 min (sessão teste). Após 7 dias (sessão reteste), todos os animais receberam uma injeção desafio de etanol para avaliação da sensibilização comportamental e, imediatamente após, sua atividade geral foi quantificada em campo aberto durante 10 min nas mesmas condições de luminosidade da sessão teste. **Resultados:** A administração aguda de etanol promoveu um aumento na locomoção total (ex: $E=475 \pm 64 > A=209 \pm 28$) e periférica, uma diminuição na habituação intra-sessão e não modificou a locomoção central em todas as condições, mas reverteu o aumento do levantar (ex: $B=77 \pm 6 > F=41 \pm 8$) e a diminuição da imobilidade (ex: $B=13 \pm 5 < F=65 \pm 17$) promovidos pela novidade. A penumbra potencializou o efeito estimulante do etanol sobre a locomoção total (ex: $G=766 \pm 127 > E=475 \pm 64$) e periférica. Por fim, a sensibilização comportamental ao etanol ocorreu apenas para a locomoção central, na presença de novidade e/ou penumbra (ex: $H=96 \pm 10 > D=68 \pm 8$ na sessão reteste). **Conclusões:** Somente a penumbra modificou os efeitos agudos do etanol. Embora tanto a exposição à novidade como à penumbra tenham potencializado a sensibilização ao etanol, esse efeito não foi sinérgico. **Palavras-Chave:** etanol, comportamento, camundongos, novidade, luminosidade

Apoio Financeiro: FAPESP

MICE SENSITIZED TO THE STIMULANT EFFECT OF ETHANOL PRESENT HIGHER LOCOMOTOR RESPONSE TO QUIMPIROLE (D2 AGONIST) ADMINISTRATION INTO THE NUCLEUS ACCUMBENS

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Rationale: Repeated ethanol (EtOH) administration may induce behavioral sensitization, defined as a progressive potentiation of EtOH stimulant effects. This process is associated with different neuroadaptations in the brain reward systems, including the dopaminergic mesolimbic pathway and the nucleus accumbens (NAcc). A previous work of our department showed that sensitized mice are hyperresponsive to a D1 agonist administered directly in the nucleus accumbens. In previous studies we showed that sensitized mice have higher density to D2 receptors than non-sensitized mice or saline controls, but it is not clear whether they are also hyperresponsive to the administration of a D2 agonist.

Objective: The goal of the present study was to evaluate the locomotor response to the intra-NAcc administration of quimpirole (D2 receptor agonist) in mice previously treated with EtOH or saline.

Method: Albino Swiss male mice received 2.2 g/kg EtOH (or saline) on alternate days during ten days (total of 5 i.p. administrations). According to their locomotor response on the last day of treatment, EtOH-pretreated mice were classified into two subgroups ("sensitized" or "non-sensitized"). 48 h after the end of treatment, these animals were challenged with 0.01 mg/side or 0.2 mg/side (0.2 ml) of quimpirole (D2 receptor agonist), on alternate days.

Results: After the intra-NAcc administration of 0.01 mg/side quimpirole all groups presented similar locomotor activity levels (mean + S.E.M: saline = 362.5 + 92.8; non-sensitized = 317.2 + 75.3; sensitized = 471.7 + 121.8). However, the intra-NAcc administration of 0.2 mg/side quimpirole induced higher locomotor stimulation in "sensitized" mice than in saline and non-sensitized mice (saline = 818.1 + 125.6; non-sensitized = 489.5 + 153.3; sensitized = 1290 + 171.9).

Conclusion: The higher response of sensitized mice to the administration of a D2 agonist suggests that the higher levels of D2 density previously observed has a functional meaning.

Palavras-Chave: sensitization, ethanol, dopamine

NICOTINE WITHDRAWAL SYNDROME AND PREVENTION WITH BACLOFEN: SOMATIC AND NEUROCHEMICAL ALTERATIONS

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Nicotine (NIC) is one of the active components of tobacco and plays a major role in tobacco addiction. The aims of the present study were: a) to evaluate the possible role of GABA_B receptor in NIC physical dependence; b) to analyze the neurochemical variations in various brain regions of mice during NIC withdrawal syndrome and its prevention with baclofen (BAC, GABA_B receptor agonist). Swiss-Webster albino mice received NIC (2.5 mg/kg, sc) four times daily, for seven consecutive days. To precipitate NIC abstinence, dependent mice were injected with the nicotine antagonist mecamylamine (MEC, 2 mg/kg, ip) 60 min after the last dose of NIC on the 8th day. A second group of dependent mice received BAC (2 mg/kg, ip) before MEC- precipitated abstinence. Somatic signs were measured for 30 min. The levels of dopamine, serotonin and its metabolites were determined by HPLC-coupled electrochemical detection in the striatum, cortex and hippocampus. BAC pre-treatment decreased the incidence of some NIC withdrawal signs, such as wet-dog-shakes ($p<0.01$) and paw tremors ($p<0.001$). The global withdrawal score was also attenuated by BAC ($p<0.001$). On the other hand, dopamine and DOPAC levels decreased in the abstinent group, while BAC reestablished these levels 10 min after MEC-precipitated nicotine withdrawal. These results indicate that BAC prevents NIC withdrawal syndrome. This would be related to changes in dopaminergic activity measured 10 min after MEC.

Palavras-Chave: Nicotine - Abstinence - GABAergic system - Neurochemical changes

Apoio Financeiro: UBACYT B021 and Fundación Florencio Fiorini

Possível relação entre perfil de ansiedade e consumo de etanol

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Objetivo: O alcoolismo apresenta-se como um distúrbio com grande heterogeneidade clínica sendo a ansiedade um possível fator predisponente para esse transtorno. Entretanto, a influência dessa comorbidade sobre o desenvolvimento da adição ainda permanece a ser elucidado. O presente trabalho buscou avaliar essa possível relação através da análise do padrão de consumo em animais classificados como ansiosos.

Métodos e Resultados: 199 camundongos *Swiss* foram expostos ao labirinto em cruz elevado (LCE) sendo calculada a porcentagem de tempo no braço aberto (TA). Os animais que apresentaram TA basal abaixo do limite de confiança estipulado (média ± 2 erros padrões: 51-2,2) foram considerados ansiosos e distribuídos entre grupo controle (CA, n=10) e experimental (A, n=30). Os animais com TA entre o limite de confiança superior e inferior e considerados como tendo ansiedade média também foram distribuídos entre grupo controle (CAM, n=10) e experimental (AM, n=30). Em seguida, foram alojados individualmente e o consumo de líquidos foi medido durante 70 dias, tendo os grupos A e AM acesso à água, etanol 5% e 10% (v/v) e, os grupos CA e CAM acesso apenas à água. Após um período de 5h de abstinência passaram por novos testes no LCE. O grupo A obteve a menor TA nas duas ocasiões (TA_{basal}: A=29 $\pm$ 1,9; AM=58 $\pm$ 0,7, t=-14,13, p<0,001; TA_{abs}: A=26 $\pm$ 4,3; AM=42 $\pm$ 4,3, t=-2,60, p<0,05). Durante as primeiras 6 semanas o consumo de etanol foi variável nos dois grupos. Não houve diferença entre as médias do consumo diário de etanol (g/kg) entre os grupos nas últimas 4 semanas (A: 8,7 $\pm$ 0,6 e AM: 9,0 $\pm$ 0,8, t=-0,33 p>0,05). Não houve correlação entre TA basal e a média do consumo diário de etanol em g/kg em ambos os grupos (A: r=0,17 e AM: r=0,08, p>0,05) nem entre TA medida na abstinência e a média do consumo diário de etanol g/kg nas últimas 4 semanas (A: r=0,31 e AM: r=0,02, p>0,05).

Conclusão: apesar dos animais classificados como ansiosos apresentarem um traço de ansiedade persistente ao longo do experimento, não houve uma maior preferência pelo etanol nesses animais podendo essa não ser uma característica predisponente ou que contribua para a manutenção do consumo.

Palavras-Chave: adição, modelo animal, ansiedade, alcoolismo, livre escolha, camundongo

Apoio Financeiro: CNPQ, CAPES

ELETOACUPUNTURA INIBE A SENSIBILIZAÇÃO LOCOMOTORA INDUZIDA PELO ETANOL EM CAMUNDONGOS VIA SINALIZAÇÃO DOS RECEPTORES CB1.

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OBJETIVO: Em um estudo prévio, verificamos que a eletroacupuntura aplicada simultaneamente nos acupontos ST-36 (*Zusanli*) e PC-6 (*Neiguan*) bloqueia a sensibilização locomotora induzida pelo etanol em camundongos. O objetivo do presente trabalho é de avaliar a influência da sinalização mediada pelos receptores CB1 nos efeitos da eletroacupuntura sobre a sensibilização locomotora induzida pelo etanol em camundongos.

MATERIAIS E MÉTODOS: Camundongos suíços machos (90 dias, 45 g) foram tratados diariamente, durante 21 dias, com etanol (2 g/Kg, i.p.). Logo após a 1ª, 7ª, 14ª e 21ª injeção, os animais eram confinados em um campo aberto por 30 min. Nas demais administrações os animais eram devolvidos para suas respectivas caixas moradias. Um grupo controle (Sal) recebeu tratamento similar, no entanto com solução salina. Após o tratamento, os animais que recebam etanol foram divididos nos seguintes grupos experimentais: EA (N=10) – estimulação simultânea por eletroacupuntura nos acupontos ST-36 (*Zusanli*) e PC-6 (*Neiguan*); Sham (N=10) – estimulação simultânea de não-acupontos localizados próximos aos acupontos ST-36 e PC-6; Naïve (N=10) – animais não submetidos à estimulação com agulhas; Imob (N=10) – animais submetidos a imobilização (10 min/dia) durante os 4 dias posteriores ao tratamento crônico com etanol (procedimento também utilizado nos grupos submetidos à estimulação com agulhas, por necessidade técnica, nesses animais, relativa à permanência local das agulhas); SR-EA (N=10) – animais tratados com SR141716A (antagonista CB1) 5 mg/kg, i.p., 30 min antes da estimulação dos acupontos e SR-Sham (N=10) – animais tratados com SR141716A e submetidos à estimulação simultânea de não-acupontos localizados próximos aos acupontos ST-36 e PC-6. A estimulação dos acupontos e dos não-acupontos consistiu de estímulos de 2 Hz e 50µA durante 10 min em cada sessão. Foi realizada 1 sessão por dia, durante os 4 dias que sucederam o tratamento crônico com etanol. Após 24 h da última sessão de eletroacupuntura, todos os animais (inclusive os que receberam salina) foram desafiados com etanol (1,6 g/Kg, i.p.) e em seguida novamente confinados por 30 min no campo aberto, onde foi avaliado o número de quadros explorados durante 30 min.

RESULTADOS: Conforme esperado, ANOVA seguida de Tukey *post hoc* mostrou que o grupo Naïve apresentou um aumento no número de quadros explorados quando comparados ao grupo Sal [$F_{(6,63)}=7,65$ $p<0,01$], um grande indicativo de sensibilização locomotora. Ainda, o grupo EA, mas não Sham, teve uma diminuição significativa do número quadros explorados quando comparados ao grupo Naïve ($p<0,05$), sugerindo que a estimulação simultânea dos acupontos ST-36 e PC-6 bloqueou a sensibilização locomotora induzida pelo etanol. No entanto, o dado mais relevante do presente trabalho é que o grupo SR-EA teve um aumento significativo no número de quadros explorados, quando comparado com os grupos Sal e EA ($p<0,01$) e não diferiu do grupo Naïve ($p=0,25$). No entanto, não houve diferenças entre os grupos SR e Sham ($p=0,86$).

CONCLUSÃO: Nossos resultados indicam que a acupuntura bloqueia a sensibilização locomotora induzida pelo etanol em camundongos, ao menos em parte, via ativação da sinalização mediada pelos receptores CB1.

Palavras-Chave: eletroacupuntura, etanol, sensibilização locomotora, receptores CB1

Apoio Financeiro: APOIO FINANCEIRO: CAPES, CNPq e FAPESP

EXPOSIÇÃO PRÉVIA AO ESTRESSE REPETIDO DIFICULTA A EXTINÇÃO DA PREFERÊNCIA CONDICIONADA POR LUGAR INDUZIDA PELA NICOTINA.

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OBJETIVO: A exposição ao estresse é um dos principais fatores que dificultam fumantes a pararem de fumar. Investigamos se a exposição repetida ao estresse dificultaria a extinção da preferência condicionada por lugar (PCL) a nicotina.

MÉTODOS E RESULTADOS: Ratos Wistar machos (180–200g), foram reunidos em 2 grupos um deles (EST) foi submetido a 2 horas de imobilização por 7 dias enquanto o grupo controle (CONT) foi mantido na gaiola-viveiro. O protocolo de PCL foi realizado em caixas com 3 compartimentos distintos (cor das paredes e textura do assoalho) em 5 etapas: 1) Pré-condicionamento (PRE): os ratos tiveram acesso aos 3 compartimentos durante 3 dias por 15 min, o tempo de permanência em cada compartimento foi determinado no 3º dia; 2) Condicionamento (COND): os ratos receberam injeção sub-cutânea de nicotina (NIC) (0,16 mg/Kg) e foram confinados isoladamente em um dos compartimentos por 30 min, na sessão seguinte os mesmos ratos receberam salina e foram confinados no outro ambiente totalizando 8 sessões; 3) Teste (TEST): os animais tiveram acesso a todos os compartimentos e o tempo de permanência em cada um deles foi determinado por 15min; 4) Extinção (EXT): os ratos foram expostos a ambos os compartimentos após receber SAL por 8 sessões. Um dia após a última exposição, foi realizado o teste de EXT, como descrito para o Teste. Os animais que não apresentaram a extinção da PCL foram submetidos a 4 sessões adicionais de EXT, sendo o teste realizado 24 horas após a última; 5) Reinstalação (REINST): 1 dia após a EXT os animais receberam injeção de NIC e foram colocados na caixa de PCL e o tempo de permanência em cada compartimento foi determinado. Foi realizada ANOVA seguido do teste F e o teste qui-quadrado (N=10-12).

RESULTADOS: Os grupos EST e CONT apresentaram reinstalação da PCL (CONT: PRE: 37±5 x REINST: 62±5; EST: PRE: 32±5 x REINST: 51±6; teste F: p<0,05). A porcentagem de ratos do grupo que EST que extinguiram a PCL após o 1º e 2º teste de EXT foi menor que CONT (CONT x EST: 83% x 50% e 100% x 75%; 1º e 2º teste, respectivamente; qui-quadrado: p<0,01).

CONCLUSÃO: A exposição prévia ao estresse dificulta a extinção da PCL induzida pela nicotina.

Palavras-Chave: Preferência Condicionada por Lugar; Estresse; Nicotina; Reinstalação.

Apoio Financeiro: FAPESP

NEONATAL FLUOXETINE (BUT NOT REBOXETINE) EXPOSURE AUGMENT THE STEREOTYPED BEHAVIORAL INDUCED BY APOMORPHINE IN FEMALE (BUT NOT MALE) ADOLESCENT RATS.

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OBJECTIVE: Major depression is a common disease in pregnant women. Nevertheless, several studies report an association between exposure to antidepressant drugs during the third trimester and abnormal development of the central nervous system. Take in to account that abnormal nigrostriatal development play a crucial role in several psychiatry disorders, included schizophrenia and aberrant habit-memory related to addictive process, in the present work was verified the influences of neonatal exposure to fluoxetine and reboxetine (selective serotonin- or noradrenaline- reuptake inhibitor, respectively) over stereotyped behavioral induced by apomorphine in male and female adolescent rats.

METHODS: At PND8-PND21, male and female pups (N=08 per group) received daily intraperitoneally injections of fluoxetine (7.5 mg/kg) and reboxetine (10 mg/kg). Control male and female pups groups received similar volume of saline. At PND49 the animals were submitted to the stereotyped movements induced by apomorphine (0.6 mg/kg) test. Stereotyped behavioral were scored from 0 (minimum stereotyped movements) to 6 (maximum stereotyped movements).

RESULTS: Non-parametrical Kruskal Wallis followed by Mann-Whitney *post hoc* statistical analysis revealed that fluoxetine (but not reboxetine) neonatal exposures augmented [$F_{(5,42)}=23.12$ $P<0.01$] the stereotyped behavioral induced by apomorphine in female adolescent rats, as compared to control group (2.2 ± 0.1 ; 1.1 ± 0.4 ; 0.8 ± 0.2). This effects was not seems in male adolescent rats (1.8 ± 0.2 ; 0.8 ± 0.3 ; 1.2 ± 0.3).

CONCLUSION: Augment of serotonergic, but not noradrenergic, transmission at neonatal period promotes a long-last augment of nigrostriatal pathways tone only in female adolescent rats.

Palavras-Chave: Antidepressivo; Comportamento; Neonatal

Apoio Financeiro: CNPq e FAPESP

Differential c-fos expression in brain regions associated with cue-induced cocaine and alcohol seeking in SHR rats

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Objectives:

The prevalence of alcohol and drug abuse is high in individuals with attention deficit hyperactivity disorder (ADHD). Thus, the identification of the neurobiological mechanisms underlying this kind of comorbidity is needed. The aim of this study was to evaluate the activation of brain regions associated with ethanol and cocaine seeking behavior in an animal model of ADHD, the spontaneously hypertensive rats (SHR).

Methods:

Initially, rats were separated in the following groups: control, ethanol 0.5, 1.2 or 2.0 mg/kg and cocaine 15 mg/kg. The conditioned place preference (CPP) was carried out in an unbiased protocol as described: day 1 habituation; day 2 pre-CPP measurement; days 3-10 conditioning (animals received drug or saline each other day); day 10 post-CPP measurement. Animals with significant CPP had their brain removed and c-fos marked cells were counted in prelimbic and infralimbic cortex, and nucleus accumbens core and shell.

Results:

Animals conditioned with ethanol 1.2 mg/kg and cocaine 15 mg/kg depicted a significant place preference to the drug-paired compartment, (260±15s pre-CPP X 390±20s post-CPP for ethanol and 258±30s pre-CPP and 410±20s post-CPP for cocaine, $p<0.05$). Only rats conditioned with cocaine have shown an increase of c-fos positive cells compared with control in both prelimbic (53.5±9 X 70.1±25) and infralimbic cortex (35.2±5 X 64.4 ±21) and either in nucleus accumbens shell (32,1±6 X 51,63±1), $p<0.05$.

Conclusions:

The SHR rats showed similar levels of alcohol and cocaine conditioned place-preference. However, preliminary results indicate that the brain regions activated by alcohol and cocaine did not necessarily overlap. These findings may contribute to the current knowledge of the brain regions implicated in cue-induced alcohol and cocaine seeking behavior and its potential relationship with ADHD.

Palavras-Chave: Cocaine, ethanol, c-FOS, place preference, SHR

ESTRESSE DE DERROTA SOCIAL PROMOVE SENSIBILIZAÇÃO COMPORTAMENTAL CRUZADA, MAS NÃO ALTERA O PONTO DE RUPTURA E O “BINGE” DA AUTO-ADMINISTRAÇÃO DE HEROÍNA

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Objetivo: A exposição ao estresse pode alterar os padrões de consumo e motivação ao uso de drogas, além de promover sensibilização comportamental cruzada. Nesse estudo foi avaliado se a exposição ao estresse de derrota social poderia promover sensibilização cruzada com a heroína e/ou modificar seus parâmetros da auto-administração.

Métodos e Resultados: Ratos Long-Evans machos adultos (180 – 200 g) foram reunidos em dois grupos estresse (EST) e controle (CONT). Os animais do grupo EST foram submetidos a 4 sessões de estresse de derrota social (confronto com animal dominante), com intervalo de 72 horas entre as sessões. O grupo controle não foi exposto ao estresse. Dez dias após a última sessão de estresse os animais, receberam injeções de salina e heroína (0,25mg/Kg; s.c.) e imediatamente após cada administração a atividade locomotora foi avaliada em intervalos de 5 min. No dia seguinte foi implantado um cateter na veia jugular para auto-administração de heroína. Em seguida os animais foram treinados para auto-administrar essa substância (0,03mg/Kg/infusão, razão fixa 3). Após a aquisição os ratos foram submetidos aos protocolos de razão progressiva (escala aritmética) para avaliação do ponto de ruptura (número de infusões (INF) e maior razão alcançada (RP) durante a sessão) e ao de “binge” por 24 horas para avaliação do consumo (mg/kg) dessa substância. Os dados foram analisados por ANOVA seguido do teste F para contraste entre pares de médias e teste *t-student*. Nossos resultados demonstraram que este tipo de estresse promoveu sensibilização cruzada com a heroína (CONT:19,7±6,3 x EST:42,4±8,5;p<0,05), porém não modificou o INF (CONT:13,9±1,3 x EST:10,8±1,4; p>0,05) e RP (CONT:118±26 x EST:72±34;p>0,05), bem como o “binge” (CONT:128,1±40,5 x EST:111,6±29,8;p>0,05) dessa substância.

Conclusões: O estresse de derrota social foi capaz de promover sensibilização comportamental cruzada com a heroína, porém não alterou os padrões de consumo e motivação a essa substância.

Palavras-Chave: auto-administração, heroína, dependência

Apoio Financeiro: CAPES

Classificação do alcoolismo pela Tipologia de Lesch no Brasil.

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Introdução: O alcoolismo é um problema de Saúde Pública, atingindo cerca de 10% da população adulta. Trata-se de uma doença heterogênea, com diferentes apresentações clínicas, e, conseqüentemente, resultados terapêuticos e recaídas variáveis, indicando vulnerabilidade biológica diferentes. Lesch e cols (1988) distinguiram quatro categorias de alcoolismo: Tipo I: modelo de alergia, com sintomas de abstinência grave e melhora importante com abstinência; Tipo II: modelo de ansiedade, usa o álcool como solução para conflitos emocionais; Tipo III: modelo de depressão, usa álcool para “tratamento” de desordens psiquiátricas, e, Tipo IV: modelo de adaptação, com história de alterações neurológicas que precedem o uso de álcool. **Objetivo:** Avaliar o emprego da classificação de Lesch em um programa ambulatorial de atendimento a alcoolistas, no estado do Espírito Santo, Brasil. **Materiais e Métodos:** 170 alcoolistas atendidos, entre dezembro de 2006 a março de 2007, foram classificados em categorias seguindo a “árvore decisória” publicada por Lesch e cols em 1990. **Resultados:** A classificação da Tipologia de Lesch mostrou: Tipo I = 21,2%; Tipo II = 29,4%; Tipo III = 28,8% e Tipo IV = 20,6%%. 148 (87,1%) eram homens, e esse gênero predominou absoluto no Tipo II de Lesch, enquanto nos pacientes classificados como Tipo III, 28,6% eram mulheres. A média de idade entre os alcoolistas foi de 46,37 anos ($\pm 9,73$), não havendo diferenças entre os diferentes tipos de alcoolismo. Os tipos de alcoolismo também não se diferiram com relação à idade de início de uso de álcool (média de 14,93 anos $\pm 4,6$), à quantidade de uso do álcool (média de 374,56 gramas/dia $\pm 309,79$), e tempo de abstinência (média de 193,36 dias $\pm 409,6$). **Conclusão:** A diferenciação do tipo clínico de alcoolismo, através da aplicação de uma classificação simples como a da Tipologia de Lesch, pode constituir-se em ferramenta importante na avaliação clínica dos alcoolistas, proporcionando significativas mudanças nas estratégias de enfrentamento individualizadas de tratamento do alcoolismo.

Palavras-Chave: Alcoolismo, Classificação, Tipologia de Lesch

C.08.031

As alterações das funções frontais em alcoolistas ocorrem primordialmente naqueles classificados como Tipo IV pela Classificação de Lesch.

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Introdução: Em 1988 Lesch e cols publicaram uma classificação de alcoolismo, distinguindo quatro categorias: Tipo I: com sintomas de abstinência grave; Tipo II: usam o álcool como solução para conflitos; Tipo III: usam álcool para “tratamento” de distúrbios psiquiátricos, e, Tipo IV: com alterações neurológicas que precedem o uso de álcool. Déficits cognitivos e mentais são descritos em alcoolistas com implicações diretas no tratamento. **Objetivo:** Avaliar funções frontais e mentais nas diferentes categorias de alcoolismo de acordo com a Tipologia de Lesch. **Materiais e Métodos:** Bateria de Avaliação Frontal (FAB) e Mini-Exame do Estado Mental (Mini-Mental) foram aplicados em 170 alcoolistas classificados em categorias de acordo com Lesch e 40 controles não-alcoolistas pareados por idade, gênero, características sócio-demográficas e escolaridade. **Resultados:** Foi encontrado um desempenho significativamente prejudicado em tarefas que requerem a função cognitiva frontal, nos pacientes classificados como Tipo IV de Lesch, comparados aos controles não-alcoolistas e também aos pacientes alcoolistas classificados como Tipos I, II e III de Lesch. Estas alterações das funções frontais não se correlacionaram com o padrão de ingestão ou com a idade de início do uso de álcool ou com a escolaridade. Os pacientes classificados como Tipo IV de Lesch também apresentaram prejuízos do estado mental, sendo o subgrupo que apresentou maior porcentagem de pontuação sugerindo demência. **Conclusão:** As alterações das funções frontais e mentais em alcoolistas não se dão de maneira generalizada, mas ocorre primordialmente naqueles classificados como Tipo IV de Lesch. A classificação clínica dos tipos de alcoolismo aliada à avaliação cognitiva frontal constituem ferramentas úteis na definição de estratégias de tratamento a serem instituídas na dependência ao álcool.

Palavras-Chave: Acoolismo, função frontal, classificação, Tipologia de Lesch

La sensibilización conductual inducida por cocaína se correlaciona con un incremento en la plasticidad sináptica en el hipocampo y una “sensibilización” en la actividad de la enzima óxido nítrico sintasa.

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El hipocampo (HP) es una estructura límbica que participa en la formación de diversos aprendizajes y memorias. Cambios en la transmisión sináptica en el HP forman parte de las neuroadaptaciones que ocurren tras la exposición a drogas de abuso. La administración repetida de cocaína (COC) induce sensibilización a sus efectos sobre la locomoción y el óxido nítrico (NO) estaría involucrado en la adquisición y mantenimiento de los efectos comportamentales de COC, ya que la inhibición de la enzima NO sintasa (NOS) atenúa el desarrollo de sensibilización. A pesar de ello, el rol del NO en los mecanismos involucrados en la sensibilización no ha sido aclarado. En este trabajo se combinaron experimentos conductuales, electrofisiológicos y neuroquímicos para: 1-determinar si la sensibilización inducida por COC está asociada con un incremento en la plasticidad sináptica, medida como una disminución en el umbral para generar potenciación a largo plazo (LTP) en rebanadas de HP, 2-establecer una correlación entre el desarrollo de sensibilización y la producción de NO, medida por la conversión de citrulina H³ a arginina H³ y 3-determinar si los cambios observados son consecuencia de la sensibilización o del síndrome de abstinencia. Ratas Wistar machos se inyectaron con COC (15 mg/kg/día ip) o salina (SAL) por 5 días, se evaluó la locomoción los días 1 y 5 y se separaron los animales sensibilizados (S). Los estudios electrofisiológicos y neuroquímicos se realizaron el día 5 (S/A) o luego de 3 días de abstinencia (C/A). Los animales S S/A mostraron una disminución en el umbral para generar LTP (SAL 102 ± 8 vs S 27 ± 4 Hz). Lo mismo se observó en los grupos C/A. El aumento en la plasticidad sináptica se correlacionó con un incremento en la actividad de NOS en el grupo S C/A (SAL 0.4 ± 0.1 vs S 2.83 ± 0.6 pmol/mg proteína). La actividad de NOS mostró un patrón de “sensibilización” en los animales S comparados con animales que recibieron una dosis de COC (S 775 ± 158 vs agudo 308 ± 51 %). Estos hallazgos indican que el NO tendría un rol importante en la alteración de la transmisión sináptica hipocámpal inducida por COC, contribuyendo al desarrollo de sensibilización y abstinencia.

Palavras-Chave: sensibilización a cocaína, plasticidad sináptica, hipocampo, óxido nítrico

Apoio Financeiro: SECYT y CONICET

EFEITO DA EXPOSIÇÃO AO ESTRESSE AGUDO NA ATIVIDADE LOCOMOTORA INDUZIDA PELA CAFEÍNA EM RATOS

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Introdução: A cafeína é uma das substâncias psicoativas mais consumidas no mundo. O estresse tem sido destacado como um fator importante na iniciação, manutenção e recaída do consumo de psicoestimulantes.

Objetivo: Investigar o efeito da exposição aguda a estresse na estimulação da atividade locomotora induzida pela cafeína.

Métodos e Resultados: Ratos Wistar machos adultos (70-75 dias) foram submetidos ao estresse de imobilização (EST) por 1 hora. Imediatamente após a exposição ao estresse os animais foram colocados na caixa de atividade para habituação por 20 minutos. Em seguida, os animais receberam injeção intraperitoneal de cafeína (CAF) (10mg/kg) ou salina (SAL) (1mL/kg) e a locomoção foi registrada durante 30 minutos. Os animais do grupo controle (CON) foram retirados da gaiola moradia e transferidos diretamente para a caixa de atividade e submetidos ao mesmo esquema de análise comportamental que aqueles submetidos ao estresse. Os dados representam a média da locomoção nos 30 minutos $\pm$ EPM (N=8 por grupo). A análise estatística consistiu da ANOVA seguida do teste Newman-Keuls. A injeção de cafeína causou aumento significativo da atividade locomotora em ambos os grupos ($p<0,001$), mas a intensidade do efeito não foi modificada pela exposição aguda ao estresse (CON-SAL: 699 $\pm$ 95; EST-SAL: 219 $\pm$ 56; CON-CAF: 2003 $\pm$ 256; EST-CAF 2276 $\pm$ 391).

Conclusões: A exposição a estresse agudo por imobilização não altera a atividade locomotora induzida pela cafeína em ratos.

Palavras-Chave: Estresse, cafeína

Apoio Financeiro: FAPESP

O CONSUMO DE ETANOL EM “BINGE” EM RATOS ADOLESCENTES PREJUDICA DE FORMA SELETIVA A MEMÓRIA DE LONGA DURAÇÃO NO TESTE DO RECONHECIMENTO DE OBJETOSCamargo, P. M.¹; Von Schaaffhausen, N. T.²; Richtzenhain MH²; dos Santos, J. G. Jr.²; Oliveira FA² -¹Universidade Federal do Estado de São Paulo - Neurofisiologia; ²FCMSCSP - Departamento de Ciências Fisiológicas

OBJETIVO: Sabe-se que o etanol provoca perda de neurônios e diminui a neurogênese hipocampal. Levando-se em consideração a importância desta estrutura nos processos de aprendizagem e memória, o objetivo deste trabalho foi avaliar o efeito da intoxicação crônica de etanol em “binge” (padrão similar ao utilizado pelos adolescentes brasileiros) em ratos adolescentes submetidos ao teste do reconhecimento de objetos.

MATERIAL E MÉTODOS: Ratos Wistar machos receberam etanol (2,5 ou 5g/kg, v.o.) em dias alternados, entre o 30º e o 60º dia de vida (adolescência). Animais controle receberam tratamento similar com solução salina. Utilizou-se um N=8 por grupo. Após 24h, os animais ficaram confinados por 10 min em um campo aberto, a fim de se habituar ao ambiente. Em seguida, foram colocados dois objetos A + B no campo aberto, e durante 3 min foi quantificado o tempo em que o animal interagiu com cada um dos objetos (sessão treino). Passadas 1,5 e 24 h da sessão treino, foi realizado o mesmo procedimento anteriormente descrito, em presença de um objeto familiar e um novo objeto (A + C) e (A + D), a fim de se avaliar a memória de curta (STM) e longa duração (LTM), respectivamente. O índice de reconhecimento foi calculado pelo tempo de exploração do novo objeto dividido pelo tempo de exploração em ambos os objetos [C/(A+C) para STM; D/(A+D) para LTM]. O mesmo cálculo foi realizado na sessão treino, a fim de se evitar possíveis vieses de preferência ou aversão ao objeto A [A/(A+B)]. O índice de exploração na sessão treino, STM e LTM foram analisados utilizando-se ANOVA de uma via, seguida de teste de Tukey post hoc quando necessário. Foi considerada diferença estatística quando $P < 0,05$. **RESULTADOS:** Conforme esperado, não houve diferença entre os grupos experimentais no que se refere ao índice de exploração na sessão treino $F(2,21)=0,09$ $P=0,92$ ($0,48 \pm 0,04$; $0,50 \pm 0,06$; $0,48 \pm 0,04$, relacionado ao grupo salina, Et2,5 e Et5, respectivamente). O mesmo foi verificado quanto ao índice de exploração durante a sessão que avaliou a STM $F(2,21)=1,99$ $P=0,17$ ($0,67 \pm 0,04$; $0,45 \pm 0,06$; $0,55 \pm 0,10$). No entanto, o grupo Et5, mas não o Et2,5, apresentou uma diminuição significativa do índice de exploração durante a sessão que avaliou a LTM, quando comparado ao índice verificado no grupo Controle $F(2,21)=3,64$ $P < 0,05$ ($0,61 \pm 0,06$; $0,44 \pm 0,06$; $0,39 \pm 0,04$).

CONCLUSÃO: O modelo utilizado no presente trabalho, a fim de mimetizar o padrão de consumo de álcool entre os adolescentes brasileiros, mostra que o padrão de consumo “binge” diminui a memória de reconhecimento de objetos de longa duração em ratos adolescentes, possivelmente por um efeito neurotóxico da droga na formação hipocampal destes animais.

Palavras-Chave: Etanol, Neurogênese Hipocampal

Apoio Financeiro: FAP - FCMSCSP

Relação entre as diferenças individuais e o hormônio corticosterona em ratos.

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O objetivo do presente trabalho foi verificar a relação entre as diferenças individuais (espontâneas e induzidas por apomorfina) e as concentrações plasmáticas de corticosterona (CORT). No experimento 1, ratos Wistar machos foram colocados na arena por 60 min (teste à novidade), os comportamentos locomotor e orofacial foram registrados e, de acordo com a média do tempo de emissão do comportamento orofacial foram classificados em respondedores altos (RA, n=9) e respondedores baixos (RB, n=7). No experimento 2, os animais foram classificados em RA e RB e, 7 dias após foram submetidos ao teste com apomorfina (APO; 2,0 mg/kg), onde receberam APO [grupos: RB-APO (n=8); RA-APO (n=9)] ou veículo [RB-VEIC (n=7); RA-VEIC (n=8)] e foram colocados imediatamente na arena por 60 min. Após o término dos experimentos, o sangue foi coletado, centrifugado e o plasma congelado – 20 °C para posterior extração e dosagem da CORT por radioimunoensaio. Os resultados do experimento 1 mostraram que o grupo RA apresentou comportamento orofacial e locomoção maiores ($74,0 \pm 38,6$ e $11,7 \pm 0,7$; média e desvio padrão do orofacial e locomoção) que o grupo RB ($55,8 \pm 3,11$ e $8,6 \pm 0,8$). Com relação a CORT, não houve diferença na concentração plasmática entre o RA ($10,1 \pm 8,0$ [g/dl]) e o RB ($16,1 \pm 10,5$ [g/dl]). No experimento 2, os grupos RA-APO ($150,0 \pm 0,0$) e RB-APO ($149,0 \pm 1,1$) emitiram maior comportamento orofacial que os RA-VEIC ($88,1 \pm 45,2$) e RB-VEIC ($78,1 \pm 49,0$), porém o RA-APO ($15,3 \pm 14,3$) apresentou maior locomoção que os RB-APO ($9,3 \pm 8,8$), RB-VEIC ($11,5 \pm 10,8$) e RA-VEIC ($10,4 \pm 9,0$). A APO aumentou a concentração plasmática de CORT nos RA-APO ($34,6 \pm 15,6$ [g/dl]) e RB-APO ($33,9 \pm 27,1$ [g/dl]) que foram diferentes dos RA-VEIC ($13,1 \pm 12,3$ [g/dl]) e RB-VEIC ($12,1 \pm 10,5$ [g/dl]). Houve uma concordância entre o teste à novidade e o teste com APO na determinação das diferenças individuais, mas não houve uma relação com a concentração de CORT. Entretanto, a concentração plasmática elevada de CORT produzida por APO talvez contribua para o desenvolvimento e manutenção da sensibilização comportamental.

Palavras-Chave: Diferenças individuais, corticosterona, apomorfina

Apoio Financeiro: PIBIC/UENF

Estudio de factores epigenéticos en un modelo comportamental de preferencia al lugar luego de la exposición a nicotina

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Una aproximación al estudio de los efectos de las drogas de abuso es describir los mecanismos que ellas desencadenan; en este sentido, nos propusimos estudiar la posible asociación entre los efectos comportamentales a largo plazo inducidos por la nicotina y la estructura de la cromatina.

Utilizando el modelo comportamental de condicionamiento preferencial a un lugar (CPP), se generaron los siguientes grupos experimentales: Animales en los cuales se evidencian las propiedades reforzadoras de la nicotina a partir de la preferencia de un contexto (CPP1/reward); animales que extinguen dicha preferencia luego de la privación de la droga (deprivado) y animales que frente a la reexposición a la droga vuelven a preferir el contexto asociado a la nicotina (CPP2/relapse). Además, se administraron animales en forma crónica y aguda. Se estudiaron los patrones de distribución nuclear y los niveles de: histona 3 acetilada (H3-Ac), histona deacetilasa 2 (HDAC2) y la proteína de unión a metil-CpG 2 (MeCP2) como marcadores de la remodelación de la cromatina. Para esto se procesaron cerebros de animales de los grupos descriptos disecando tres áreas de la vía mesocorticolímbica: núcleo accumbens, estriado y corteza prefrontal efectuándose las técnicas de inmunohistoquímica y western blot.

Resultados preliminares no mostraron cambios en los niveles de expresión de la H3-Ac ni de HDAC2 en los grupos agudo y crónico respecto del salino. Por otro lado se evidenció un cambio cualitativo en el patrón de distribución de HDAC2 y MeCP2 en dos de las áreas estudiadas en los grupos CPP1 y CPP2 respecto del salino y del deprivado.

Estos estudios sugieren que ciertos factores epigenéticos podrían estar involucrados en el desarrollo y mantenimiento de los efectos comportamentales generados por la nicotina. Los cambios observados indicarían que existe una remodelación de la cromatina inducida por la nicotina en aquellos animales que mostraron un CPP1 positivo y aquellos que manifestaron un CPP2 positivo.

Palabras-Chave: Epigénesis - Nicotina - CPP - HDAC2

Apoio Financeiro: Agencia Nacional de Promoción Científica y Tecnológica, Consejo Nacional de Investigaciones Científicas y Técnicas

A EXPRESSÃO SENSIBILIZAÇÃO COMPORTAMENTAL A NICOTINA EM RATOS ADOLESCENTES É DEPENDENTE DA DOSE.

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OBJETIVOS: A sensibilização comportamental reflete neuroadaptações relacionadas ao desenvolvimento da farmacodependência. Poucos trabalhos investigam esse fenômeno em modelos animais de adolescência. Demonstramos anteriormente a ausência de expressão de sensibilização após a administração de 0,4 mg/kg de nicotina em ratos adolescentes. Nosso objetivo foi investigar se a expressão da sensibilização comportamental à nicotina em ratos adolescentes é dependente da dose.

MATERIAIS E MÉTODOS: Ratos Wistar machos adolescentes (dia pós-natal 28-42) foram aleatoriamente reunidos em seis grupos distintos. Para o desenvolvimento da sensibilização, três grupos foram pré-tratados com uma injeção diária de nicotina (NIC) (0,4 mg/Kg) pela via subcutânea (s.c.) e outros três com salina (SAL) s.c. por sete dias. Três dias após o término do pré-tratamento a expressão da sensibilização foi avaliada. Para tanto os animais foram colocados na caixa de atividade locomotora por 20 minutos para habituação. Em seguida todos os grupos receberam SAL e a atividade locomotora foi registrada por 15 minutos. Em seguida, cada grupo pré-tratado com SAL ou NIC recebeu injeção s.c. de NIC (0,1 ou 0,2 ou 0,4 mg/Kg) e a locomoção foi avaliada por 15 minutos. Assim os seguintes grupos foram formados: SAL-NIC 0,1; SAL-NIC 0,2; SAL-NIC 0,4; NIC-NIC 0,1; NIC-NIC 0,2; NIC-NIC 0,4. Os valores representam a média $\pm$ EPM (5-6 ratos por grupo) das unidades de locomoção acumuladas em 15 minutos e foram analisados por ANOVA bifatorial para medidas repetidas, seguido pelo teste F para contraste entre os pares de médias.

RESULTADOS: O grupo NIC-NIC 0,2 (2026 $\pm$ 323) apresentou maior atividade locomotora do que o grupo SAL-NIC 0,2 (1068 $\pm$ 285), $p < 0,05$, evidenciando a presença da sensibilização comportamental. Nenhuma diferença na atividade locomotora foi observada entre os grupos SAL-NIC 0,1 (787 $\pm$ 216) vs NIC-NIC 0,1 (1163 $\pm$ 220) e SAL-NIC 0,4 (949 $\pm$ 175) vs NIC-NIC 0,4 (1170 $\pm$ 288).

CONCLUSÃO: A expressão da sensibilização comportamental à nicotina foi evidenciada apenas para dose de 0,2 mg/Kg, dessa forma a expressão da sensibilização à nicotina em ratos adolescentes é dependente da dose de nicotina. **Palavras-Chave:** Nicotina; Dependência; Adolescência; Sensibilização Comportamental.

Apoio Financeiro: PIBIC/CNPq

Adaptaciones neuronales inducidas por nicotina: receptores nicotínicos y proteínas de andamiaje

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Como consecuencia de la estimulación con nicotina, se han descripto numerosas adaptaciones neuronales interpretadas como respuestas homeostáticas inducidas por la droga. La propuesta del siguiente trabajo es examinar los niveles de expresión de los receptores nicotínicos conjuntamente con su entorno molecular, en particular, con proteínas de andamiaje con dominio Post Synaptic Density-95, discs large, zona occludens (PDZ) luego de la estimulación con nicotina.

Los ensayos se realizaron en cultivos primarios de neuronas estriatales de ratas de un día postnatal. Luego de 11 días *in vitro*, se ensayaron los siguientes protocolos de exposición a nicotina: **A.** exposición única (15min); **B.** exposición repetida y **C.** exposición crónica (20hs). Para cada uno de los protocolos las células fueron fijadas a distintos tiempos y se evaluó cualitativamente, mediante inmunocitoquímica, la presencia de receptores nicotínicos (positivos para la subunidad beta2) y proteínas de la familia PDZ (PDZ-f).

La estimulación con nicotina produjo un aumento de la intensidad en la señal de beta2 en todos los tratamientos respecto del salino. En el protocolo **A.**, dicha intensidad volvió a los valores basales a las 3hs. En el grupo **B.** se observó, además, un cambio en el patrón de distribución de beta2. La intensidad de la señal de PDZ-f aumentó después de la exposición a la nicotina en los grupos tratados en forma prolongada (**B.** y **C.**).

En forma preliminar, se describe el efecto *in vitro* de la nicotina sobre su receptor y una familia de proteínas que podrían estar involucradas en la estabilización de dichos receptores en la membrana. El cambio en la intensidad y la distribución de beta2 podría ser considerado como una adaptación celular generada como respuesta a la presencia de la droga. Dado que el aumento en los niveles de PDZ-f sólo se evidenció en los grupos **B.** y **C.**, es posible que su rol funcional esté directamente asociado a la respuesta bajo condiciones de estimulación prolongada para mantener aquella adaptación neuronal.

Palavras-Chave: Nicotina, cultivos primarios, receptores nicotínicos, PDZ-f

Apoio Financeiro: Agencia Nacional de Promoción Científica y Tecnológica, Consejo Nacional de Investigaciones Científicas y Técnicas

Efeitos de diferentes doses de anfetamina sobre a atividade locomotora e sua sensibilização em animais jovens e adultos

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Objetivos: A hiperfunção dopaminérgica representa uma das principais hipóteses fisiopatológicas relacionadas à esquizofrenia. Essa doença que usualmente se manifesta entre a adolescência e o início da vida adulta apresenta marcante comorbidade com o abuso de psicoestimulantes. Assim, buscamos verificar o efeito estimulante agudo de diferentes doses de anfetamina e sua sensibilização comportamental (SC) em animais jovens e adultos. Nesse aspecto, o fenômeno de sensibilização comportamental tem sido preconizado como um possível modelo fisiopatológico de farmacodependência.

Métodos: Camundongos jovens (jv) e adultos (ad) receberam intraperitonealmente salina (s) ou anfetamina (a) nas doses de 1, 2 ou 4 mg/kg, formando os seguintes grupos: s-jv, a1-jv, a2-jv, a4-jv, s-ad, a1-ad, a2-ad e a4-ad. Imediatamente após, os animais foram observados em campo aberto (CA) para a avaliação do efeito agudo da droga. Após 7 dias, os animais foram desafiados com salina ou 2 mg/kg de anfetamina, formando os seguintes grupos: s-s jv, s-a jv, a1-a jv, a2-a jv, a4-a jv, s-s ad, s-a ad, a1-a ad, a2-a ad e a4-a ad. Em seguida, foram novamente observados em CA para a avaliação da SC.

Resultados: Com relação ao efeito estimulante agudo da droga, observou-se um aumento da locomoção promovido pelas doses de 2 ou 4 mg/kg de anf somente nos animais adultos. Já nos animais jovens, apenas a dose de 4 mg/kg de anf foi efetiva em promover esse aumento [s-jv(106,9±6,6), a4-jv(184,4±27,0), s-ad(99,9±8,5), a2-ad(153,6±13,5) e a4-ad(221,9±28,4)]. No que diz respeito à SC, os animais adultos pré-tratados com as doses de 2 ou de 4 mg/kg de anfetamina apresentaram SC ao efeito estimulante promovido pela injeção desafio de 2 mg/kg. Em relação aos animais jovens, a SC foi observada somente nos animais pré-tratados com 4 mg/kg de anfetamina [s-s jv(66,1±11,7), s-a jv(60,2±7,3), a4-a jv(160,6±38,6), s-s ad(74,5±8,5), s-a ad(66,5±12,5), a2-a ad (142,6±21,2) e a4-a ad (157,8±23,7)].

Conclusão: Nossos resultados sugerem que, nesse modelo comportamental, os animais adultos são mais sensíveis que os camundongos jovens tanto ao efeito estimulante da anfetamina, quanto a sua sensibilização.

Palavras-Chave: hipóxia neonatal, sensibilização comportamental, dependência química, anfetamina

Apoio Financeiro: FAPESP, CEPID e AFIP.

A INTERAÇÃO SOCIAL MODIFICA A PREFERÊNCIA POR LUGAR INDUZIDA POR ETANOL EM CAMUNDONGOS

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Objetivos: A preferência condicionada por lugar (place preference-PP) tem se destacado como um modelo animal muito utilizado para o estudo da farmacodependência. Paralelamente, a interação e o aprendizado social contribuem de forma determinante para o desenvolvimento da dependência química em humanos. Este trabalho tem como objetivo comparar o desempenho de camundongos submetidos ao etanol no paradigma de PP após o condicionamento individual ou em duplas. **Métodos:** Camundongos Swiss (3 meses) foram injetados com salina (SAL), etanol (ET-1,8g/kg) ou haloperidol (HAL-2mg/kg), sendo pareados a um dos compartimentos do aparelho com droga (10 min) e, 6 hs após, pareados com SAL no outro compartimento (10 min). Esse procedimento foi realizado por 4 dias consecutivos. Formando os grupos SAL ISO e ET ISO (animais condicionados isolados com SAL ou ET respectivamente), ET INT SAL, ET INT ET e ET INT HAL (animais ET condicionados com um animal SAL, ET ou HAL, respectivamente). 24 hs após a última sessão de condicionamento, os animais foram submetidos, isoladamente, à sessão teste (15 min), com livre acesso ao aparelho. Quantificou-se a cada 5 min o tempo de permanência em cada compartimento. Os resultados mostraram que: 1) O grupo SAL ISO não desenvolveu PP. 2) O grupo ET ISO apresentou uma tendência a desenvolver PP, manifestada no final do teste. 3) O grupo ET INT SAL apresentou uma potencialização da PP, sendo o único grupo a permanecer mais tempo no compartimento pareado com a droga (522,25+51,9 seg) do que no compartimento pareado com salina (228,1+37,1 seg) durante toda a sessão teste. 4) O grupo ET INT ET apresentou tendência à PP manifestada nos 5 min iniciais do teste. 5) O grupo ET INT HAL mostrou aversão ao compartimento pareado. **Conclusão:** A interação social é capaz de modificar bidirecionalmente a PP induzida por ET. Nesse sentido o estado do animal “acompanhante” é muito importante para a direção dessa modificação: quando o estado comportamental do animal “acompanhante” é normal a PP é potencializada, quando o estado comportamental do animal é eufórico, essa potencialização deixa de ocorrer e quando esse estado é depressivo a PP se transforma em aversão.

Palavras-Chave: Place Preference, interação social, etanol

Apoio Financeiro: CNPq

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DNA methyltransferase expression in mice with different profiles of locomotor activity after chronic treatment with ethanol

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Objectives: Recent studies indicate that global DNA methylation in patients with alcoholism is increased. Considering that the developmental process of behavioral sensitization to ethanol does not occur evenly, we investigated the expression of DNA methyltransferases (DNMTs) in the pre-frontal cortex and striatum of mice with different profiles of locomotor activity, after chronic ethanol administration.

Methods and Results: Male Swiss mice received 2.2 g/kg ethanol or saline intraperitoneally daily for 21 days, being tested weekly for locomotor activity. Ethanol-treated mice were classified into high sensitized, low sensitized and intermediate subgroups (n=10) according to their locomotor activity levels during treatment. Real time reverse transcription polymerase chain reaction assays were used to measure levels of mRNAs encoding the gene of enzymes in the pre-frontal cortex and striatum tissues. We observed significant increases in DNMT3a ($p < 0.01$) and 3b ($p < 0.01$) mRNA levels in the pre-frontal cortex of low sensitized animals in relation to high sensitized animals. No significant differences were observed in the DNMT1 ($p_e=0.55$, $p_{cpr}=0.84$) expression in both tissues neither in the DNMT3a ($p=0.46$) and 3b ($p=0.16$) expression in the striatum.

Conclusions: Since methylation of DNA is an important epigenetic factor in the regulation of gene expression, these epigenetic modifications may have important implications in the development of behavioral sensitization to ethanol in mice. **Palavras-Chave:** ethanol, sensitization, DNA methyltransferases, mice

Apoio Financeiro: FAPESP, CNPq and AFIP

A influência do tempo decorrido entre a administração de apomorfina e o teste comportamental no desenvolvimento da sensibilização em ratos

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Trabalhos mostram que a sensibilização pode ocorrer após uma única exposição à apomorfina (APO) enquanto em outros, a sensibilização só ocorre após várias exposições à droga. O objetivo do presente trabalho foi verificar a influência do tempo decorrido entre a administração de APO (2,0 mg/kg) e o início do teste comportamental no desenvolvimento da sensibilização e também a influência da reversão dos esquemas de administração na manutenção da sensibilização. No experimento 1, ratos Wistar machos após receberem APO (APO-0MIN, n=7) ou veículo (VEIC-0MIN, n=5) foram colocados imediatamente na arena onde a locomoção foi registrada por 40 min durante 3 dias (fase de indução). Após 7 dias de retirada de droga, houve a fase reversa, onde os animais foram colocados na arena 20 min após receberem os tratamentos durante 3 dias. No experimento 2 ocorreu o inverso; na fase de indução, os animais receberam APO (APO-20MIN, n=6) ou veículo (VEIC-20MIN, n=5) 20 min antes de serem colocados na arena e na fase reversa foram colocados na arena imediatamente após receberem os tratamentos. Os resultados mostraram que na indução do experimento 1, o grupo APO-0MIN apresentou maior locomoção que o VEIC-0MIN nos dias 1 (6,8 + 2,5 e 5,3 + 2,6; média + desvio padrão dos grupos APO e veículo, respectivamente), 2 (11,5 + 6,3 e 5,0 + 2,4) e 3 (13,8 + 7,3 e 3,8 + 2,6). Na fase reversa, o APO-0MIN manteve maior locomoção que o VEIC-0MIN nos dias 1 (11,3 + 6,0 e 5,5 + 2,3), 2 (14,7 + 7,4 e 4,4 + 3,0) e 3 (17,7 + 8,8 e 4,3 + 2,8). Já na indução do experimento 2, o APO-20MIN apresentou maior locomoção que o VEIC-20MIN somente no dia 3 (8,0 + 3,0 e 4,4 + 2,4), porém na fase reversa, o APO-20MIN apresentou maior locomoção que o VEIC-20MIN nos dias 1 (8,9 + 3,5 e 5,4 + 2,7), 2 (9,5 + 3,3 e 5,0 + 2,8) e 3 (10,8 + 4,7 e 5,0 + 3,0). A sensibilização ocorreu em ambos os grupos APO só que primeiro no grupo zero min. Porém, com a continuidade das administrações, houve a expansão temporal da resposta sensibilizada.

Palavras-Chave: Apomorfina, Atividade Locomotora e Resposta Sensibilizada.

Apoio Financeiro: UENF e FAPERJ

INVESTIGAÇÃO DOS EFEITOS DA KETAMINA NOS MODELOS DE ANSIEDADE E DEPRESSÃO.

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Objetivo: Investigar os efeitos da ketamina (Ket) nos modelos animais de ansiedade e depressão.

Métodos: Foram utilizados camundongos *Swiss* machos. Foram realizados os experimentos de Plus maze, campo aberto, nado forçado e suspensão da cauda, para a investigação de possíveis efeitos de ansiedade e depressão. Os animais foram divididos em três grupos com 10 animais cada: Controle (salina); Ket 20mg/Kg e Ket 50 mg/Kg. As drogas foram administradas intraperitonealmente, 30 minutos antes do início dos experimentos.

Resultados: Os resultados são apresentados como Média $\pm$ Erro Padrão da Média e a análise estatística foi feita pela ANOVA. As diferenças foram consideradas estatisticamente significativas em $p < 0,05$. Os resultados mostraram que a Ket em ambas as doses não foram capazes de alterar os parâmetros analisados no Pluz Maze (TPBA: salina $148,4 \pm 6,457$; Ket 20: $145,0 \pm 7,860$; Ket 50: $144,5 \pm 6,224$. PTBA: salina $51,00 \pm 2,733$; Ket 20: $47,60 \pm 2,709$; Ket 50: $50,00 \pm 2,022$. NEBA: salina $7,800 \pm 0,6464$; Ket 20: $7,300 \pm 0,6675$; Ket 50: $8,625 \pm 0,5957$. PEBA: salina $48,90 \pm 2,807$; Ket 20: $47,00 \pm 2,147$; Ket 50: $51,40 \pm 1,222$). A Ket também não alterou os parâmetros analisados no campo aberto (Atividade Locomotora: salina $58,60 \pm 5,147$; Ket 20: $51,40 \pm 4,067$; Ket 50: $49,30 \pm 4,203$. Rearing: salina $7,300 \pm 1,506$; Ket 20: $6,300 \pm 1,033$; Ket 50: $7,200 \pm 1,191$. Grooming: salina $17,30 \pm 2,813$; Ket 20: $13,40 \pm 3,307$; Ket 50: $19,80 \pm 2,776$). No entanto, no nado forçado a Ket na dose de 20mg/Kg foi capaz de reduzir significativamente o tempo de imobilidade (salina: $115,7 \pm 11,75$; Ket 20: $73,30 \pm 6,448$) e Ket na dose de 50 mg/Kg os animais morreram. No teste da suspensão da cauda a ket na dose de 50mg/Kg reduziu significativamente o tempo de imobilidade (salina: $116,9 \pm 3,683$; Ket 20: $107,2 \pm 13,14$; Ket: $55,10 \pm 8,989$). Nenhum efeito significativo foi observado na dose de 20 mg/Kg.

Conclusão: Verificamos que o tratamento agudo de Ket nos testes de Plus maze e campo aberto parece não possuir atividade ansiolítica nem excitatória do Sistema Nervoso Central. Porém, nos testes do nado forçado e suspensão da cauda a diminuição no tempo de imobilidade sugere uma possível atividade antidepressiva. **Palavras-Chave:** Ketamina, ansiedade, depressão

Apoio Financeiro: CAPES, CNPq e Funcap

CONDITIONED REINFORCING PROPERTIES OF TOLUENE: GENDER AND PRE-NATAL LEAD EXPOSURE

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Objectives: Lead exposure and solvent abuse are prevalent problems in urban areas. Pre-natal lead exposure influences drug-related behaviors in animal models. Toluene was shown to produce conditioned place preference in male rats. Also, studies have shown gender differences in response to psychoactive drugs. The purpose of this study was to examine the effects of pre-natal lead intoxication on the reinforcing properties of toluene using the conditioned place preference paradigm (CPP), and evaluate differences between the sexes. **Methods:** 79 Wistar rats, males and females, 5 months old, formed 4 groups: control-air (C), control-toluene (Pb0), pre-natal exposed to lead 8 mg (Pb8) and 16 mg (Pb16). The CPP apparatus consisted of 3 compartments, one dark with smooth floor, one light with grid floor and one neutral. CPP consisted of Pre-test, 10-day conditioning phase and Test. In the conditioning phase, animals were exposed alternatively to approximately 2000 ppm toluene or medicinal air, for five minutes, in pairs, inside a glass apparatus. Controls received only air. On toluene days, animals were placed for 15 minutes in the non-preferred compartment and on air days in the preferred one. CPP score was calculated by subtracting time spent on toluene-paired compartment in the Test minus the Pre-test (in seconds). Data were analyzed by ANOVA-2W, followed by SNK, and are presented as mean±sem. Differences were considered when $p < 0.05$. **Results:** No significant differences in the CPP score were found between lead-intoxicated animal and normal animals ($F(2,25)=0,257, p=0,774$). Toluene-treated animals showed significant conditioning ($48 \pm 14s$) when compared to controls ($-20 \pm 26s$) ($F(1,75)= 5,462, p=0,022$). Different behavioral patterns were found for each sex, as males exhibited stronger toluene conditioning than females (78 ± 19 vs $18 \pm 20s, p=0,037$). Females showed higher locomotion during the Test (23 ± 1 vs $16 \pm 0,9s, F(1,75)=26,357, p<0,001$). **Conclusions:** Pre-natal lead intoxication doesn't seem to influence toluene's conditioning ability in our experiment. On the other hand, sex may be an important feature for toluene's abuse properties, as males presented stronger conditioning.

Palavras-Chave: Toluene abuse, lead exposure, gender

Apoio Financeiro: UFCSPA, CNPq, AAPeFaTo

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Efeitos da administração do antagonista D1, SCH 23390 e D2, sulpiride sobre a supersensibilidade dopaminérgica

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Investigou-se os efeitos da administração de SCH 23390 (SCH-0,01, 0,02 e 0,05 mg/kg) e do sulpiride (SUL-10, 30 e 100 mg/kg) sobre a supersensibilidade avaliada pela apomorfina (agonista D1/D2-APO-2mg/kg). Ratos Wistar foram divididos em dois experimentos. No primeiro, administrou-se SCH e APO e os animais divididos em: SCH-veículo (SCH-VEIC; n=8), SCH-0,01 (n=6), SCH-0,02 (n=8), SCH-0,05 (n=7), (APO-VEIC; n=6), APO (n=6), durante cinco dias no tratamento farmacológico (TF), sendo o número de cruzamentos registrados por 30 minutos. Seguindo um período de retirada de droga de 5 dias, administrou-se APO (teste de sensibilidade; TS). O controle veículo recebeu veículo. No segundo, os ratos foram divididos, obedecendo ao mesmo protocolo, em grupos que receberam SUL (SUL-VEIC; n=8, SUL-10; n=7; SUL-30; n=6, SUL-100; n=6), e APO (APO-VEIC; n=6; APO; n=6). No experimento 1, no TF, ANOVA seguida do teste de Duncan mostrou que no dia 3, 4 e 5, o APO ($507,83 \pm 56,07$; $717,00 \pm 65,28$; $903,00 \pm 63,74$) apresentou n° de cruzamentos maior que os demais. Os SCH-0,02 ($21,91 \pm 14,41$), SCH-0,05 ($27,30 \pm 10,32$) apresentaram um menor n° de cruzamentos do que os demais. O SCH-0,01 ($51,63 \pm 18,84$) apresentou um n° de cruzamentos menor que os demais exceto dos SCH-0,02 e SCH-0,05. No TS, os APO ($692,00 \pm 93,83$) e SCH-0,05 ($624,57 \pm 116,17$) apresentaram um n° de cruzamentos maior que os demais. No experimento 2, no TF, os resultados mostraram que nos dias 3, 4 e 5, o APO ($507,83 \pm 56,07$; $717,00 \pm 65,28$; $903,00 \pm 63,74$) apresentou n° de cruzamentos maior que todos os demais. O SUL-100 ($66,87 \pm 28,97$) apresentou um n° de cruzamentos menor do que os SUL-VEIC ($116,77 \pm 38,62$), SUL-10 ($117,31 \pm 39,59$), SUL-30 ($122,43 \pm 37,53$) e APO-VEIC ($131,67 \pm 34,81$). No TS, o SUL-100 ($340,17 \pm 118,26$) apresentou um n° de cruzamentos maior do que os demais, exceto do APO ($692,00 \pm 93,83$). As administrações de APO produziram sensibilização comportamental. As doses elevadas de SCH e SUL produzem o desenvolvimento da supersensibilidade, entretanto, o SCH produziu maior magnitude de supersensibilidade do que o SUL.

Palavras-Chave: SCH23390, SULPIRIDE, APOMORFINA, LOCOMOÇÃO, SUPERSENSIBILIDADE

Apoio Financeiro: FAPERJ/UENF

The effects of naltrexone on the expression of behavioral sensitization to ethanol in maternally-separated male and female mice.

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Objectives: Long maternal separation (LMS) alters the opioid system, which is involved with behavioral sensitization to ethanol (EtOH). This study examined the effects of an opioid antagonist, naltrexone, on the expression of behavioral sensitization to ethanol in male and female mice.

Methods: From day 2 to 14, whole litters were submitted to LMS (3h/day) or not (animal facility rearing, AFR). On day 90, male and female AFR and LMS mice received 7 i.p. injections of saline (SAL) or EtOH (2.0 g/kg) spaced by 48h. Five days after the end of treatment, animals were submitted to 4 challenges spaced by 48h. The challenges were: SAL/SAL, SAL/EtOH, naltrexone (NTX, 1mg/kg)/SAL and NTX /EtOH given in two i.p. injections spaced by 30 min.

Results: Male and female mice developed behavioral sensitization to ethanol. In the challenges, LMS and AFR male mice showed higher locomotor activity to the SAL/EtOH challenge (AFR:SAL=846.7±87.2, EtOH=1050.4±177.3; LMS:SAL=820.3±235.5, EtOH=954.4±136.2) compared to SAL/SAL challenge (AFR:SAL=534.7±67.2, EtOH=500.5±78.2; LMS:SAL=339.0±90.3, EtOH=405.7±43.2) ($p<0.05$). The locomotor activity of AFR mice was similar between NTX/EtOH (SAL=675.9±103.5, EtOH=735.9±122.6) and SAL/SAL challenge ($p>0.05$) with a trend for NTX to decrease the stimulant effect of ethanol ($p=0.06$); LMS mice showed higher locomotor activity in the NTX/EtOH (SAL=973.2±345.7, EtOH=698.4±108.4) than in the SAL/SAL challenge ($p<0.01$). In females, LMS and AFR mice were similar in the challenges. EtOH pre-treated mice showed higher locomotor activity in the SAL/EtOH than SAL pre-treated mice (AFR: 761.1±104.0; LMS=866.9±164.8) ($p<0.05$) and higher locomotor response to NTX/EtOH (AFR=1551.9±239.3; LMS=1554.8±432.6) than to SAL/SAL challenge, but no difference from SAL/EtOH challenge ($p>0.05$).

Conclusions: LMS did not influence the locomotor response to EtOH and NTX did not block the behavioral sensitization in female mice; in male mice, NTX tend to decrease EtOH-induced locomotor activity in AFR, but not in LMS mice.

Palavras-Chave: behavioral sensitization, maternal separation, ethanol, gender differences

Apoio Financeiro: AFIP, CNPq, FAPESP

DETECÇÃO PRECOCE E INTERVENÇÃO BREVE PARA O USO DE RISCO E NOCIVO DE DROGAS: AVALIAÇÃO DAS CRENÇAS, ATITUDES E HABILIDADES ENTRE PROFISSIONAIS E ALUNOS DA ÁREA DE SAÚDE.

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Objetivo: Avaliar as crenças, atitudes e habilidades de profissionais e alunos da área de saúde em relação à detecção precoce e intervenção breve (IB) para uso de risco e nocivo de drogas.

Método: 142 profissionais de saúde da Atenção Primária (APS) de Curitiba, Lapa e São José dos Pinhais e 20 alunos do curso de graduação em Terapia Ocupacional (TO) da Universidade Federal do Paraná foram treinados para fazer detecção precoce do uso de risco e nocivo de drogas utilizando o ASSIST (*Alcohol Smoking and Substance Involvement Screening Test*) e IB. Antes do treinamento, os participantes responderam 58 questões sobre crenças, atitudes e habilidades relativas ao usuário de drogas, à detecção precoce e à IB. As respostas foram avaliadas e agrupadas de acordo com seus conteúdos obtendo-se 12 novas afirmativas. ANOVA de uma via seguida por teste de Duncan foi utilizada para comparar as médias de porcentagem de discordância para as afirmativas entre os grupos de participantes. Uma análise fatorial considerando-se as 58 questões com todos os participantes foi realizada para detectar as respostas correlacionadas entre si e promover a interpretação dos fatores extraídos.

Resultados: Os participantes apresentaram valores altos para as médias dos percentuais de discordância para crenças e atitudes negativas e valores altos de concordância para as positivas. Os alunos relataram falta de habilidade [$F(5,141)=2,48$ $p<0,04$], crença na eficácia do tratamento [$F(5,129)=2,93$ $p<0,02$] e acham que é papel do profissional lidar com usuários de risco [$F(5,144)=2,89$ $p<0,02$]. Os profissionais de Curitiba relataram menos desconforto ao lidar com usuários de drogas [$F(5,139)=2,66$ $p<0,02$]. Da análise fatorial foram extraídos 18 fatores que forneceram conclusões semelhantes às obtidas pela análise das 12 afirmativas.

Conclusão: Em geral, independente do grupo participante, as crenças e atitudes foram positivas em relação ao procedimento de detectar usuários de drogas com intervenção precoce. Os alunos da TO concordaram mais que é papel do profissional lidar com usuários de risco, provavelmente porque não conhecem ainda as dificuldades e a complexidade de lidar com usuários de drogas.

Palavras-Chave: abuso de drogas, detecção precoce, intervenção breve

Apoio Financeiro: CnPq- Conselho Nacional de Desenvolvimento Científico e Tecnológico

DETECÇÃO PRECOCE E INTERVENÇÃO BREVE PARA O USO DE RISCO E NOCIVO DE DROGAS: AVALIAÇÃO DAS BARREIRAS PARA IMPLEMENTAÇÃO NA ATENÇÃO PRIMÁRIA À SAÚDE EM TRÊS MUNICÍPIOS DO PARANÁ.

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Objetivo: Avaliar as barreiras para a implementação de um projeto para detecção precoce e intervenção breve (IB) para uso de risco e nocivo de drogas.

Método: 85 profissionais de saúde da Atenção Primária (APS) de Curitiba, Lapa e São José dos Pinhais foram treinados para fazer detecção precoce do uso de risco e nocivo de drogas utilizando o ASSIST (*Alcohol Smoking and Substance Involvement Screening Test*) e IB. Antes e após o treinamento (n=45), os participantes responderam questões sobre crenças, atitudes e habilidades relativas ao usuário de drogas, à detecção precoce e à IB. As respostas foram avaliadas e agrupadas segundo seus conteúdos em 12 novas afirmativas. Uma análise fatorial com todos os participantes foi realizada para detectar as respostas correlacionadas entre si e promover a interpretação dos fatores extraídos. A comparação entre as respostas obtidas antes e depois do treinamento foi feita por teste de Wilcoxon para as 12 afirmativas e os 18 fatores. Foram realizadas entrevistas com os gestores das unidades de APS dos municípios e uma reunião de grupo focal, sendo seus depoimentos transcritos, interpretados e reunidos de acordo com seus conteúdos.

Resultados: Para as 12 afirmativas, notou-se que os profissionais de Curitiba passaram a acreditar menos na eficácia da IB ($p<0,05$), acharam mais desconfortável lidar com usuários de drogas ($p<0,002$) e acharam o tempo insuficiente para lidar com os usuários de risco ($p<0,02$). Os profissionais de São José dos Pinhais acharam que o tempo foi insuficiente para lidar com o problema ($p<0,02$), acharam menos importante detectar ($p<0,005$) e acharam que o treinamento promoveu melhora nas suas habilidades ($p<0,001$). Tanto os profissionais (P) que participaram do grupo focal como os gestores (G) entrevistados relataram como principais barreiras falta de tempo (P: 68%, G: 59%), de estrutura física (P: 53%, G: 27%) e de profissionais (P: 26%, G: 36%).

Conclusão: O treinamento melhorou o conhecimento, as habilidades e a confiança em detectar e intervir precocemente em usuários de drogas. A implementação foi prejudicada devido as seguintes barreiras: falta de tempo, de estrutura física e de profissionais.

Palavras-Chave: drogas de abuso, intervenção breve, detecção precoce.

Apoio Financeiro: CnPq - O Conselho Nacional de Desenvolvimento Científico e Tecnológico

Effects of prenatal exposure to the cannabinoid agonist WIN 55,212-2 on Growth-Associated Protein-43 expression in rat hippocampus.

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There is growing evidence that endocannabinoid system plays a modulatory role in specific processes of brain development such as cell proliferation, migration, neuritic elongation and guidance and synaptogenesis. The aim of this study was to assess the effects of prenatal exposures to cannabinoid CB1/CB2 receptor agonist WIN55,212-2 (WIN) on the expression of growth-associated protein 43 (GAP43), a marker of growth cones and axons, on hippocampus. GAP43 plays important roles in synaptogenesis, axonal path finding as well as regulation of cytoskeletal organization in the nerve ending. Pregnant Wistar rats were treated, from gestational day 5 to 20, at a daily WIN dose (0.75mg/kg) which causes no evident signs of toxicity or teratogenesis. Control rats (C) were injected with vehicle (0.3%Tween80/saline). One pup per litter was decapitated at postnatal day 1 (P1) and it's brain was fixed by immersion in 4% paraformaldehyde in PBS for 4 hs. Frozen brains were sliced in a cryostat and expression of GAP43 analyzed by immunohistochemistry. The body weight of dams, length of pregnancy, litter size at birth and body weight at P1 were monitored in order to evaluate possible effects of WIN exposure on reproduction and postnatal development. Prenatal exposure to WIN did not influence dam weight gain from days 0 to 20 of pregnancy (M $\pm$ SEM; C=45.7 $\pm$ 3.3; WIN=37.9 $\pm$ 1.5%), pregnancy length (C=21.3 $\pm$ 0.3; WIN=22 $\pm$ 0 days), litter size at birth (C=13.7 $\pm$ 1.8; WIN=11.7 $\pm$ 0.9) and body weight at P1 (C=6.7 $\pm$ 0.6; WIN=6.9 $\pm$ 0.2g). Exposure to WIN significantly reduced the expression of GAP43 in the stratum radiatum (SR) of de CA3 region (C= 35.1 $\pm$ 1.7; WIN= 24.9 $\pm$ 0.8%Optical Density; p<0,05). No significant changes were observed in GAP43 staining at the stratum pyramidale (SP) and stratum oriens (SO) of the CA3 or SR, SP and SO of the CA1 region. Since prenatal exposure to WIN resulted in a significant reduction of GAP43 in the CA3 SR, there might be some deleterious synaptic changes in the rat hippocampus. This work suggests that reduction of GAP43 expression might contribute to cognitive and neuroplastic defects associated with prenatal exposure to cannabinoids, previously reported.

Palavras-Chave: cannabinoids, hippocampus, growth-associated protein-43, prenatal exposure.

Apoio Financeiro: PICT 31964, UBACYT M-072

**EFEITO PRÓ-DEPRESSIVO DO CHÁ DE AYAHUASCA EM CAMUNDONGOS
SUBMETIDOS AO TESTE DO NADO FORÇADO.**

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OBJETIVO: O chá de ayahuasca vem sendo utilizado popularmente no Brasil durante rituais religiosos. Sabe-se que os principais princípios psicoativos do chá são a harmina e a dimetiltryptamina. A harmina apresenta uma ação inibitória sobre a enzima monoaminooxidase A (MAO-A), enquanto que a dimetiltryptamina atua como agonista dos receptores serotoninérgicos 5-HT₂. Neste sentido, o objetivo do presente trabalho foi de verificar um possível efeito antidepressivo do chá de Ayahuasca em camundongos submetidos ao teste do nado forçado.

MATERIAIS E MÉTODOS: Para avaliar um possível efeito antidepressivo do chá de Ayahuasca, foram utilizados os testes do campo aberto e nado forçado. Camundongos suíços machos (90 dias, 45 g), foram tratados com o chá por via oral, 24 h, 6 h e 1 h antes da realização dos testes comportamentais, formando os seguintes grupos experimentais: Cont (*N*=8) – animais tratados com solução salina; Aya1,25 (*N*=8) – animais tratados com chá de Ayahuasca na dose de 1,25 mL/Kg; Aya2,5 (*N*=8) – animais tratados com chá de Ayahuasca na dose de 2,5 mL/Kg; Aya10 (*N*=8) – animais tratados com chá de Ayahuasca na dose de 10 mL/Kg. Vale a pena ressaltar que a primeira dose equivale aproximadamente àquela comumente ingerida pelos usuários durante os rituais.

RESULTADOS: ANOVA seguida de Newman Keuls *post hoc* mostrou que todas as doses empregadas do chá aumentaram consideravelmente o tempo em que os animais permaneceram em posição de imobilidade durante o teste do nado forçado, quando comparado aos animais tratados com salina [$F_{(3,28)}=4,82$ $p=0,01$]. No entanto, somente as doses de 2,5 e 10 mL/Kg foi capaz de diminuir o número de quadros explorados [$F_{(3,28)}=27,78$ $p=0,01$] e aumentar o tempo que o animal permaneceu parado durante o teste do campo aberto [$F_{(3,28)}=3,61$ $p=0,01$], comparados aos animais tratados com salina e com o chá na dose de 1,25 mL/Kg.

CONCLUSÃO: Nossos resultados sugerem que o chá de Ayahuasca, administrado em camundongos em dose similar aquela utilizada popularmente em rituais, apresenta um efeito pró-depressivo, haja vista que a mesma foi capaz de aumentar o tempo de imobilidade no nado-forçado, sem causar alterações evidentes nos parâmetros avaliados no teste do campo aberto. Uma possível explanação para os efeitos encontrados seria que a harmina (MAO-A) impediria a metabolização da dimetiltryptamina (agonistas 5-HT₂) no trato gastrointestinal, que por sua vez, ao atuar em receptores 5-HT_{2A} no sistema nervoso central, poderia promover comportamento pró-depressivo observado no presente trabalho.

Palavras-Chave: Chá de Ayahuasca, Campo Aberto, Nado Forçado, Efeito Pró-Depressivo

Apoio Financeiro: Cnpq

Diferenças no desempenho da função mental entre homens e mulheres alcoolistas

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Dos 20 milhões de alcoolistas norte-americanos, a metade apresenta déficit cognitivo sendo que, destes 10 milhões, mais de 2 milhões possuem debilidades que exigem custódia médica contínua. No Brasil, 11,2% da população apresenta critérios de inclusão diagnóstica como dependente do álcool, ainda assim, dados brasileiros que caracterizam e relacionam alcoolistas e demência são escassos. **Objetivo:** Avaliar as funções mentais dos alcoolistas atendidos no PAA/HUCAM/CCS/UFES entre Janeiro de 2007 e Abril de 2008. **Métodos:** Foram aplicados em 263 alcoolistas questionários referentes a dados sócio-demográfico (nome, idade, gênero, estado civil, município onde reside, grau de escolaridade e situação trabalhista); consumo de álcool (quantidade ingerida, idade de início de uso, tempo de abstinência) e o Mini Exame do Estado Mental (Mini-Mental) como forma de avaliação cognitiva para sugestão de demência.

Resultados: Dos 263 alcoolistas entrevistados, 87,1% eram homens e 12,9% eram mulheres. A pontuação média da amostra total de alcoolistas no Mini-Mental foi de 24,8(DP±4,47), dos homens alcoolistas foi de 25,24(DP±4,13) e das mulheres alcoolistas foi de 21,76(DP±5,49). Ajustados os escores do Mini-Mental de acordo com a escolaridade do paciente, 27,1% dos alcoolistas apresentavam pontuação sugestiva de demência. Quando separados por gênero, evidenciou-se que 24% dos homens alcoolistas tiveram resultado sugestivo para demência, ao passo que 48,5% das mulheres alcoolistas obtiveram esse resultado. Apenas a escolaridade mostrou-se estatisticamente significativa como preditor a demência. **Conclusão:** Este trabalho evidenciou que aproximadamente 25% dos alcoolistas apresentam déficit cognitivo sugestivo de demência, sendo esta prevalência duas vezes maior nas mulheres alcoolistas do que nos homens alcoolistas. Maiores investigações devem ser feitas a fim de estimar a real prevalência de demência entre os alcoolistas, as diferenças de desempenho cognitivo entre os gêneros e, se confirmadas, abordagens terapêuticas diferenciadas devem ser orquestradas para o grupo de alcoolistas com prejuízo severo das funções mentais com o intuito de se obter maior sucesso terapêutico. **Palavras-Chave:** Alcoolismo; demência induzido quimicamente

Desempenho ocupacional e drogadição em adolescentes

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Objetivo: Nas duas últimas décadas o elevado consumo de substâncias psicoativas vem sendo um dos maiores desafios da contemporaneidade. O objetivo deste estudo é identificar na literatura científica as principais alterações dos componentes do desempenho ocupacional de adolescentes usuários de substâncias psicoativas. **Metodologia:** Desenvolveu-se uma pesquisa bibliográfica nas bases de dados da Bireme, PubMed, Biblioteca Digital da UNICAMP, além do acervo digital da revista de Terapia Ocupacional da USP, da revista Chilena de Terapia Ocupacional e da revista de Terapia Ocupacional Galicia, no idioma inglês, espanhol e português, nos últimos 10 anos. Utilizaram-se como descritores os termos: Saúde do Adolescente, Transtornos Relacionados ao Uso de Substâncias, Terapia Ocupacional. Além de obras de referência no campo da Neurologia e Terapia Ocupacional. **Resultados:** A *American Academy of Pediatrics* estabelece seis estágios no envolvimento do adolescente com substâncias psicoativas: abstinência, uso experimental/recreacional, abuso inicial, abuso, dependência e recuperação. Os comprometimentos advindos do abuso referem-se a a **Palavras-Chave:** adolescentes, comportamento, desempenho ocupacional, dependência química, terapia ocupacional

Wane of mesencephalic 5-HT could underlie the negative affective states observed in rats under diazepam withdrawal

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Objectives: This study was designed to evaluate i) the role of dorsal periaqueductal gray (dPAG) on the modulation of the anxiety induced by withdrawal (WD) from diazepam (Dz), ii) the role of 5-HT receptors of the dPAG and dorsal raphe nucleus (DR) on the modulation of this state. **Methods:** Male Wistar were submitted to 18-day oral intake of sucrose (Suc, 5%) or Dz (10 mg/kg). A cannula and a chemitrode aimed to the DR and dPAG, respectively, were implanted on day 15. After 30 min or 48 h from the last drug intake the aversive thresholds for the electrical stimulation (EE) of the dPAG for freezing (Fz) and escape (Esc), and the ultrasonic vocalizations (USV's) elicited in these two conditions, were registered. Two hours after WD sessions the animals were submitted to a local injection of 5-HT in the dPAG or DR and the aversive thresholds were again determined. Additional groups were tested in the elevated plus-maze (EPM) in order to assure the effectiveness of the chronic regimen to promote WD. **Results:** Except for the EPM, raw-data underwent square-root transformation. Two-way RM ANOVA showed significant differences in the EE thresholds of rats tested under Dz effects (Suc: Fz*5.59±0.25, Esc*7.78±0.44; Dz: Fz*9.18±0.40, Esc*11.96±0.49). Decreased 22-kHz emissions in Dz group (Suc: 4.67±0.65; Dz: 2.50±0.31) and later increases on WD (Suc: 5.09±0.44; Dz: 8.15±0.86) were noted during Fz. Despite WD from Dz does not change the aversive thresholds, increased levels of anxiety was achieved in EPM test (% time OA; Suc: 26.53±3.12; Dz: 8.25±0.55). ANOVA showed significant effects of 5-HT on Fz and Esc thresholds after injections into dPAG (Suc: Fz*6.07±0.54, Esc*7.55±0.88; 5-HT: Fz*7.95±0.45, Esc*11.84±1.64; Dz WD: Fz*5.96±0.24, Esc*8.12±0.60; 5-HT: Fz*8.17±0.48, Esc*11.13±0.73) in both Suc and Dz groups. Main effects of 5-HT into DR was achieved only in the Esc response of Dz pre-treated rats (Suc: 9.84±1.64, Dz: 13.35±0.73). **Conclusions:** i) In spite of rats under Dz WD have showed similar overt responses after EE of the dPAG, an increased level of anxiety- but not panic-like state, probably mediated by dPAG 5-HT neurons themselves and a DR-dPAG pathway, was present.

Palavras-Chave: Diazepam withdrawal, anxiety, dPAG, dorsal raphe, 5-HT

Apoio Financeiro: FAPESP (04/02859-0)

**REINSTALACION DE LA CONDUCTA DE PREFERENCIA A UN CONTEXTO
PREVIAMENTE ASOCIADO CON COCAINA: IMPORTANCIA DE LA DURACION DEL
ESTRESOR**

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La prueba de condicionamiento apareado a un contexto (CPP) es usada para medir tanto las propiedades reforzantes como la reinstalación de la conducta de búsqueda de la droga luego de un período de abstinencia (1). El objetivo del presente estudio fue inducir la reinstalación de la preferencia por un sitio previamente apareado con cocaína mediante estrés por inmovilización y determinar la relación entre la duración del estresor y su capacidad para inducir dicha conducta.

Ratas Wistar macho de 2 meses de edad fueron inyectadas con 10 mg/kg de cocaína y confinadas a uno de dos contextos en sesiones alternadas droga/vehículo, evaluándose dos días después la preferencia por uno u otro contexto en una situación libre de drogas (test). La fase de extinción consistió en apareamientos sucesivos con vehículo en ambos contextos. La reinstalación de la preferencia por el contexto previamente asociado con cocaína se evaluó sometiendo a todos los animales a estrés por inmovilización durante 15, 30 ó 60 min en el contexto neutro (central) y registrando el tiempo de permanencia en todos los contextos. En otro grupo de animales se evaluó la reinstalación mediante la administración de 5 mg/kg de cocaína.

Los resultados demuestran que la administración repetida de la droga en forma contingente con el confinamiento indujo preferencia condicionada, la cual fue extinguida luego de apareamientos sucesivos con vehículo. La inmovilización durante 30 y 60 min (y no 15 min) fue suficiente para inducir la reinstalación de la preferencia por el sitio previamente apareado con cocaína, observándose la mayor discriminación entre ambos contextos cuando la duración del estresor fue de 60 min. Del mismo modo, la cocaína fue capaz de inducir reinstalación de la preferencia por el contexto previamente asociado a la droga. Estos resultados demuestran la dependencia de la duración del estresor en la conducta de reinstalación a cocaína, y apoyan la hipótesis de mecanismos comunes en la intercambiabilidad de las respuestas inducidas por drogas psicoestimulantes y estrés.

(1) Mueller, D & Stewart, J. Behavioral Brain Research 115: 39-47 (2000).

Palavras-Chave: cocaína - estres - reinstalacion - preferencia condicionada -

Apoio Financeiro: FONCyT - CONICET - SECyT

INFLUÊNCIA DA KETANSERINA SOBRE OS EFEITOS ESTIMULANTE E ANSIOLÍTICO DO ETANOL EM CAMUNDONGOS

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Objetivos: O objetivo do trabalho foi avaliar a influencia da ketanserina, antagonista 5-HT_{2A}, sobre o desenvolvimento de sensibilização induzida pelo etanol e sobre o seu efeito ansiolítico.

Métodos e Resultados: Camundongos Swiss machos (n=118; 15 a 23 por tratamento) foram avaliados sem droga (basal) quanto à porcentagem de tempo no braço aberto (efeito ansiolítico) no labirinto em cruz elevado (LCE) e à ambulação na caixa de atividade (CA). Sete dias depois, cada animal foi agudamente administrado (i.p.) com um dos seguintes tratamentos: salina+salina (S), salina+etanol 2g/Kg (E), salina+ketanserina 1,25mg/Kg (K1), ketanserina 1,25mg/kg+etanol 2g/kg (E+K1), salina+ketanserina 5mg/Kg (k2), ketanserina 5mg/kg+etanol 2g/kg (E+k2), e avaliados no LCE e CA 10 minutos após a segunda injeção. Receberam esse mesmo tratamento diariamente sendo novamente avaliados no 7º e 21º dias. Os dados estão apresentados em média e erro padrão e analisados por ANOVA seguida de Newmann Keuls. Etanol cronicamente aumentou significativamente a ambulação em relação ao basal e sem significância em relação ao agudo (basal: 68±3,2; agudo: 82±9,5; 21dias:102±12,2) e a co-administração E+K1 e E+K2 bloqueou esse aumento (basal: E+k1=58±3,2; E+k2=69±6,0; agudo: E+k1=29±5,4; E+k2=16±3,0; 21dias: E+K1=61±12,9; E+K2=52±11,8). O etanol e a associação E+K1 e E+K2 cronicamente preveniram a ansiogenese do reteste observada nos grupos S, K1 e K2 (agudo: E=59±2,7; S=51±4,5; E+K1=48±7,3; E+K2=49±9,7; 21dias: E=63±5,7; S=37±5,6; E+K1=51±7,1; E+K2=50±6,9).

Conclusão: A ativação dos receptores 5-HT_{2A} aumenta a liberação de dopamina no núcleo accumbens (NAcc), sendo importante no efeito estimulante do etanol e no desenvolvimento de sensibilização comportamental. O bloqueio desses receptores diminuiria a liberação de dopamina no NAcc, impedindo o desenvolvimento da sensibilização ao efeito estimulante do etanol. O efeito ansiolítico do etanol não é influenciado por esse receptor serotoninérgico.

Palavras-Chave: etanol, ketanserina, sensibilização

Apoio Financeiro: CAPES

Combined exposure to nicotine and ethanol in adolescent mice: Effects on the central cholinergic system.

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Co-occurrence of tobacco smoking and alcohol consumption during adolescence is frequent and well documented. However, little is known about the basic neurobiology of the combined exposure in the adolescent brain. Since nicotine is a cholinergic agonist and it has been shown that ethanol interferes with nicotinic acetylcholine receptors (nAChRs), the current work will focus on cholinergic systems. Particularly, we assessed nAChR binding, choline acetyltransferase (ChAT) activity, a constitutive marker for cholinergic nerve terminals, and [³H]hemicholinium-3 (HC-3) binding to the high affinity choline transporter, which responds to cholinergic synaptic stimulation. From the 30th to the 45th postnatal day, C57BL/6 mice (15-19 mice per group, equally divided by sex) were exposed to nicotine free base (NIC) and/or ethanol (ETOH). Mice were distributed into 4 groups: 1) Concomitant NIC [50µg/ml in 2% saccharin to drink] and ETOH [25%, 2g/kg i.p. injected every other day] exposure; 2) NIC exposure; 3) ETOH exposure; 4) Vehicle. Brain tissues were analyzed on the 45th postnatal day. In the cerebral cortex, adolescent ethanol exposure had no effect on nAChRs. In contrast, nicotine produced nAChR upregulation (NIC>VEH: P=0.04) while the combined NIC+ETOH treatment elicited an even more pronounced (NIC+ETOH>VEH: P=0.0006) effect. In the midbrain, neither ethanol nor nicotine exposure had effects on nAChRs. The NIC+ETOH treatment, however, elicited a robust nAChR upregulation (NIC+ETOH>VEH: P=0.001). Regarding ChAT activity, treatment effects differed for males and females in the cerebral cortex. Male NIC mice presented an increase in ChAT activity (NIC>VEH, P=0.01). However, ethanol reversed the nicotine-induced effect. In contrast, female NIC mice presented decreased ChAT activity (NIC<VEH: P=0.04). In the midbrain, ETOH exposure elicited increased ChAT activity (ETOH<VEH, p=0.005). There were no alterations in HC-3 binding. The results obtained in this study indicate that the central cholinergic system is a site at which nicotine and ethanol interact. This interaction might underlie the association between tobacco and alcohol consumption during adolescence. **Palavras-Chave:** Adolescence, choline acetyltransferase, choline transporter, nicotinic cholinergic receptors

Apoio Financeiro: CNPq, FAPERJ, SR2-UERJ, Philip Morris USA Inc. and Philip Morris International

GABAergic disturbances in the basolateral amygdala can underlie increased acoustic evoked potentials observed in the central nucleus of the inferior colliculus of diazepam withdrawn-rats.

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Objectives: It has been suggested that the basolateral nucleus of the amygdala (AMbl) regulates the processing of auditory aversive information in the inferior colliculus (IC). Withdrawal (WD) from diazepam (Dz) increases anxiety and Fos-immunolabelling in the IC. This study examined the influence of GABA systems in the AMbl on the processing of acoustic information in the IC of rats under Dz WD, assessed by the recording of auditory-evoked potentials (AEP). **Methods:** Male Wistar rats were submitted to 18-day oral intake of sucrose (Suc, 5%) or Dz (10 mg/kg). A cannula and a bipolar electrode aimed to the AMbl and the central nucleus of the IC, respectively, were implanted on day 15. After 30 min and 48 h from the last drug intake the AEP were recorded. GABA modulation on the AEP was analysed 30 min after local AMbl injections (0.2 ml) of the GABA_A agonist muscimol (MUS 1 nmol) or the glutamic-acid decarboxylase inhibitor semicarbazide (SMC, 6 mg), 2 h after WD sessions. AEP were recorded as the voltage difference between the tips of the electrode. The data acquisition sweep was on for 10 ms before the onset of the sound stimulation and 200 ms after it. A software was programmed to sum up the 100 individual AEP. **Results:** Raw-data underwent square-root transformation. Later, two-way RM ANOVA was used. No changes were observed in rats tested under Dz effects (7.95 ± 0.59) compared with Suc group (8.24 ± 0.50). Significant difference was noted on WD (Suc: 8.22 ± 0.72 , Dz: 11.52 ± 1.00). MUS did not change WD effects in both Suc and Dz groups. SMC increased AEP in control rats (Suc: 8.22 ± 0.72 , SMC: 12.92 ± 0.50) and decreased this measure in rats WD from Dz (WD: 11.52 ± 1.00 , Dz: 9.51 ± 0.74). **Conclusions:** i) Chronic Dz treatment do not change the amplitude of AEP in the IC, revealing that its anxiolytic properties do not interfere with the processing of the auditory information; ii) the same animals, tested on WD, have increased amplitude of AEP showing that WD increases the negative valence of a previously innocuous acoustic stimuli; iii) it is likely that changes in GABA systems on the AMbl can contribute to the increased levels of anxiety observed in rats withdrawn from Dz.

Palavras-Chave: Diazepam withdrawal, anxiety, GABA, basolateral amygdala, inferior colliculus, auditory evoked-potentials

Apoio Financeiro: FAPESP (04/02859-0)

INTERAÇÃO ENTRE O MODAFINIL E A COCAÍNA EM UM MODELO ANIMAL DE DEPENDÊNCIA QUÍMICA

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Este trabalho teve como objetivo investigar se o modafinil produziria *per se* sensibilização comportamental (um modelo animal de dependência), bem como se haveria sensibilização cruzada entre o modafinil e a cocaína. Foram usados camundongos Swiss fêmeas (30-35g – 3 meses), distribuídos em 5 grupos (n=12): S-S-S, S-S-C, S-M64-C, M64-M64-C, M128-M64-C. Os animais foram habituados (3 dias) no campo aberto (CA) recebendo salina (S) i.p. No dia do primeiro teste os grupos S-S-S, S-S-C e S-M64-C receberam salina e os grupos M64-M64-C e M128-M64-C receberam modafinil (M) nas doses de 64 e 128 mg/kg, respectivamente. Após 7 dias, os animais foram desafiados no CA: os grupos S-S-S e S-S-C receberam salina (ip) e os grupos S-M64-C, M64-M64-C e M128-M64-C receberam M 64 mg/kg (ip). Após 12 dias todos os grupos, com exceção do S-S-S, receberam cocaína 10 mg/kg (ip). As injeções foram administradas 30 minutos antes dos testes, com exceção do desafio cocaína (5 min antes do teste). Os animais permaneceram 10 min no CA para quantificação da atividade locomotora (LO). A análise estatística foi feita por ANOVA seguida do teste de Duncan. Na primeira observação, verificou-se que os grupos M64-M64-C e M128-M64-C (285 ± 38 e 501 ± 40) apresentaram um aumento da LO em relação ao grupo S-S-S (145 ± 34). No desafio M, os grupos S-M64-C, M64-M64-C e M128-M64-C (308 ± 53 , 435 ± 69 e 625 ± 63) exibiram LO maior ($p < 0,05$) que aquela do grupo S-S-S (104 ± 19). Além disso, o grupo M128-M64-C apresentou LO aumentada em relação ao grupo S-M64-C, revelando o desenvolvimento de sensibilização comportamental. No desafio cocaína, todos os grupos desafiados com a droga apresentaram aumento da LO em relação ao grupo S-S-S. Ainda, o grupo M128-M64-C apresentou um aumento significativo da LO ($p < 0,05$) em relação ao grupo S-S-C, revelando a sensibilização cruzada entre modafinil e cocaína. Os resultados indicam que o modafinil induz sensibilização aguda dependente de dose em camundongos. Essa sensibilização prévia pode potencializar os efeitos estimulantes da cocaína (sensibilização cruzada). **Palavras-Chave:** Modafinil, sensibilização

Apoio Financeiro: CAPES

AÇÃO ANSIOLÍTICA DO PROPRANOLOL E ONDANSETROM EM RATOS SUBMETIDOS AO TESTE DO PLUS MAZE APÓS ABSTINÊNCIA DE COCAÍNA SUBCRÔNICA.

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Objetivos: Estudos prévios têm demonstrado que a abstinência ao uso repetido de cocaína frequentemente provoca sintomas de ansiedade (1) e diversos fármacos têm sido testados na tentativa de reverter tais sintomas. Assim, o presente trabalho teve como objetivo investigar as alterações comportamentais de ratos submetidos ao teste do *Plus maze* 24h após a retirada de cocaína, bem como verificou se o ondansetrom e o propranolol seriam capazes de reverter o provável comportamento de ansiedade dos animais.

Métodos: A cocaína (20 mg/kg, i.p.) foi administrada durante 7 dias consecutivos a ratos *Wistar* machos (150-200g) e as alterações comportamentais foram avaliadas 24h após a última administração. Para tanto, os animais foram submetidos ao teste comportamental *Plus Maze*. Trinta minutos antes da realização do teste, os animais receberam salina (1ml/kg), propranolol (10mg/kg, i.p.) ou ondansetrom (4mg/kg, i.p.). Para a análise estatística foi utilizado ANOVA, seguida de Newman Keuls *post hoc*.

Resultados: Os resultados mostraram que a abstinência ao tratamento repetido com cocaína alterou todos os parâmetros no teste do *Plus Maze*, quando comparados ao grupo controle, os quais foram revertidos pela administração prévia de ondansetrom e propranolol: número de entradas nos braços abertos (Controle: 3,30±0,40; Coca: 2,0±0,46; Coca + Ondansetrom: 5,37±0,56; Coca + Propranolol: 5,50±0,67; p<0,05); número de entradas nos braços fechados (Controle: 4,80±0,51; Coca: 8,22±0,68; Coca + Ondansetrom: 6,43±0,72; Coca + Propranolol: 5,0±0,62; p<0,05); tempo de permanência nos braços abertos (Controle: 53,75±2,85; Coca: 20,17±4,75; Coca + Ondansetrom: 76,5±5,40; Coca + Propranolol: 46,2±6,24; p<0,05); tempo de permanência nos braços fechados (Controle: 203,7±10,60; Coca: 256,6±10,32; Coca + Ondansetrom: 202,9±7,33; Coca + Propranolol: 264,4±9,52; p<0,05).

Conclusão: Os resultados indicam que a abstinência ao tratamento repetido com cocaína provocou ansiedade nos animais, que foi revertida tanto pelo ondansetrom quanto pelo propranolol, caracterizando uma ação ansiolítica dessas drogas durante a abstinência de cocaína.

Referências: 1- Addictive Behaviors 30 (2005) 1125–1134

Palavras-Chave: Cocaína, abstinência, propranolol e ondansetrom

Apoio Financeiro: CAPES e CNPq

Previous elevated plus-maze exposure is required for the anxiety-inducing place preference for the open-arms in morphine-withdrawn rats

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Objectives: This study was designed to evaluate the power of the negative-affective states elicited during withdrawal (WD) from low doses of chronic-morphine (MF) regimen in promotes place preference (PP) in rats conditioned in a conflict paradigm in which drug effects were paired with an aversive context, in this case, the distal part of the open arms (OA) of the elevated plus-maze (EPM). The influence of previous exposure to EPM on conditioning was also evaluated. Additional groups were tested under MF effects. **Methods:** Male Wistar rats (180-200 g) were submitted to 10 days of chronic s.c. injections of saline (SAL) or MF (10 mg, 2 ml/kg). Immediately after each injection animals were confined on the distal parts of the OA of the EPM, in order to experience the drug effects on this aversive context. The traditional (% OA time, % of OA entries and number of closed arms (CA) entries), OA scrutiny (distance run, number of entries and time spent in open extremities) and ethological measures (head-dipping and rearing behaviors) were recorded as a representative index of anxiety, OA PP and drug-seeking behavior, respectively. The frequency of stretch-attending postures (SAP) was taken as a measure of risk-assessment behavior (RA). The number of CA entries was taken as an index of locomotor activity. Animals were tested 30 min or 48 h after the last injections. **Results:** Three-way ANOVA (EPM: naive/experienced; treatments: SAL/MF; condition: drug effects/WD) was used. No significant differences were obtained in naive rats tested under MF effects or on WD. Significant PP ($p < 0.05$) was obtained in experienced rats tested under MF effects or on WD (% time OA: $F_{1,68}=49.24$; % OA entries: $F_{1,68}=30.32$). Increases in motor activity were found only in rats tested under morphine effects ($F_{1,68}=10.57$). Increases in the frequency of RA behavior were found only in rats withdrawn from ($F_{1,68}=8.46$). **Conclusions:** Rats naive for the EPM do not develop PP for the distal parts of the OA. However, PP was obtained in experienced animals tested under MF WD showing that WD-inducing anxiety could underlie drug-seeking behavior in rats conditioned in a familiar context.

Palavras-Chave: Morphine withdrawal, anxiety, place-preference, plus-maze

Apoio Financeiro: FAPESP (04/02859-0), CNPQ

CRISES DE PÂNICO EM FUMANTES: AVALIAÇÃO DA AÇÃO AGUDA DA NICOTINA EM UM MODELO EXPERIMENTAL DE PÂNICO

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Diversos estudos epidemiológicos apontam uma prevalência na incidência da síndrome do pânico na população de fumantes. Esta constatação, entretanto, ainda não recebeu confirmação experimental. Em vista disso, o presente estudo avaliou se a administração aguda de nicotina aumenta a susceptibilidade de ratos a deflagrarem a corrida selvagem (CS), que é tida como uma manifestação de pânico e é induzida por estimulação acústica de alta intensidade. Inicialmente, a sensibilidade individual para a manifestação de CS em resposta a estímulo sonoro de 112dB, aplicado por no máximo 60s, foi avaliada três vezes em um estoque de ratos machos Wistar (325-475g). A primeira avaliação se fez sem nenhum tratamento; a segunda e a terceira com administração de estircnina (0,5 mg/Kg, i.p.), droga que permite a detecção de animais com susceptibilidade intermediária ao pânico por meio da facilitação de CS. A medida da latência acumulada (em segundos) de CS, ao longo das três avaliações, serviu como parâmetro de sensibilidade individual. Este resultado prévio permitiu a constituição de dois grupos com níveis equivalentes de sensibilidade à CS, sendo um deles tratado com nicotina 0,4mg/Kg, s.c. (grupo NIC; n=10), e o outro, com solução salina em volume equivalente (grupo SAL; n=10), injetados 15 minutos antes de novas reavaliações de susceptibilidade ao pânico. Antes destes tratamentos, as médias das latências acumuladas foram de 148, 90 s + 12,01 (EPM) e de 138,10 s + 13,93 nos grupos NIC e SAL respectivamente; e de 141,10 s + 16,99 e de 132,20 s + 19,38 nos mesmos, após os tratamentos (diferenças não significantes, teste ANOVA de duas vias, nível de significância de 5%). Conclui-se que, nas condições do estudo, a administração de nicotina não afeta a sensibilidade dos ratos à CS, sugerindo a importância de seu uso crônico na facilitação ao pânico.

Palavras-Chave: pânico, tabagismo, nicotina, modelos experimentais, corrida selvagem

Apoio Financeiro: FAPESP

EFEITO DE UM ANTAGONISTA DO RECEPTOR 5-HT₂ SOBRE O CONSUMO DE ETANOL EM UM MODELO ADIÇÃO DE LIVRE ESCOLHA EM CAMUNDONGOS.

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Objetivos: avaliar o efeito da mianserina, antagonista 5-HT₂, sobre o consumo de etanol em camundongos adictos e não-adictos caracterizados segundo modelo de adição desenvolvido em nosso laboratório (1)

Métodos e Resultados: Camundongos (n=60) tiveram acesso a etanol 5 e 10% e água em um paradigma de livre escolha que consiste em 4 fases: Livre escolha (10 semanas), abstinência (2 semanas, acesso somente a água), re-apresentação (2 semanas) e adulteração com quinino das soluções de etanol (2 semanas). Animais controle (n=10) tiveram acesso apenas a água. Eles foram mantidos isolados com comida *ad libitum*. Os camundongos foram caracterizados como: adictos (A) (n=13, preferência pelo etanol sem redução do consumo na fase de adulteração), bebedor pesado (P) (n=15, preferência pelo etanol com redução do consumo na fase de adulteração) e bebedor leve (L) (n=27, sem preferência pelo etanol). Após a exposição ao modelo, os animais receberam salina ou doses de mianserina (0.0(M0); 2,5(M1); 5.0(M2) e 10.0(M3) mg/Kg) usando-se o método do quadrado latino de distribuição e com quatro dias de abstinência entre cada dose. Trinta minutos depois eles tiveram acesso as soluções de etanol e a água e o consumo foi medido 24H depois. M2 reduziu o consumo de etanol em todos os grupos. No grupo A quando comparado com M0, M1, M3 e S (F(4,95)=4.800; p<0.002); no grupo P quando comparado com M0, M1 e M3 (F(4,112)=3.287, p<0.02); no grupo L quando comparado com M1, M3 e S (F(4,102)=2.932, p<0.03). No entanto, não diminuiu o consumo de água.

Conclusão: A mianserina pode ter agido sobre os receptores 5HT_{2C} na área tegmental ventral, antagonizando-os. Esses receptores possuem alta atividade basal e agem diminuindo a liberação de dopamina. Assim a mianserina aumentaria a liberação de dopamina no núcleo accumbens, diminuindo o consumo de etanol em todos os grupos. Acredita-se então que a mianserina na dose de 5mg/Kg tenha substituído o papel reforçador do etanol. Esse efeito foi específico sobre a procura do etanol, visto que não houve diminuição no consumo de água.

Referências: 1- J Neural Transm.2006 Sep;113(9):1305-21. **Palavras-Chave:** livre escolha, etanol, mianserina, modelo animal

Apoio Financeiro: CAPES; Fundação araucária

Efeitos da pré-habituação sobre a sensibilização comportamental induzida pela administração única e pareada de morfina

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Objetivo: Estudos anteriores têm demonstrado que a sensibilização comportamental (SC – modelo animal de dependência) pode ocorrer após uma única injeção da droga de abuso, principalmente quando é pareada a um novo ambiente. Este trabalho tem como objetivo verificar se a novidade ambiental é necessária para a ocorrência desse fenômeno.

Método: 74 camundongos EPM-M2 foram submetidos à pré-habituação ao campo aberto por três dias consecutivos. Os animais receberam injeção subcutânea (s.c.) de salina vinte minutos antes das exposições. No quarto dia, os animais foram distribuídos em cinco grupos partindo do mesmo nível basal de atividade geral: Sal, Mor5, Mor10, Mor15, Mor30, os quais receberam (s.c.), respectivamente, salina, 5, 10, 15 ou 30 mg/kg de morfina, e vinte minutos depois foram expostos ao campo aberto para a quantificação da atividade geral durante cinco minutos. Essa sessão foi denominada de indutora. Após sete dias de intervalo, todos os animais foram desafiados com 15 mg/kg de morfina, sendo, vinte minutos da injeção expostos ao campo aberto por cinco minutos para nova quantificação da atividade geral (sessão desafio).

Resultados: A análise ANOVA de uma via seguida do teste de Duncan revelaram que a administração de morfina nas doses 10; 15 e 30 mg/kg (mas não na dose de 5 mg/kg) produziu elevação significativa da atividade locomotora quando comparada com os animais que receberam salina (grupo H-Sal) [F (4,69) = 12,835; p<0,001]. Todavia, nenhuma diferença entre os grupos foi observada na sessão desafio, quando todos os animais foram observados após a injeção de 15 mg/kg de morfina [F (4,69) = 2,098; p>0,05] demonstrando a ausência de desenvolvimento de sensibilização comportamental.

Conclusão: Considerando que resultados anteriores obtidos em nosso laboratório demonstram de maneira conspícua o desenvolvimento de sensibilização em condições experimentais idênticas àsquelas descritas neste estudo, com a exceção da pré-habituação (isto é, injeção indutora pareada a um campo aberto para os animais), nossos resultados sugerem que a novidade ambiental é fundamental para o desenvolvimento de sensibilização comportamental à injeção única de morfina.

Palavras-Chave: morfina; sensibilização comportamental; dose única; habituação

Apoio Financeiro: CAPES

C.09.001

SCIATIC NERVE REGENERATION IS ENHANCED IN GALECTIN-3 KNOCK-OUT MICE

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Success of peripheral nerve regeneration depends mainly on a permissive environment; although the mechanisms underlying its molecular events are still not elucidated. Schwann cells are considered one of the best candidates for this success. These cells and invading macrophages are responsible for myelin/axonal debris clearance, creating an appropriate route for a successful regeneration. It is known that, after injury, Schwann cells express galectin-3 and this has been correlated with phagocytosis; in the presence of galectin-3, there is an inhibition of Schwann cells proliferation in vitro. In the present study we explored in vivo the effects of the absence of galectin-3 in Wallerian degeneration and nerve fiber regeneration. For that, we crushed sciatic nerves of galectin-3 knock-out and wild-type mice and followed the pattern of degeneration and regeneration from 48 hours up to 3 weeks. We analyzed the number of myelinated fibers, axon area, fiber area, myelin area, G-ratio and immunofluorescence for macrophages in DAPI counterstained sections. In our results, knock-out mice presented a much faster regeneration when compared to wild-type animals. We concluded that the absence of galectin-3 allowed a better regenerataion of the crushed nerve. These results are discussed in terms of an increase on Schwann cell proliferation, combined with a possible increase in the release of neurotrophic factors, which would favor neuron survival and axon sprouting, followed by faster myelination, culminating in a better morphological and functional outcome. **Palavras-Chave:** Sciatic nerve, Degeneration, Regeneration, Galectin-3, Crush, Mice

Apoio Financeiro: CNPq, CAPES, FAPERJ

C.09.002

RESPONSE OF GABAERGIC INTERNEURONS TO HUMAN TBI

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Traumatic brain injury (TBI) is a public health problem with high mortality and or neuropsychological, neurological and psychiatric sequelae. These can be the result among others, of alterations in connectivity due to primary or secondary lesion. The GABAergic dysfunction play an important role in TBI as it has been demonstrated in other pathologies. The purpose of the present work was to establish the behavior of GABAergic interneurons in human cerebral cortex after TBI. Eleven contusion samples were valued, identifying the expression of calcium-binding proteins CaBPs. The study was made in sectors where the cytoarchitecture was altered valued with NeuN. The antibodies showed some sectors with remarkable loss of neuropil and positive somas for the CaBPs, coincident with sectors of total loss of IR for NeuN; as well as others in which changes of IR were less remarkable and corresponding to sectors with conserved cytoarchitecture. In these sectors, it was found that there were no changes in the amount of PV positive neurons, increase of CB positive neurons in layers III and V, and increase in CR positive neurons in the supragranular layers. These variations can reflect a dynamic activity as a result of the lesion associated to changes in the excitatory circuits of neighboring hyperactivated glutamatergic neurons maybe due to impact or secondary events like hypoxia-ischemia. Temporal evolution of these changes may allow to establish a direct relationship within TBI and emotional and cognitive dysfunctions.

This results are part of the project "Estudio cualitativo y cuantitativo del trauma craneoencefalico en humanos", financed by COLCIENCIAS - code 1106-04-16329 **Palavras-Chave:** TBI, Calcium binding proteins, NeuN

Apoio Financeiro: financed by COLCIENCIAS - code 1106-04-16329 Colombia

Treino de marcha em esteira reduz atrofia muscular do sóleo em ratos com transecção da medula espinal

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O objetivo deste estudo foi avaliar os efeitos do treino de marcha em esteira sobre a área das fibras musculares do sóleo após completa transecção da medula espinal (TME) em ratos. Ratos wistar adultos foram divididos em grupos: Controle-Sham, TME Sedentário e TME Treinado. A TME foi realizada ao nível de T8-9 e no 5º dia pós-lesão o grupo TME Treinado foi submetido a 8 semanas de treinamento de marcha na esteira com suporte parcial do peso corporal e auxílio manual quando necessário. Após este período, os animais (n = 4 por grupo) foram anestesiados e transcardiacamente perfundidos com solução fixadora. O músculo sóleo direito foi dissecado e uma pequena amostra da região central de cada músculo foi embebida e polimerizada em resina. Secções transversais semi-finas (1 µm) foram obtidas com ultramicrotomo e coradas com azul de toluidina 1%. Após, imagens das fibras musculares foram capturadas e digitalizadas utilizando um microscópio acoplado a uma câmera digital e ao programa *Image Pro Plus 6.0*. A análise morfométrica planar foi utilizada para determinar a área transversal das fibras musculares (100 fibras) e um histograma de frequência (500 µm² de intervalo) foi produzido. As medidas foram analisadas por ANOVA de uma via e pelo *Post hoc* Duncan (P < .05). O grupo TME Sedentário teve uma maior porcentagem de fibras musculares pequenas (500 – 1000 µm²; 38 ± 8.8 %; P < .05) do que os grupos Controle (2 ± .9%) e TME Treinado (18 ± 5.6%). Além disso, houve uma redução na porcentagem de fibras musculares largas (1000 – 3000 µm², P < .05) no grupo TME Sedentário quando comparado com o grupo Controle. Entretanto, não houve diferenças significativas na porcentagem de fibras largas (2500 – 3000 µm²) entre os grupos TME Treinado (1.8 ± 1.4%) e Controle (6.5 ± 2.4%). Os resultados evidenciam que o treino de marcha em esteira reduz a extensão da atrofia muscular no sóleo, facilitando a plasticidade muscular em ratos após TME. Esses resultados estão em acordo com publicações prévias e encorajam o uso do treino de marcha em esteira em programas de reabilitação após LME. **Palavras-Chave:** Transecção medular; Atrofia muscular; Músculo sóleo; Treino de marcha; Morfometria planar

Apoio Financeiro: Esse trabalho teve auxílio do CNPq (476880/2007-6), da CAPES e do FINEP/Rede IBN 01.06.0842-00.

C.09.004

Canabidiol influencia a recuperação da Lesão Medular de Ratos Produzida por Criogenia.

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Objetivos: Analisar a lesão medular de ratos produzida por aplicação local de um jato de nitrogênio líquido (N₂). Testar o efeito do Canabidiol no pós-lesão.

Métodos: Ratos *Wistar* machos (250g–280g) foram anestesiados e em seguida realizada incisão, para exposição das vértebras T8–T12. Foi realizada laminectomia em T10 e aplicação do N₂ na medula espinhal. Os animais foram divididos em três grupos que receberam injeções intra-peritoneais uma vez ao dia, durante 6 dias: Grupo Veículo (n=4), Grupo Canabidiol-10mg/kg (CBD10, n=4); Grupo Canabidiol-20mg/Kg (CBD20, n=4). Os animais foram avaliados nos períodos pré-teste, um (1ºPO), três (3ºPO) e sete (7ºPO) dias após o procedimento experimental, utilizando a escala de avaliação locomotora de Basso, Beattie e Bresnahan (BBB) e o teste de retirada de cauda (TF). Os dados foram analisados por MANOVA de medidas repetidas (variáveis tempo e tratamento), seguida de uma one-way ANOVA para cada tempo. No caso de interações, foi realizada ANOVA de medidas repetitivas de um fator, seguida do teste de comparação múltipla de Duncan.

Resultados: A Escala BBB indicou que todos os grupos apresentaram deficiência no 1ºPO ($F_{(1,6)}=10,417$; $P=0,018$) e uma melhora com o tempo (Veículo, $F_{(2,9)}=18,60$; $P=0,01$; 10mg, $F_{(2,9)}=159,60$; $P<0,01$; 20mg, $F_{(2,9)}=55,69$; $P<0,01$). No 3ºPO o CBD atenuou os efeitos da lesão (10mg, $F_{(2,9)}=6,30$; $P=0,019$; 20mg $F_{(7,24)}=812,17$; $P<0,01$). No 7ºPO foi observado melhora nos animais que receberam CBD 10mg ($F_{(1,6)}=7,41$; $P=0,03$) e 20mg ($F_{(1,6)}=10,41$; $P=0,018$). No 7ºPO, foi observado um aumento do tempo de retirada de cauda no grupo CBD 10mg ($F_{(2,9)}=6,46$; $P=0,018$) comparado ao veículo e CBD 20mg.

Conclusão: A aplicação de nitrogênio líquido induziu lesão homogênea. Animais que receberam canabidiol após a lesão apresentaram melhora na recuperação motora. Estes resultados estão de acordo com o efeito neuroprotetor do canabidiol descrito na literatura.

Palavras-Chave: Canabidiol; lesão medular; criogenia.

Apoio Financeiro: CAPES, FAPESP e CNPQ.

Gap junction blockade improves recovery after traumatic/ischemic spinal cord injury

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Introduction: Paraplegia secondary to spinal cord injury (SCI) remains a devastating complication. Gap junction channels (GJs), through which neighboring cells communicate, are thought to play an active role in the expansion of neurodegeneration following a number of central nervous system (CNS) insults. Here we report that gap junction blockade improves recovery after SCI.

Objectives: The aim of this work is to determinate the “window” of neurodegeneration post-spinal cord injury and to critically determinate the contribution of gap junctions in the ischemic injury.

Methods: Sprague-Dawley female rats (~250 g) were used. MES induced injury around T10. Histopathology was performed in 20 um thick spinal slices stained with cresyl violet (CV) and CD11b (marker of inflammation). Samples of RNA and protein were collected from T10 (epicenter), rostral (T8) and caudal (T12) regions and global assays included microarray, proteomics, RT-PCR and western blot assays. Functional assays (EMG) were performed up to 7 days after SCI.

Results: MES induced watershed in the spinal cord leading to injury (without fracture) around T10 (epicenter). Carbenexolone 75 mg/kg (I.P.) dramatically reduced the extent of the lesion around T10 and improved functional recovery. Of interest, Cx43 was upregulated in untreated animals and downregulated in carbenexolone-treated animals.

Conclusion: Gap junction channel play an important role in the development of secondary damage in ISCI.

Financial Support: NYS SCIRB, CNPq, CAPES and FAPERJ.

Palavras-Chave: Spinal Cord Injury, Gap junction, Carbenoxolone

Apoio Financeiro: NYS SCIRB, CNPq, CAPES and FAPERJ

BRAIN DAMAGE MARKERS LEVELS AFTER SEVERE TRAUMATIC BRAIN INJURY

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OBJECTIVE: Following traumatic brain injury (TBI) several biomarkers may have clinical utility in stratifying injury severity level, predicting adverse secondary events or outcomes, and monitoring the effectiveness of therapeutic interventions. Extracellular S100B, NSE, GFAP, lactate and glucose are proteins biomarkers that reflect CNS injury. To determine whether S100B, neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP), lactate and glucose are a potentially reliable biomarker for TBI in humans, the present study examined levels of these proteins in cerebrospinal fluid (CSF) from adults with severe TBI.

METHODS: CSF was analyzed in 22 subjects in an attempt to detect CNS injury. Samples were analyzed for S100B, NSE, GFAP, lactate, and glucose using commercial kits. Samples were taken in patients with severe head injury receiving intracranial pressure (ICP) monitoring. Controls were CSF from pregnant that not has neurological diseases.

RESULTS: In all subjects, there was a notable increase in CSF S100B, NSE, GFAP, lactate, and glucose in the first few days post-TBI. CSF S100B levels (5.88 ± 0.65 ng/ml and 0.24 ± 0.002 ng/ml; $p = 0.0001$); NSE (125.2 ± 25.32 ng/ml and 1.02 ± 0.3 ng/ml; $p = 0.0001$); GFAP (3.99 ± 3.6 ng/ μ L and 0 ± 0 ng/ μ L; $p=0.0001$); lactate (4.34 ± 2.9 mmol/L and 1.48 ± 0.55 mmol/L; $p=0.0001$); glucose (97.25 ± 36.97 mg/dL and 49.06 ± 22.08 mg/dL; $p=0.0001$) levels in subjects after TBI and controls respectively.

DISCUSSION: This study indicates a higher increase of S100B, NSE, GFAP, lactate and glucose CSF levels after severe TBI in humans. Parallel increases of these molecules may indicate a pivotal role of the pathophysiology of post-traumatic brain edema, secondary cell destruction and chronic cell loss following severe TBI and may open new targets for post-traumatic prognostic and therapeutic interventions.

Palavras-Chave: CNPq, CAPES and the FINEP research grant “Rede Instituto Brasileiro de Neurociência (IBN-Net)” # 01.06.0842-00

Apoio Financeiro: brain damage markers; traumatic brain injury; cerebrospinal fluid

NEUROTROPHIC FACTORS LEVELS AFTER SEVERE TRAUMATIC BRAIN INJURY

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OBJECTIVE: Following traumatic brain injury (TBI) several biomarkers may have clinical utility in stratifying injury severity level, predicting adverse secondary events or outcomes, and monitoring the effectiveness of therapeutic interventions. The study objective was to assess whether biomarkers concentrations obtained at the time of injury are associated TBI. Serial in cerebrospinal fluid (CSF) concentrations of BDNF, GDNF, and NGF were measured in 21 adults with acute TBI.

METHODS: CSF was analyzed in 22 subjects in an attempt to detect CNS injury. Samples were analyzed for BDNF, GDNF, and NGF using commercial kits. Samples were taken in patients with severe head injury receiving intracranial pressure (ICP) monitoring.

RESULTS: In all subjects, there was a notable increase in BDNF in the first few days post-TBI, while GDNF and NGF have not statistical significance when compared with controls. CSF BDNF levels (254.3 ± 13.35 picog/ml and 191.1 ± 4.62 picog/ml; $p = 0.002$); GDNF (386.6 ± 15.6 ng/ μ L picog/ml and 345.3 picog/ml; $p = 0.0932$); and NGF (72.33 ± 2.9 ng/ μ L

and 73.02 ± 2.56 ; $p=0.1942$) levels in subjects after TBI and controls respectively.

DISCUSSION: This study indicates a higher increase of BDNF CSF levels after severe TBI in humans. Increases of these molecules may indicate a pivotal role of the pathophysiology of post-traumatic brain edema, secondary cell destruction and chronic cell loss following severe TBI and may open new targets for post-traumatic prognostic and therapeutic interventions. BDNF concentration in CSF may be used as is a useful marker of brain damage following severe TBI and could be correlates with a favorable neurologic outcome. Nevertheless, more studies allow us to determine the putative role of CSF BDNF levels as brain markers of injury/activity in TBI.

Palavras-Chave: neurotrophics factors; traumatic brain injury; cerebrospinal fluid

Apoio Financeiro: CNPq, CAPES and the FINEP research grant “Rede Instituto Brasileiro de Neurociência (IBN-Net)” # 01.06.0842-00

PARTICIPATION OF THE TRPA1 RECEPTOR IN OVERACTIVITY BLADDER INDUCED BY SPINAL CORD INJURY IN RAT

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Objectives: Overactivity bladder (OAB), which can be induced by spinal cord injury (SCI), is characterized by the presence of involuntary contractions of the detrusor muscle during the storage phase. Following SCI, the urinary bladder becomes hyperreflexic due to the emergence of spinal micturition reflex pathways, involving C-fiber bladder afferent neurons expressing TRPV1 and TRPA1 receptors (1). Although several studies address the TRPV1 involvement in OAB, TRPA1 participation remains to be elucidated. Thus, the present study evaluates the TRPA1 role in OAB by the analysis of histological and inflammatory parameters and bladder contractility to TRPV1 and TRPA1 agonists and receptors expression in rat urinary bladder.

Methods and Results: Adult male Wistar rats weighing 270-300 g were used during the experiments. The spinal cord was injured at 10th thoracic vertebrae, with a Fogarty 2F catheter. We evaluated the humid weight, the contractile responses to cinnamaldehyde (300 µM, TRPA1 agonist) and capsaicin (0.1 µM, TRPV1 agonist), the TRPA1 and TRPV1 expression and the inflammatory process in urinary bladder in different periods after SCI. SCI induced an increase in strips urinary bladder weight [day (d)2: 71.9 ± 6.4%; d7: 221.8 ± 20.8%; d14: 222.6 ± 22.9%, d28: 246.5 ± 32.4%)] and in the contractility to cinnamaldehyde (d14: 51.6 ± 15.3%, d28: 76.3 ± 22.5%) and to capsaicin (d14: 98.1 ± 46.4%; d28: 154.8 ± 34.4%) relative to sham animals (P<0.05). The TRPA1 and TRPV1 expression increased after 7th day and mieloperoxidase enzyme activity increased (about 70-fold) on 2nd day after SCI. Also, bladder SCI-histological analysis showed inflammatory cells migration on 2nd day and pronounced edema, vessels congestion, reduction of urothelial cells in all periods after SCI.

Conclusion: Our results show that SCI induces changes in the urinary bladder such as hypertrophy, inflammation, enhanced contractility to capsaicin and cinnamaldehyde and enhanced TRPV1 and TRPA1 expression. Besides the development of an appropriate model to evaluate OAB by SCI, these results reveal an important role of TRPA1 receptors in OAB.

References: 1- Prog Brain Res, 152:59-84, 2006.

Palavras-Chave: , spinal cord injury, TRPA1 and TRPV1 receptors, urinary bladder, overactivity bladder

Apoio Financeiro: CAPES, CNPq, FINEP, FAPESC, PRONEX

Participação dos receptores B1 e B2 para as cininas na hiperatividade da bexiga urinária induzida pela lesão medular traumática em ratos.

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Objetivos: A hiperatividade da bexiga urinária, a qual pode ser decorrente de uma lesão da medula espinhal é uma patologia caracterizada pela presença de contrações involuntárias do músculo detrusor da bexiga durante a fase de enchimento. O presente estudo visa avaliar o envolvimento dos receptores B1 e B2 para as cininas na hiperatividade da bexiga urinária induzida pela lesão medular. **Métodos e Resultados:** Ratos Wistar machos (270 -300g) foram submetidos à lesão medular ao nível da 10ª vértebra torácica através da inserção de um cateter Fogarty 2F. Para garantir a eficácia da lesão medular, foram utilizados somente animais cuja atividade locomotora não ultrapassou um escore 3 da escala BBB (escore 0 a 21). Diferentes períodos após a cirurgia, a contratilidade da bexiga e a expressão dos receptores B1 e B2 foram analisadas. Em animais naive ou falso-operados, a adição do agonista de receptores B2 bradicinina (0,0001-10 µM), porém não do agonista de receptores B1 des-Arg⁹-BK (0,0001-10 µM) induziu contração da bexiga urinária. Interessantemente, a lesão medular induziu um aumento significativo na frequência e amplitude da atividade contrátil basal da bexiga, potencializou a resposta contrátil à bradicinina (dia 2: 161 ± 41; dia 7: 80 ± 8; dia 14: 218 ± 75; dia 28: 124 ± 31 %) e desencadeou um efeito contrátil à des-Arg⁹-BK (dia 2: 266 ± 100%; dia 7: 469 ± 117%; dia 14: 100 ± 43%; dia 28: 800 ± 112%) (P<0.05). Além disso, houve aumento na expressão dos receptores B1 e B2 após a lesão medular. Por outro lado, a incubação prévia das preparações com os antagonistas de receptores B1 ou B2 (30 nM) não foi capaz de inibir a resposta contrátil induzida pela des-Arg⁹-BK ou pela bradicinina respectivamente, na bexiga urinária de animais 28 dias após a lesão medular. **Conclusão:** Nossos resultados mostram que a lesão medular altera a atividade basal da bexiga urinária, potencializa o efeito contrátil à bradicinina, desencadeia um efeito contrátil à des-Arg⁹-BK e aumenta a expressão dos receptores B1 e B2. O envolvimento de receptores B1 e B2 na hiperatividade da bexiga urinária induzida por lesão medular sugere um novo alvo terapêutico para esta patologia. **Palavras-Chave:** Hiperatividade da bexiga urinária, receptores B1 e B2, lesão medular traumática.

Apoio Financeiro: CAPES, CNPq, FINEP, FAPESC, PRONEX

C.09.010

alteraciones neuropsicológicas en pacientes con trauma craneoencefálico

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El trauma craneoencefálico es un grave problema de salud pública, tanto en países desarrollados como en vía de desarrollo, es la principal causa de discapacidad y muerte en la población económicamente activa.

Método: se estudiaron 46 pacientes con TCE moderado a severo, edad promedio de 31,6 años (*DE* 12,3); 14 de ellos con lesión con predominantemente frontal, 18 lesión predominantemente temporal y 14 con lesión difusa. Se comparó su desempeño con los criterios de la batería de evaluación neuropsicológica breve en español "NEUROPSI".

Resultados: 1) los pacientes con trauma craneoencefálico presentan alteraciones principalmente en la atención memoria (más del 60% presentaron puntaje por debajo del rango normal) y funciones ejecutivas (67 % de los pacientes obtuvieron puntaje por debajo del rango normal) 2) los pacientes con lesiones más difusas presentaron puntajes más bajos en la escala de Glasgow al momento agudo del trauma: lesión de predominio frontal 12,7 *DE* 2, 09, lesión de predominio temporal 11,6 *DE* 2,6; lesión difusa 9,25 *DE* de 3,49. El análisis reveló que el grupo de lesión difusa se diferenciaba significativamente de los otros dos grupos con un grado de significancia de 0.05. 3) No se encontró relación entre el grado en la escala de Glasgow y el grado de deterioro cognitivo posterior, no se observaron diferencias en los puntajes en las de la pruebas del NEUROPSI, 4) Los pacientes con lesiones mayores signos de lesión frontal derecha mostraron peor desempeño en pruebas de praxis construccional, memoria visoespacial y atención sostenida; pacientes con lesiones izquierdas presentan mayor alteración en la inhibición de respuestas de mayor saliencia.

Conclusiones: en la mayoría de los casos el trauma craneoencefálico afecta las mismas funciones cognitivas de manera global (memoria atención y funciones ejecutivas) pero genera patrones muy variados con relación a las alteraciones específicas de cada una de las mismas. El puntaje en la escala de Glasgow es útil para la toma de decisiones en el momento agudo del trauma pero no como indicador de la respuesta cognitiva posterior.

Palavras-Chave: trauma craneoencefálico, neuropsi

Apoio Financeiro: Universidad del Valle.

D.01.001

ELECTROPHYSIOLOGICAL RESPONSES TO CHEMICAL STIMULATION OF THE ANTENNULE OF THE FRESHWATER PRAWN *MACROBRACHIUM AMAZONICUM*

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Objectives: Chemoreception is used by many aquatic organisms in feeding behavior, spatial orientation and communication. This sense has been widely investigated in crustaceans, partly due to the ecological, technological and economic applications of such research, and also because of the relative simplicity of their nervous system, which makes them excellent experimental models. The main objective of the research undergone by our group was to analyze the electrophysiological responses *in vivo* from the antennules of the freshwater prawn *Macrobrachium amazonicum* when exposed to chemical stimulants and to elaborate models of how chemoreception functions in these animals.

Methods: The animals were captured and kept in the laboratory under controlled conditions. During the experiments they were sedated in cold water and immobilized in a anatomically designed acrylic support positioned inside a recipient filled with cold crustacean saline. The electrophysiological recordings were made using glass covered tungsten microelectrodes positioned inside the peduncle of the antennule. The antennule was isolated in a recipient with constant water flow in which the chemical stimulants were applied. Solutions of ammonium chloride and urea in concentrations of 1, 10 and 100 mM were used. Each recording lasted up to two minutes, with intervals of two minutes between each test.

Results: Distinct excitatory responses were recorded for each stimulus. We observed that the frequency of spikes was proportional to the concentration of the stimuli, but that the statistical temporal properties of the response were similar for different concentrations of the same stimulus.

Conclusion: The results indicate that the chemoreceptive system of *Macrobrachium amazonicum* probably operate very similarly to that of other decapod crustaceans, in which it is believed that the concentration of a given substance is codified by the frequency of action potentials whilst the identification of the stimulus is coded in the temporal properties of the response.

Palavras-Chave: Chemoreception, electrophysiology, prawn.

Apoio Financeiro: CNPQ - PIBIC, IBNnet, UFPA.

D.01.002

The elusive crypt olfactory receptor neuron: Evidence for its stimulation by amino acids and cAMP pathway agonists

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Objectives and methods. Crypt olfactory receptor neurons (ORNs) are a third type of chemosensory neuron along with ciliated and microvillous ORNs in the olfactory epithelium of fishes, but their functional role is still unknown. To investigate their odorant response properties and possible transduction pathways, we recorded crypt ORN activity with calcium imaging and the patch clamp technique in its cell-attached mode in combination with odorant and agonist stimulation.

Results. While bile salts and putative fish pheromones did not elicit responses with either method, the cells frequently responded to amino acids with excitation and intracellular calcium signals. 8Br-cAMP and IBMX plus forskolin stimulated over 40% of crypt ORNs and triggered calcium signals in a similar percentage. Furthermore, crypt ORNs were immunoreactive to an antiserum against adenylate cyclase III.

Conclusions. Our data suggest the presence of a cAMP transduction pathway in teleost crypt ORNs, which might transduce odorants such as amino acids. While responses to putative fish pheromones were not detected, this proposed function of crypt ORNs cannot be ruled out at present.

Palavras-Chave: crypt cell, fish, olfaction, chemosensory, olfactory receptor neuron, odorant, transduction

Apoio Financeiro: PBCT-Conicyt project ACT-45,

D.01.003

Los Lípidos Cuticulares y su Papel como Semioquímicos en la vinchuca *Triatoma infestans*: Comportamiento Sexual y de Agregación

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Objetivos: Los lípidos cuticulares están implicados en la comunicación química en insectos. Algunos son feromonas de contacto con funciones como comportamiento de agregación, sexual, involucrando quimiorreceptores de contacto, ubicados en todo el cuerpo (1). La vinchuca *Triatoma infestans* permanece durante el día en akinesis agrupada con otros conespecíficos, activándose durante la noche en búsqueda de hospedadores, pareja (2,3). Analizamos si las señales de contacto son usadas en agregación, estudiándolo frente a fracciones lipídicas. Investigamos si los machos dependen del contacto de la antena o cuerpo para reconocer una hembra.

Métodos: Para analizar la agregación se expusieron larvas de *T. infestans* a cada compuesto a ensayar. Se realizaron experimentos con lípidos cuticulares de larvas del 5^{to} estadio y sus respectivas fracciones, diacilglicerol, triacilglicerol, ácidos grasos libres (AGL), alcoholes, ceras, hidrocarburos. Se investigó la respuesta frente a algunos componentes de AGL. Para investigar el comportamiento sexual se le presentó a un macho de *T. infestans* una hembra muerta con o sin lípidos cuticulares. Se midieron cópulas e intentos.

Resultados: En agregación, las larvas se agruparon significativamente sobre un papel con lípidos totales y solamente frente a la fracción AGL. La respuesta de agregación frente a los principales componentes de los AGL, ácido hexadecanoico (C16:0), octadecanoico (C18:0) y hexacosanoico (C26:0) fue variada a diferentes dosis. Para el C18:0 fue significativa con 1 equivalente ($P < 0,001$) (=extracto de un adulto). Para C26:0 la respuesta varió entre la agregación y rechazo significativos. Frente al C16:0 se distribuyeron al azar. Los machos copulan o intentan copular con hembras muertas luego de contactarla con sus patas o antenas. Copulan pero en menor proporción con hembras sin sus lípidos pero pintadas con extracto de lípidos de hembra.

Conclusiones: La existencia de componentes en la cutícula de *T. infestans* evidencian comunicación química en los comportamientos de agregación y de cópula.

Referencias: 1-Ann.Rev.Entomol.27:149-172, 1982; 2-Physiological Entomology 23:33-37,1998; 3-Mem.Inst.Oswaldo Cruz 90:645-648,1995.

Palabras-Chave: comunicación química, agregación, comportamiento sexual, lípidos cuticulares, *Triatoma*

Apoio Financeiro: Universidad de Buenos Aires, CONICET-UNLP

D.02.001

¿TIENE EL COLICULO INFERIOR UN ROL EN EL MANTENIMIENTO DE LA VIGILIA?

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Objetivo: El colículo inferior (CI) además de sus clásicas funciones auditivas tendría un papel en funciones de vigilancia. Es nuestro objetivo intentar demostrar, mediante la estimulación e inhibición del CI, si éste tiene la capacidad de modular los niveles de vigilia y/o de sus signos electrográficos. **Métodos:** Cobayos adultos fueron preparados para ser registrados en condiciones agudas (anestesiados, n = 14) o implantados crónicamente con electrodos para registros polisomnográficos (n = 5). En los preparados agudos se utilizó uretano como anestésico (1,5 mg/Kg) y se colocaron electrodos para registros del electroencefalograma (EEG), electrograma hipocámpico, electrocardiograma y frecuencia respiratoria. Se realizaron orificios en el cráneo para descender electrodos de estimulación o cánulas de inyección al CI. En los preparados agudos se realizaron las siguientes maniobras: 1. estimulación eléctrica (5 a 500 mA con distintos protocolos). 2. inyección unilateral de bicuculina (antagonista GABA_A; 5 mM) o hipocretina-1 (neuropéptido involucrado en funciones de vigilia; 250 mM). En los preparados crónicos se inyectó bilateralmente muscimol (agonista GABA_A, 25 mM). Inyecciones control (salina) fueron realizadas en ambos preparados. **Resultados:** En preparados agudos la estimulación eléctrica y química generó desincronización del EEG, ritmo theta hipocámpico, aumento de la frecuencia cardíaca y respiratoria. Este efecto se produjo principalmente estimulando la zona ventral del CI. En los registros crónicos, la inhibición del CI disminuyó el porcentaje de tiempo de vigilia en relación con los controles ($37,9 \pm 5,1$ % Vs. $64,9 \pm 4,5$ %; $p < 0,01$). **Conclusiones:** Estos datos sugieren que el CI tendría un rol regulatorio sobre el estado de vigilia. *Parcialmente financiado por TWAS.*

Fibras y receptores hipocretinérgicos en el colículo inferior del cobayo.

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Introducción. Neuronas hipocretinérgicas del hipotálamo postero-lateral median comportamientos que ocurren en conjunción con diversos estados motivacionales. El colículo inferior (CI) es un núcleo mesencefálico auditivo que procesa la frecuencia e intensidad del sonido, así como la localización del sonido en el espacio. Otras funciones tales como mediar el despertar electrocortical evocado por sonidos y regular la expresión de respuestas defensivas, también han sido descritas en este núcleo. Nuestra hipótesis de trabajo es que el sistema hipocretinérgico regula el procesamiento de la información auditiva durante estados motivacionales, como por ejemplo, en situaciones de huida o lucha, jerarquizando el procesamiento de sonidos relevantes para el animal en ese momento. Nuestro objetivo inicial fue determinar las bases anatómicas de la interacción entre el sistema hipocretinérgico y el CI. **Métodos.** Cobayos adultos fueron preparados para procedimientos de inmunohistoquímica. Se utilizaron anticuerpos primarios contra hipocretina-1, hipocretina-2, receptor de hipocretina tipo-1 y tipo-2. Se realizó doble inmunohistoquímica para reconocer GABA y la enzima sintasa de óxido nítrico conjuntamente con los anticuerpos anti-hipocretinas o anti-receptores. Las reacciones antígeno-anticuerpo fueron reveladas por el método ABC-DAB y por inmunofluorescencia. **Resultados.** Fibras hipocretinérgicas fueron observadas en toda la vía auditiva, siendo el CI el núcleo más innervado. Los receptores fueron observados en todo el CI, pero con mayor densidad en la región ventral del núcleo central. Las neuronas GABAérgicas presentaron el receptor hipocretinérgico tipo 1 mientras que la mayoría de neuronas nitrérgicas no presentaron este receptor. **Conclusiones.** Fibras y receptores hipocretinérgicos están presentes en el CI. Los receptores se encuentran principalmente en las neuronas GABAérgicas de la región ventral del CI. *Parcialmente financiado por TWAS.*

Palavras-Chave: inferior colliculus, hypocretin, auditory system, guinea-pig, GABA

Apoio Financeiro: TWAS

D.02.003

Objective measurement of human temporal auditory acuity

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Temporal auditory acuity is the ability to discriminate rapid changes in the envelope of the sound, it is essential for speech understanding. A more comprehensive approach is to measure the threshold for detecting changes in the amplitude of a sound as a function of the rapidity of the changes. The function relating threshold to modulation rate is known as a temporal modulation transfer function (TMTF). In this paper we obtain an electrophysiological characterization of TMTF assessed by the sweep envelope following responses (EFR) technique. Ten healthy adults ranged in age from 25-35 years participated in this experiment. Amplitude modulation was applied to a uniform white-noise carrier presented at 50 dB HL, with a 100% of depth at frequencies from 20 to 200 Hz. Response were amplified with gain 1000 and bandpass filtered 1-300 Hz. An F ratio was employed to test whether the response amplitude was significantly different from the noise. In the waking subjects all responses were statistically different from the background EEG noise, with the exception of the region between 65 and 75 Hz. In sleep, the responses remained highly significant except below 33 Hz, and near the broader amplitude minimum at 66 Hz. Also, there was a deep valley in the amplitude response near 27 Hz during both sleep and wakefulness. The 35-50 Hz peak amplitude of the TMTF wasn't significantly larger during wakefulness than during sleep for all the subjects. EFR technique would be very helpful to have a simple objective test of temporal auditory acuity that could be used to assess patients who cannot give reliable data from psychophysical testing.

Palavras-Chave: envelope following responses, temporal auditory acuity, temporal modulation transfer function

D.02.004

Effect of carrier frequency on maturation of Envelop Following Response

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Objective: Codification of the temporal pattern of amplitude modulated acoustic stimuli is extremely important for comprehension of spoken language. Using rats as experimental model, we studied the effect of amplitude modulation frequency on Auditory Steady State Responses using a continuous sweep of modulation frequency (MF) through the band of interest frequencies (envelop following response, EFR).

Methods: Responses were recorded in animals with different ages (15, 20, 25 and 70 days after birth). Input-Output functions were constructed and effect of temporal parameter was measured at saturating intensities. It was measured amplitude of the response to broad band amplitude modulated white noise and amplitude modulated pure tones of 2 and 4 kHz.

Results: EFR typically is a non-monotonic function of the modulation frequency. After a minimum reached at 55 Hz approximately (worst modulation frequency), amplitude of the response increased up to a maximum value (best modulation frequency, BMF). Further rises of MF dropped the response. White noise was a more effective stimulus than pure tone to evoke EFR. For every tested frequency, white noise elicited larger responses than pure tones. For different pure tones, the higher carrier frequency, the larger amplitude of the response. On the other hand, amplitude for the best modulation frequency increased with the age of the animal. Besides, EFR/modulation frequency function shifted to left when stimulus changed from white noise to pure tone. Also, shifting was observed comparing pure tones of different frequencies. Magnitude of the shifting decreased when animals became adults.

Conclusion: The effect of modulation frequency on auditory response is modulated by maturation of auditory system –i.e. amplitude of Envelop Following Response increase and the effect of modulation frequency decreased when animals become adults. Maturation temporal patterns depend on the carrier frequency of the stimulus. These results contribute to understand how temporal structure of acoustic stimuli is coded by auditory system.

Palavras-Chave: maturation, evoked potentials, envelop following response, rats

Apoio Financeiro: Cuban Neuroscience Center

D.02.005

Effect of contralateral acoustic stimulation in cochlear sensitivity in *Chinchilla laniger*

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Objective. Determination of the effect in cochlear sensitivity of auditory-efferent activation through contralateral acoustic stimulation in chinchilla. **Materials and methods.** We used 10 adult chinchillas (*Chinchilla laniger*), anesthetized with xilazine and ketamine. The surgical approach to the cochlea was through a posterior craniotomy of the tympanic bulla. A silver-wire electrode in the round window membrane was used to record auditory nerve compound action potentials (CAP) and cochlear microphonics. The experimental protocol consisted of contralateral tones of 500 ms duration followed after 5-10 ms by 15 ms ipsilateral tones. CAP amplitudes and latencies were measured in response to the ipsilateral tones at different frequencies and intensities, in presence and absence of contralateral stimuli. **Results.** Activation of olivocochlear efferent fibers through contralateral acoustic stimulation produced CAP changes for tones at frequencies between 1-9 kHz. Efferent activation caused CAP amplitude reductions that reached up to 14 dB, that were dependent on the intensity and frequency of sound stimuli. The reductions in CAP amplitude were greater for contralateral stimulus frequencies similar to those of the ipsilateral stimulus, normally reaching a maximum at the same frequency (maximum reduction $\pm$ SD = 10.4 ± 2.8 dB). Efferent activation produced CAP latency increments, which were also dependent on tone intensity and frequency. **Conclusion.** Contralateral acoustic activation of the efferent olivocochlear pathway in anesthetized chinchillas produces a tonotopic modulation of cochlear sensitivity; that is, CAP reductions were maximal for contralateral stimulus frequencies equal to those of the ipsilateral tone. These results show the existence of a tonotopic distribution of efferent olivocochlear fibers in most of the extent of the chinchilla cochlea.

Palavras-Chave: auditory efferent, olivocochlear, chinchilla, contralateral supression.

Apoio Financeiro: Supported by FONDECYT 1080227 and Fundación Guillermo Puelma (CV).

D.02.006

Efficiency of the automatic detection of multiple auditory steady state responses measured with ROC methodology

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The purpose of this study was to evaluate comparatively using Receiver Operating Characteristics (ROC) curves, the efficiency of the automatic detection of Multiple Auditory Steady State Responses (MSSR) with an established methodology such as click Auditory Brainstem Responses (cABR). For the MSSR recorded we used the new semi-automatic system designed and validated on the V AUDIX version. Electrophysiological recordings were obtained from 35 normal newborns between 1 and 2 weeks of born. We studying a sample of 139 cABR and 139 MSSR recorded. Each sample was constituted by 69 recorded with response and 70 recorded without response both obtained at a fixed intensity of 40 dB nHL. For the automatic detection of cABR two statistics were calculated: the Standard Deviation Ratio (SDR) between the signal and the background noise and the Correlation Coefficient Ratio (CCR) between two replicates, whereas for the MSSR the statistics in the frequency domain Hotelling T^2 (HT2) and circular T^2 (CT2) were used. The MSSR showed better efficiency with higher areas under the ROC curve (HT2: 0,98 and 1 for 0,5 and 2 KHz; CT2: 0,96 and 0,99 for 0,5 and 2 KHz) compared to the cABR (0,92 and 0,91 for CCR and SDR). For a fixed false alarm rate of 20% the MSSR recorded without response can be correctly detecting in the 96-97% of cases for 0.5 KHz and in the 100% of cases for 2 KHz, while the cABR only can be detected in the 84% of cases. Although these evoked responses (cABR and MSSR) could be identified efficiently by the different statistics, the MSSR showed better efficiency compared to the cABR.

Palavras-Chave: auditory brainstem response, steady state response, ROC curves, hearing screening, signal detection methods, automatic detection.

O COMPLEXO NUCLEAR COCLEAR DO SAGÜI (*Callithrix jacchus*): CARACTERIZAÇÃO CITOARQUITETÔNICA E DISTRIBUIÇÃO DE PROTEÍNAS LIGANTES DE CÁLCIO.

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Objetivos: O complexo nuclear coclear (NC), a primeira estação da via auditiva ascendente, é um conjunto de células que forma uma proeminência nas imediações do sulco bulbo-pontino. Em primatas, pode ser dividido em dois núcleos principais, o NC dorsal e o NC ventral (NCV). Este por sua vez subdivide-se em núcleo coclear ventral anterior (NCVA) e núcleo coclear ventral posterior (NCVP). O objetivo deste trabalho foi delimitar o NC, com base na citoarquitetura e expressão imuno-histoquímica das proteínas ligantes de cálcio: calbindina (CB), cal-retinina (CR) e parvalbumina (PV) no sagüi (*Callithrix jacchus*), um pequeno primata neo-tropical, típico da região Nordeste Brasileiro. **Métodos:** 04 sagüis adultos jovens, provenientes do Núcleo de Primatologia da UFRN (Licença IBAMA no. 1/24/92/0039-00), foram anestesiados e submetidos à perfusão transcardíaca, com solução salina tamponada heparinizada seguida de solução de paraformaldeído 4% em tampão fosfato 0,1M, pH 7,4. Os encéfalos foram removidos e submetidos à microtomia por congelamento, obtendo-se secções coronais de 20 µm coletadas em 10 séries. Secções de uma série através da ponte foram coradas pelo método de Nissl (Thionina) para demarcar a citoarquitetura. Outras foram submetidas à imuno-histoquímica (protocolo ABC) a fim de revelar a presença de CB, CR e PV. **Resultados:** Grande quantidade de células, fibras e terminais imunorreativos a CB foram encontrados em todos os núcleos do NC, apresentando distribuição aparentemente uniforme no sentido rostrocaudal. Células, fibras e terminais fortemente imunorreativos a CR também foram visualizados em todo NC. Fibras imunorreativas foram vistas em maior concentração no NCAV, seguido do NCPV, seguido do NCD. Células, fibras e terminais imunorrativos a PV também foram vistos distribuídos em toda extensão rostrocaudal do núcleo, sendo que as fibras aparentemente apresentavam um gradiente rostrocaudal de concentração. **Conclusão:** Embora não tenham um papel funcional estabelecido na via auditiva, as proteínas ligantes de cálcio podem ser uma ferramenta auxiliar na delimitação do NC do sagüi. **Palavras-Chave:** COMPLEXO NUCLEAR COCLEAR, SAGÜI (*Callithrix jacchus*), PROTEÍNAS LIGANTES DE CÁLCIO.

Apoio Financeiro: CNPq; CAPES; PROPESQ-UFRN

O COMPLEXO NUCLEAR COCLEAR DO SAGÜI (*Callithrix jacchus*): CARACTERIZAÇÃO NEUROQUÍMICA.

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Objetivos: Em primatas, o complexo nuclear coclear (NC) compreende dois núcleos principais, o NC dorsal (NCD) e o NC ventral (NCV). Este por sua vez subdivide-se em núcleo coclear ventral anterior (NCVA) e núcleo coclear ventral posterior (NCVP). O objetivo deste trabalho foi caracterizar a expressão imuno-histoquímica para proteína nuclear neuronal específica (NeuN) e substâncias neuroativas: encefalina (ENK), serotonina (5-HT), glutamato (GLU), descarboxilase do ácido glutâmico (GAD), e colina acetil transferase (ChAT) no sagüi (*Callithrix jacchus*), um pequeno primata neo-tropical, típico da região Nordeste Brasileiro. **Métodos:** Secções obtidas de 7 sagüis adultos jovens, provenientes do Núcleo de Primatologia da UFRN (Licença IBAMA no. 1/24/92/0039-00), foram utilizadas neste trabalho. Os animais foram anestesiados e submetidos à perfusão transcardíaca, com solução salina tamponada heparinizada seguida de solução de paraformaldeído 4% em tampão fosfato 0,1M, pH 7,4. Os encéfalos foram removidos e submetidos à microtomia por congelação, obtendo-se secções coronais de 20 µm coletados em 10 séries. Um série através da ponte foi imunocorada para NeuN, a fim de delimitar a citoarquitetura. Outras foram submetidas à imuno-histoquímica (protocolo ABC) para revelar a presença das substâncias supracitadas. **Resultados:** Células, fibras e terminais imunorreativos a GAD, GLU e ENK foram encontrados no NCVA, NCVP e NCD. Já quanto a 5-HT foi verificada presença de fibras e terminais imunorreativos em todos os sub-núcleos do NC. Maciça presença de células imunorreativas a ChAT foram visualizadas no NCAV e poucas células mergulhadas em densa rede de fibras no NCPV e NCD. **Conclusão:** A expressão imuno-histoquímica para NeuN delimitou a citoarquitetura dos sub-núcleos do NC e a expressão imuno-histoquímica das demais substâncias ativas contribuiu para delimitar os sub-núcleos e caracterizar o NC do sagüi. **Palavras-Chave:** COMPLEXO NUCLEAR COCLEAR, SAGÜI (*Callithrix jacchus*), CARACTERIZAÇÃO NEUROQUÍMICA

Apoio Financeiro: CNPq; CAPES; PROPESQ-UFRN

D.03.001

Therapeutic effect of the radial optic neurotomy on experimental glaucoma

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Glaucoma is a leading cause of blindness, characterized by specific visual field defects due to the loss of retinal ganglion cells and optic nerve head damage. Recently, we have developed a new experimental model of glaucoma in rats through chronic injections of hyaluronic acid (HA) in the anterior chamber of the eye. HA injections induce an increase of the intraocular pressure (IOP), a progressive retinal dysfunction and a significant loss of ganglion cells and fibers of the optic nerve. The radial optic neurotomy (RON), a surgical strategy originally used for the treatment of the retinal central vein thrombosis, consists in the section in depth of the head of the optic nerve, decompressing the scleral channel and improving the arterial and venous blood flow. The aim of this work was to analyze the therapeutic benefits of RON in the model of experimental glaucoma in rats induced by HA. HA (25 μ l, 10 mg/ml) was weekly injected in both eyes of *Wistar* rat anterior chamber. After 3 weeks of ocular hypertension, the surgical procedure (RON) was performed in one eye from each animal and the contralateral eye was submitted to a sham operation. Retinal function (electroretinogram (ERG) and visual evoked potential (VEP)) was assessed after 10 weeks of ocular hypertension. Moreover, the cell number in the ganglion cells layer (GCL) was quantified. RON significantly reduced the decrease in the scotopic ERG a-wave (sham: $42,20 \pm 6,11 \mu$ V, RON: $50,26 \pm 6,03 \mu$ V, $p < 0,05$) and b-wave amplitude (sham: $177,75 \pm 23,02 \mu$ V, RON: $195,54 \pm 24,67 \mu$ V $p < 0,05$), in VEP wave 4 (sham: $50,31 \pm 15,08 \mu$ V, RON: $84,04 \pm 15,08 \mu$ V $p < 0,01$) and in the number of cells in the GCL (sham: $6,65 \pm 0,86$ cell/ 100μ m, RON: $11,27 \pm 0,35$ cell/ 100μ m $p < 0,05$). These results indicate that RON abrogated histological and functional alterations induced by ocular hypertension, and support the potential use of RON as a new surgical strategy in the treatment of the glaucoma.

Palavras-Chave: Glaucoma, hyaluronic acid, radial optic neurotomy

Apoio Financeiro: Agencia Nacional de Promoción Científica y Tecnológica, UBA

D.03.002

Modeling of Human Judgment of Visual Image Complexity using Multivariate Imaging Techniques

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A set of 49 images was applied as a systematic methodology to characterize human perception of image complexity, based on correlation between the human scores and a series of measurements of geometrical properties of the presented images. The experiment requested subjects to assign scores (0-10) to each presented images according to their subjective judgment of image complexity. The images included a set of simple graphic elements (oriented bars varying in number and angles plus salt-and-pepper noise). A total of Thirty five subjects with ages ranging from 30-50 years were considered, all they were post-graduated in exact sciences. The results showed interesting correlations between the subjective scores and three objective computational measurements of the presented patterns, paving the way to further investigations upon visual complexity using modeling based on data mining.

Palavras-Chave: Image Complexity, image perception, Data Mining.

Apoio Financeiro: USP

D.03.003

Discomfort elicited by moving striped patterns in patients with migraine

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Objectives: The goals of this study were to investigate, in migraine patients and healthy volunteers: 1) discomfort elicited by observing moving striped patterns; 2) association between degree of visual discomfort and clinical features.

Methods: Forty-eight subjects participated in the study: 18 healthy volunteers (HV) and 30 patients diagnosed with migraine (MG). Visual stimuli consisted of horizontal square wave gratings moving up or down, within an overall circular shape. Temporal frequency, spatial frequency and duty cycle were varied in images, across ten conditions. Subjects were asked to grade the discomfort produced by the images.

Results: There were no significant differences in age ($p=0.126$) or sex ($p=0.288$) between HV and MG groups. History of motion sickness was more common in the MG group (21/30) than in the HV group (7/18) ($p=0.034$). There were significant differences in discomfort in eight of the ten conditions ($p<0.05$). Discomfort was independent of history of motion sickness in a Generalized Estimating Equations model. There was a significant correlation ($\rho=0.51$; $p=0.004$) between median degree of aversion across conditions and number of migraine attacks in the past month.

Conclusion: Moving striped patterns were more uncomfortable to MG than to HV. Differences in visual processing may explain these findings. Similarly to other studies, history of motion sickness was more common in MG than in HV, but visual discomfort was independent of motion sickness. These results suggest that different mechanisms underlie these two findings in MG.

Palavras-Chave: migraine, visual discomfort , visual stimuli, visual movement

D.03.004

Reciprocally connected cells in the lobula of the crab: neural circuits underlying binocularity of the Lobula Giant Neurons of *Chasmagnathus*

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Runner crabs perform directional visually-elicited escape responses, which implicate binocular processing of information. We have characterized four types of Lobula Giant neurons (LG's) involved in motion processing in *Chasmagnathus*, which are central elements of the visually guided escape circuit. LG's arborize tangentially in one or two strata of the lobula have wide visual fields and are binocular. Information from ipsilateral eye converges from the retina through columnar elements but it is unknown how contralateral eye information reaches them. Two models were proposed to explain visual information transference between eyes. One states that columnar elements input to ipsilateral LG's while sending collateral projections that input into the cognate LG at the opposite eye. The second model postulates that twins LG elements located one in each lobula are reciprocally connected. To find evidence of the neuronal circuit underlying binocularity in the crab, we perform intracellular recordings from LG's in monocular and binocular conditions followed by Neurobiotin staining. We show that at least for one of the LG's groups, the Bistratified Lobula Giant type 1 neurons (BLG1), binocular integration would be done by reciprocal connection between twin elements. Each BLG1 would receive columnar projections from the ipsilateral medulla and would project through the midbrain to contact its cognate at the contralateral eye. Pairs of elements with similar visual responses but differing in the type of terminal processes (spines or blebs) and in the presence or no of the somata were stained in the same lobula. These morphological differences were in agreement with differences found in the amplitude of AP and latency to flashes of light. Additionally, 50% of the BLG1 recorded showed AP of two amplitudes in the same cell. This fact, combined with staining of pairs of BLG1's suggests the existence of electrical connections between BLG1's. Therefore, all this evidence indicates that BLG1's achieve binocular responses throughout their reciprocal connections. Whether the same mechanism accounts for the binocular properties of other types of LG neurons remains to be investigated. **Palavras-Chave:** vision-motion detection-binocular integration-crabs

Apoio Financeiro: UBA X-173, CONICET

D.03.005

Differential cells subpopulations in the RGC-5 cell line characterized by morphology and calcium responses to ATP.

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RGC-5 cells are a new cell line derived from postnatal rat retinal ganglion cells. They express typically retinal ganglion cell markers as Thy-1 protein and Brn 3c mRNA. Furthermore, they do express the mRNA for NMDA, GABA, Trk A, Trk B, Trk C receptors and BDNF, NGF, NT4 and NT3 neurotrophins. In addition, these cells can morphologically differentiate to a neuronal phenotype by addition of Staurosporine, an unspecific Protein Kinase C inhibitor.

Objetives: Although the RGC-5 cells are derived from a single clone; we investigate whether these cells constitute a morphologically homogeneous population.

Methods: the identification of morphological different populations was done by flow cytometry and immunocytochemistry. The intracellular levels of calcium were assessed by fluorescence microscopy with Fura-2 calcium chelator.

Results: A least three morphologically different populations of non differentiated RGC-5 in culture were identified by flow cytometry and ICC. RGC-5 cells show differential calcium responses to ATP stimulation, some of them, exhibit a high amplitude positive response; other cells display a low amplitude positive response, while a third population shows a null response. These differences could be a direct consequence of heterogeneous expression of different ATP receptors in these cells. Surprisingly, RGC-5 cells do not respond to glutamate stimulation (100 and 200 μ M). Staurosporine administration (25ng/mL) induces morphological differentiation that correlates with a change in the flow cytometry pattern at 24 h post staurosporine administration. Interestingly, after 48 h of staurosporine treatment, cells died by apoptosis.

Conclusions: The results strongly support the idea that this cell line is not a homogeneous population and this observation may result an important feature to be considered for future studies and for analyzing biochemical responses in these cells.

Palavras-Chave: RGC-5; Retina; calcium

Apoio Financeiro: CONICET- FONCyT- Fundación Florencio Fiorini - CECyT- U.N.C.

Morphological and Morphometric Analysis of the Optic Nerve in Induced Experimental Hydrocephalus in Rats

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Hydrocephalus is a syndrome caused by the imbalance between the production and absorption of the cerebrospinal fluid with the enlargement of cerebral ventricles [1]. Hydrocephalus unrelated to tumor and hereditary/genetic diseases is the third most frequently observed causes of optic atrophy [2]. Recurrent elevation of intracranial pressure can have serious visual consequences for a child [3]. The aim of the present study was to evaluate the morphological and morphometric alterations of pups rat optic nerves submitted to the experimental hydrocephalus. Forty rats were used for hydrocephalus induction and 10 rats were used as controls. Hydrocephalus induction was performed by means of injection of kaolin suspension into the cisterna magna. Two and 3 weeks after induction, the animals were anesthetized and killed by intracardiac perfusion with a fixative mixture of 4% glutaraldehyde and 2% paraformaldehyde. Their brains were removed and the right optic nerve was dissected and postfixed, embedded in araldite and stained with toluidine blue. To analyze the densities of damaged myelinic fibers, astrocytes and oligodendrocytes six random fields on an optical microscopic were used. For statistical analysis the GraphPad 3.0 program was used, and the data obtained were analysed by the Kruskal – Wallis test with post – hoc Dunn test. The morphometric analysis showed an tendency in increased oligodendrocytes density in the optic nerve of the hydrocephalic animals when compared with controls. Therefore it did not show significant differences. Quantitative measures of the density of the damaged myelinated nervous fibers showed a significant statistical increased in the optic nerves of hydrocephalic rats when compared with matched controls. The difference was bigger as bigger the time of progression of hydrocephalus (table 1). The progression time of hydrocephalus can be a more important factor than dilation degree of ventricles in the injury of the optic nerves in young rats. Key words: experimental hydrocephalus, optic nerve. References [1] Exp Neurol. 154: 157, 1998 [2] Clin. Exp. Ophthalmol 28: 34, 2000 [3] J. Neurosurgery. 39: 599, 1996 Table 1: Densities (means and standard deviation) of oligodendrocytes, astrocytes and damaged fibers in optic nerve of controls (14 days and 21 days), mild hydrocephalic rats (hydro m 14 and 21) and severe hydrocephalic rats (hydro s 14 and 21).

Parameter	Control 14 (mean ± sd)	Control 21 (mean ± sd)	Hydro m 14 (mean ± sd)	Hydro s 14 (mean ± sd)	Hydro m 21 (mean ± sd)	Hydro s 21 (mean ± sd)
Oligodendrocytes	13.30 ± 5.968	12.03 ± 2.653	19.23 ± 3.064	16.77 ± 3.718	14.71 ± 3.280	17.28 ± 2.817
Astrocytes	19.33 ± 1.775	15.86 ± 1.459	21.10 ± 4.076	19.96 ± 3.880	19.18 ± 4.683	19.48 ± 4.735
Damaged fibers	252.2 ± 81.81	352.0 ± 45.80	451.0 ± 99.81	386.5 ± 127.2	533.5 ± 107.0	604.7 ± 111.6

Palavras-Chave: experimental hydrocephalus, optic nerve

Apoio Financeiro: CNPq

D.03.007

Characterization of auditory and visual areas in the forebrain and thalamus of the barn owl (*Tyto alba*)

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Objectives:

Owls possess a sophisticated visual and auditory system. Visual information is provided through two main pathways: the tectofugal and the thalamofugal pathways. The telencephalic recipient of the avian thalamofugal pathway, called visual Wulst, is highly developed in owls and seems functionally akin to the primary visual cortex of mammals. Since there is only limited information about the neuroanatomy/chemistry of the Wulst and other visual and auditory centers in the telencephalon and thalamus, we investigated these areas by histological, immunohistochemical and retrograde tracing methods in the barn owl.

Methods:

Under deep anesthesia, five Barn owls were perfused and their brains were cut coronally on a freezing microtome. Standard histological staining methods such as Nissl-, Gallyas and cytochrome oxidase staining as well as standard immunoperoxidase and fluorescence techniques using antibodies to NeuN, substance P, parvalbumin, calbindin, GAD 65/67, GABA, DARPP-32, and NMDAR1 receptors were performed. In one owl, the retrograde tracer FluoroGold was injected in the visual Wulst.

Results:

The different layers of the huge visual Wulst could be best outlined by DARPP-32, NMDAR1 and cytochrome oxidase staining. Primary sensory areas like the relatively small-sized visual entopallium and the large auditory Field L2 were characterized by the absence of DARPP-32. Parvalbumin staining outlined a relatively small nucleus rotundus and a huge OPT, the relay nuclei of the tectofugal and thalamofugal system, respectively, as well as a highly developed auditory nucleus ovoidalis. Retrograde tracing experiments together with Nissl and NeuN staining indicated that the OPT consists of several distinct subnuclei.

Conclusions:

Results indicate that the thalamofugal system of barn owls is more prominently developed than the tectofugal system, which is reflected by the respective size of their thalamic relay nuclei as well as their telencephalic targets. The large size of the auditory Field L and the auditory thalamic relay nucleus ovoidalis should motivate further studies to investigate more intensively the role of these areas in auditory processing.

Palavras-Chave: Visual, auditory, avian, thalamus, evolution

Apoio Financeiro: FAPESP, FAPEMIG, FINEP

D.03.008

IS SPEED ENCODED IN THE OWL VISUAL WULST?

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Objectives: The visual wulst is the avian telencephalic recipient area of the thalamofugal projection. In owls, this area is known to have a close functional resemblance with primate V1. In the present study, we sought to determine whether visual wulst neurons are tuned to stimulus speed.

Methods: We isolated 131 neurons from four awake burrowing owls (*Athene cunicularia*) and studied their responses to drifting sinusoidal gratings of different spatial and temporal frequencies. The degree of response covariation between these two dimensions was used to classify cells as: “speed-tuned” (positive covariation), “independent” (no covariation), “intermediate,” or “negative” (negative covariation). Covariation was quantified by a speed-tuning parameter from a modified two-dimensional Gaussian function with which the spatiotemporal frequency response profiles were fitted (J. Neurosci., 26:2941, 2006).

Results: We found that 80% of our sampled cells were significantly modulated by the stimulus. Of these, 84.3% (n=86) passed our goodness-of-fit criteria. Three units (3.5%) were classified as “speed-tuned,” 56 (65%) as “independent” and 22 (55.6%) as “intermediate.” Five other units (5.8%) were categorized as “negative.” A higher mean value of the speed-tuning parameter was noted for directional cells when compared to bidirectional cells, but this difference was not statistically significant (Wilcoxon rank sum test, $p=0.20$). There was also no statistical difference between these two cell types with regards to their respective distribution within each of the speed tuning categories (Fisher’s exact test, $p=0.21$).

Conclusions: Even though the majority of cells had independent spatiotemporal tuning, our finding that intermediate and fully formed speed-tuned cells are present in the owl visual wulst is compatible with the idea that this area is important for processing the speed of moving objects independently of their spatial attributes.

Palavras-Chave: speed, motion processing, owl, visual telencephalon

Apoio Financeiro: FAPEMIG, FINEP, CNPq

D.03.009

CONTRAST RESPONSE FUNCTIONS IN THE VISUAL WULST OF THE AWAKE OWL: A SINGLE-CELL STUDY.

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Objectives: Visual contrast is a fundamental image feature. In the present study, we ask how this feature is encoded by neurons in the visual wulst of the owl.

Methods: We isolated 53 neurons from three awake burrowing owls (*Athene cunicularia*), and studied their responses to drifting sinusoidal gratings as a function of contrast intensity. All measurements were made through the dominant eye and with gratings centered on the cell receptive field and optimized for direction of motion, spatial frequency, and speed. In order to characterize the contrast response function of each cell quantitatively, we used four different mathematical functions: linear, logarithmic, power and hyperbolic ratio. To determine which of the four functions provided the best fit, we used the square of the fitting correlation coefficient (R^2) and the Aikake's Information Criterion (AIC).

Results: Cells usually responded monotonically and non-linearly to visual contrast. Typically, responses increased sharply, often linearly, at low contrast values, and compressed, at times saturated, at higher values. The shape of the contrast response profile was highly variable from cell to cell. Quantitatively, our results show that 73% of the recorded cells were significantly modulated by contrast. Of these, the function which best fitted the data was hyperbolic ratio for 39% of our cell sample, logarithmic for 33%, power for 15% and linear for 13%.

Conclusions: Our study indicates that the response to contrast of most visual wulst neurons is non-linear, being best modelled by hyperbolic ratio and logarithmic functions. This non-linearity is consistent with the notion that some kind of contrast gain control is present in the owl visual wulst. **Palavras-**

Chave: contrast response, visual wulst, owl

Apoio Financeiro: FAPEMIG, FINEP, CAPES

D.03.010

SENSIBILIDADE AO CONTRASTE DE ADULTOS PARA ESTÍMULOS POLARES E CARTESIANOS EM CONDIÇÃO ESCOTÓPICA

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Objetivo: Estudos neurofisiológicos mostram que estímulos em coordenadas cartesianas (grade senoidal) e polares (radial) são processados por áreas visuais diferentes. O objetivo deste estudo foi determinar e comparar a sensibilidade ao contraste para estes padrões e verificar se o sistema visual (SV) apresenta sensibilidade diferente para tais estímulos em condição escotópica.

Método: Participaram deste estudo oito voluntários de ambos os sexos com faixa etária de 19 a 25 anos, com acuidade visual normal ou corrigida e livres de patologias visuais identificáveis. Mensurou-se oito curvas de sensibilidade para grade senoidal (FSC) e oito para frequência radial (FSCr), utilizando as frequências de 0,25; 0,5; 1,0; 2,0; 4,0; 8,0; 12,0 ciclos por grau de ângulo visual (cpg). As medidas foram realizadas com o método psicofísico da escolha forçada, binocularmente, com pupila natural. Os estímulos foram gerados em um monitor LG de 19 polegadas, controlado por um microcomputador e um *hardware* Bits ++ (Cambridge Research Systems). Durante o experimento, eram apresentados pares de estímulos circulares, com diâmetro de 7,2 graus de ângulo visual, e o participante tinha que escolher aquele que continha uma das frequências de teste (grade senoidal ou radial) acima em detrimento de um padrão homogêneo com a luminância média de 0,04 cd/m². A ordem de apresentação dos estímulos era aleatória e controlada pelo software.

Resultados: A análise de variância (ANOVA) para medidas repetidas mostrou diferenças significantes entre os estímulos [$F(1, 158) = 14,1$; $p < 0,001$]. Já o Teste Post-hoc Tukey HSD revelou diferença significativa apenas entre as frequências espaciais e radiais de 4 cpg ($p < 0,001$). Os resultados mostraram que o sistema visual humano em condições escotópicas foi da ordem de 1,7 vezes mais sensível para grade senoidal do que para estímulos radiais.

Conclusão: Estes resultados revelam que o SV apresenta sensibilidade diferente para estímulos definidos em coordenadas polares e cartesianas, sugerindo que os mesmos são processados por mecanismos distintos.

Palavras-Chave: sensibilidade ao contraste; grade senoidal; radial; escotópica

Apoio Financeiro: CNPQ

D.03.011

AVALIAÇÃO DA PERCEPÇÃO VISUAL DA FORMA DE PACIENTES COM DEPRESSÃO UTILIZANDO CENAS VISUAIS COMPLEXAS

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Objetivo:

O episódio depressivo faz parte dos transtornos de humor e acarreta alterações na atenção, memória, aprendizagem, redução da motivação, auto-estima baixa, padrões de sono e alimentares desregulados, perda da libido, prejuízo no funcionamento do sistema imunológico, dentre outros. O presente trabalho teve por objetivo investigar possíveis alterações na percepção visual da forma em pacientes com episódio depressivo utilizando como instrumento cenas visuais complexas.

Método:

Participaram deste estudo 40 voluntários adultos de ambos os sexos com idades entre 19 e 62 anos (Média Geral = 40,12 e Desvio Padrão = 12,7), 20 deles isentos de patologia neuropsiquiátrica (Grupo Controle – GC) e 20 com o diagnóstico de episódio depressivo (Grupo Experimental – GE) segundo avaliação de médico psiquiatra de acordo com a CID-10 (Classificação Internacional de Doenças – décima revisão). Foram utilizados vinte e quatro cenas (quadros do pintor Salvador Dalí fotografados na dimensão 15 x 10 cm) selecionadas baseadas em estudos anteriores com pacientes com esquizofrenia. As fotografias dos quadros foram apresentadas sucessivamente, uma após a outra, sem limite de tempo para observação/resposta. Após a apresentação de cada fotografia, o participante foi instruído a indicar a primeira figura que lhe chamou atenção. Cada figura indicada pelo voluntário foi mensurada em milímetros, e em seguida os valores foram transformados em grau de ângulo visual através da equação $Tang\ a = \frac{\text{Tamanho da figura (mm)}}{\text{Distância do observador (300 mm)}}$.

Resultados:

Os dados foram analisados utilizando uma ANOVA para medidas repetidas Repeated Measures ANOVA. Os resultados mostraram diferença significativa entre os dois grupos [$F(1, 38) = 4,28$; $p = 0,04$]. A média do tamanho das figuras escolhidas pelos participantes do GE ($M = 10,22$ graus de ângulo visual) foi maior do que a média das figuras escolhidas pelos participantes do GC ($M = 8,12$ graus de ângulo visual). Além disso, os tamanhos das figuras observadas pelo GE foram maiores em 22 dos 24 quadros observados.

Conclusões:

Os resultados sugerem que os episódios depressivos alteram a percepção visual de cenas visuais complexas. **Palavras-Chave:** Percepção visual da forma, cenas visuais complexas, depressão

Apoio Financeiro: CNPq e CAPES

D.03.012

Suppression effects outside the classical receptive field in the visual wulst of the burrowing owl (*Athene cunicularia*)

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Objective: In the primary visual cortex (V1) of mammals, neurons can be modulated in a selective way by stimuli outside their classical receptive field. In the present study, we ask whether such contextual effects characterize neurons in the visual wulst of the owl. This question is particularly interesting given the close functional analogies that have been found between V1 and the owl visual wulst, which makes the latter an attractive model to learn more about the evolution of the visual system.

Methods: Using standard extracellular recording techniques, we measured the responses of isolated neurons to moving sinusoidal gratings of 10 different sizes (0.5, 0.7, 1, 2, 3, 3.6, 6, 9, 12 and 18 degrees of visual angle). Manual mapping was initially used to determine the location of the receptive field center. All measurements were made through the dominant eye, with gratings centered on the cell receptive field and optimized for direction, contrast, spatial frequency, and speed.

Results: Cell responses typically increased with stimulus diameter, reaching a peak at about 2 degrees of visual angle (spatial summation), followed by a decrease of variable magnitude (suppression), which plateaued for large grating sizes. For a subset of cells, this plateau was well below baseline activity, a behavior typical of Hubel and Wiesel's hypercomplex cells.

Conclusion: The suppressive effects observed outside the classical receptive field of neurons in the owl visual wulst are analogous to those reported in V1. Further work will be needed to understand the mechanisms underlying such effects in the owl.

Apoio Financeiro: CNPq, FINEP

D.03.013

alterações na função de sensibilidade ao contraste de ALCOOLISTAS EM RECUPERAÇÃO

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Objetivo: A função de sensibilidade ao contraste (FSC) é uma das principais técnicas na avaliação do sistema visual humano quer seja do ponto de vista teórico, quer seja do ponto de vista clínico. O presente estudo utilizou a FSC para caracterizar a percepção visual de alcoolistas em fase de recuperação para estímulos do tipo grade senoidal.

Método: Participaram do estudo oito sujeitos na faixa etária de 39 a 56 anos, quatro sem histórico de abuso de álcool (Grupo Controle, GC) e quatro alcoolistas em recuperação (Grupo Experimental, GE). Todos os participantes apresentavam acuidade visual normal ou corrigida. Os participantes do GE foram selecionados pelo tempo de consumo de álcool (média de 20 anos de consumo) sem registros de dependência de outras substâncias psicoativas, exceto o tabaco. Todos os participantes do GE freqüentavam grupos de apoio a dependentes do álcool e estão em abstinência de no mínimo oito anos. Os estímulos com freqüências espaciais de 0,25; 1,0; 2,0 e 8,0 cpg (ciclos por grau de ângulo visual) eram circulares, tendo um diâmetro de 7,25 graus de ângulo visual. Os mesmos foram apresentados em um Monitor LG 19 polegadas, controlado por um microcomputador com placa de vídeo de 128 megabytes, entrada VGA e DVI, conectado a um bits ++ (Cambridge Research Systems). As medidas foram realizadas binocularmente, utilizando o método psicofísico da escolha forçada com os participantes a uma distância de 150cm dos estímulos. Foram apresentados pares de estímulos, sendo que um deles continha a freqüência espacial, enquanto o outro era um padrão homogêneo com a luminância média (42,6 cd/m²). A tarefa dos participantes foi escolher sempre o círculo com a freqüência espacial.

Resultados: As análises com a ANOVA para medidas repetidas mostraram diferenças significantes entre os grupos [$F_{(1, 94)} = 27,09$]; $p < 0,001$]. A análise com o teste post-hockey Turkey HSD mostrou diferenças significantes entre os grupos apenas na freqüência de 8 cpg ($p < 0,001$).

Conclusões: Os resultados sugerem alterações na FSC relacionadas ao uso crônico do álcool, principalmente na freqüência espacial mais alta onde a diferença foi estatisticamente significativa. **Palavras-Chave:** Sensibilidade ao contraste, alcoolistas

Apoio Financeiro: CNPq e CAPES

D.03.014

Padrão de distribuição de neurônios reativos a NADPH diaforase e proteínas ligantes de cálcio nas áreas visuais VI e VII do córtex visual do sagüi (*Callithrix jacchus*).

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No presente trabalho, pretendemos avaliar o padrão de distribuição de neurônios reativos a NADPH diaforase e proteínas ligantes de cálcio nas áreas visuais VI e VII do córtex visual do sagüi (*Callithrix jacchus*).

Quatro sagüis machos adultos foram perfundidos com solução salina heparinizada 0.9% e paraformaldeído a 4%. Seus cérebros foram crioprotégidos com solução de sacarose a 20%, separados em três blocos e congelados. Em dois animais, o bloco posterior, contendo o córtex visual, foi seccionado a 20 µm no plano coronal com o auxílio de um criostato. Secções adjacentes foram então submetidas ao método de Nissl para delimitação das camadas corticais, à histoquímica para revelação da atividade da enzima NADPH-d e à imunohistoquímica para os ligantes de cálcio CB, CR e PV. Nos dois outros animais, o tecido foi seccionado a 100 µm e reagido para NADPH-d. Dois tipos distintos de neurônios reativos a NADPH-d foram observados: um mais reativo, com arborização dendrítica exuberante (tipo I), mais presente na substância branca, e um menos reativo e bem mais numeroso (tipo II), predominantemente localizado nas camadas supragranulares do córtex. Já os neurônios positivos para os ligantes de cálcio localizam-se especialmente nas camadas supragranulares do córtex. Os neurônios do tipo I encontrados na substância branca possuem arborização dendrítica bem mais complexa que aqueles encontrados no córtex, em geral projetando seus dendritos para o interior deste.

A distribuição de ambas as subclasses de neurônios segue o padrão descrito para outras espécies de primatas não humanos (Cereb. Cortex 6: 524, 1996; Cereb. Cortex 10: 160, 2000), sugerindo que o padrão de organização cortical é filogeneticamente conservado neste grupo. A morfologia e distribuição distintas podem estar relacionadas a aspectos diferenciais de circuitaria cortical.

Palavras-Chave: Interneurônios; organização cortical; córtex visual; sagüi

Apoio Financeiro: AASDAP; CNPq; FINEP

Efeito do envelhecimento sobre o processamento visual de estímulos elementares

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Este estudo mensurou a sensibilidade ao contraste visual para três padrões elementares: freqüências angulares de 3, 24, 48 e 96 ciclos/360° e freqüências radiais e grades senoidais de 0,25; 1; 2 e 8 cpg. Considerando que estes estímulos são processados por áreas visuais diferentes, o objetivo foi caracterizar alterações no processamento destes padrões relacionados ao envelhecimento. Participaram do estudo 14 voluntários, sete adultos na faixa de 20-30 anos (G20; M=23,8 Sd= 2,6) e sete idosos na faixa de 60-70 anos (G60; M=64,2 Sd=3,0). Todos os participantes estavam livres de patologias visuais identificáveis e com acuidade visual normal ou corrigida. As medidas foram realizadas com o método psicofísico da escolha forçada, binocularmente, a 150 cm da tela do monitor. Os estímulos foram apresentados em um monitor LG de 19 polegadas, controlado por um microcomputador e um hardware Bits ++ (Cambridge Research Systems). Durante o experimento, foram apresentados pares de estímulos e o participante tinha que escolher o estímulo que continha uma das freqüências de teste acima em detrimento de um padrão homogêneo com a luminância média de 42 cd/m². Todos os estímulos eram circulares, com diâmetro de 7,2 graus de ângulo visual. As análises (ANOVA para medidas repetidas) mostraram diferenças significantes relacionadas à idade entre os dois grupos para os estímulos grade senoidal [$F_{(3, 498)} = 132.66$, $p < 0,01$], radial [$F_{(3, 426)} = 3.3976$, $p < 0,01$] e angular [$F_{(3, 496)} = 23.507$, $p < 0,01$]. O teste post-hoc Tuckey indicou diferenças significantes entre o G20 e o G60 nas freqüências angulares de 3 e 96 ciclos/360° e na freqüência radial e de grade senoidal de 8,0 cpg ($p < 0,01$). Observou-se ainda que a sensibilidade média do G20 e do G60 para freqüências angulares foi 5 vezes maior do que para grades senoidais e 8 vezes maior do que para freqüências radiais. Estes resultados sugerem que o envelhecimento altera diferentemente o processamento de estímulos elementares definidos em coordenadas cartesianas e polares, sugerindo ainda que estes padrões sejam processados por áreas visuais distintas.

Palavras-Chave: Sensibilidade ao contraste, Envelhecimento, Estímulos elementares, Percepção

Apoio Financeiro: PIBIC / CNPQ , UFPB

DETECÇÃO DE ESTÍMULOS VISUAIS ANGULARES ESCOTÓPICOS E FOTÓPICOS EM PACIENTES COM ESQUIZOFRENIA

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O presente estudo teve como objetivo mensurar a sensibilidade ao contraste de portadores de esquizofrenia para estímulos de frequências angulares em condições de luminância escotópica ($0,06 \text{ cd/m}^2$) e fotópica (42 cd/m^2). Participaram da pesquisa cinco voluntários de ambos os sexos, com diagnóstico de esquizofrenia e idades entre 20 e 45 anos. Todos os voluntários estavam isentos de patologias visuais identificáveis, com acuidade visual normal ou corrigida e utilizando os medicamentos antipsicóticos sistematicamente. Foram mensuradas 40 curvas de limiares de contraste para as frequências angulares de 3, 24, 48 e 96 ciclos/ 360° , sendo 20 na condição fotópica e 20 na condição escotópica. Os estímulos foram apresentados em um monitor LG de 19 polegadas, controlado por um microcomputador e um hardware bits ++ (Cambridge Research Systems). As medidas foram realizadas binocularmente a 150 cm da tela do monitor. Usou-se um delineamento experimental com medidas repetidas e o método psicofísico da escolha forçada. Durante o experimento, foram apresentados, sucessiva e aleatoriamente, pares de estímulos, um com um padrão homogêneo à luminância média ($0,04$ ou 42 cd/m^2) e o outro com uma frequência angular. A tarefa do participante foi escolher sempre o estímulo com a frequência angular. Todos os estímulos eram circularmente simétricos, com diâmetro de 7,2 graus de ângulo visual. As análises estatísticas (ANOVA para medidas repetidas) apontaram um efeito significativo entre frequências [$F_{(3, 594)}=97,89$, $p < 0,001$], entre condições de luminância [$F_{(1, 198)}=135,97$, $p < 0,001$] e uma interação entre frequência e luminância [$F_{(3, 594)}=70,97$, $p < 0,001$]. O teste post-hoc Tuckey HSD apontou diferenças significantes nas frequências de 3, 48 e 96 ciclos/ 360° ($p < 0,001$). Os resultados mostram ainda que os participantes foram da ordem de 1,2 vezes mais sensíveis nas condições de luminância fotópica do que na escotópica. Os resultados indicam que a sensibilidade ao contraste para frequências angulares de pacientes com esquizofrenia é diferente nas duas condições de luminância.

Palavras-Chave: Esquizofrenia, Sensibilidade ao Contraste, Luminância Fotópica, Luminância Escotópica

Apoio Financeiro: CNPQ

PRIVAÇÃO AUDITIVA ALTERA A PERCEPÇÃO VISUAL DE ESTÍMULOS ELEMENTARES?

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Objetivo:

Estudos discutem que a privação parcial ou total de uma modalidade sensorial altera a resposta de outras modalidades, que tentam naturalmente se reorganizar para se adaptarem a esta condição. Este estudo teve como objetivo medir a função de sensibilidade ao contraste para estímulos visuais elementares de frequência espacial (FSC) e radial (FSCr) em crianças surdas.

Método:

Participaram do estudo oito crianças com surdez profunda bilateral (perda auditiva ≥ 90 dB), de ambos os sexos, com idades entre 8 e 11 anos. Todas as crianças apresentavam acuidade visual normal ou corrigida. Os estímulos de frequências espaciais e radiais de 0,25; 2,0 e 8,0 cpg (ciclos por grau de ângulo visual) eram circulares, com diâmetro de 7,25 graus de ângulo visual. As medidas foram obtidas com o método psicofísico da escolha forçada, com visão binocular, a uma distância de 150 cm de um monitor LG de 19 polegadas. O procedimento consistiu na apresentação sucessiva de pares de estímulos, um contendo uma das frequências espaciais ou radiais acima e o outro apenas a luminância média de 42,6 cd/m². A tarefa das crianças foi escolher sempre o estímulo que continha uma das frequências. As instruções sobre os experimentos foram explicadas para as crianças surdas em LIBRAS (Língua Brasileira de Sinais).

Resultados:

A ANOVA para medidas repetidas mostrou diferença significativa entre a FSC de estímulo espacial e a FSCr de estímulo radial ($F_{1, 190} = 269,94$; $p < 0,001$). O teste post-hoc Tukey HSD revelou diferença significativa entre os dois estímulos em todas as frequências testadas ($p < 0,001$). Isto é, os resultados mostraram que as crianças surdas foram da ordem de 2,0; 2,5 e 2,1 vezes mais sensíveis para frequências espaciais do que para frequências radiais nas frequências de 0,25; 2,0 e 8,0 cpg, respectivamente.

Conclusão:

Estes resultados sugerem que a percepção visual de crianças surdas é mais sensível para frequências espaciais do que para frequências radiais, indicando que estes estímulos são processados por mecanismos visuais distintos.

Palavras-Chave: percepção visual; função de sensibilidade ao contraste; surdez; crianças.

Apoio Financeiro: FACEPE e CNPq

D.03.018

DETECÇÃO VISUAL DE ESTÍMULOS ANGULARES APÓS O CONSUMO MODERADO DE ÁLCOOL

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Objetivo: Caracterizar a função de sensibilidade ao contraste para estímulos de frequências angulares (FSCa) de 3, 24 e 96 ciclos/360° em adultos sem (Grupo Controle, GC) e após a ingestão moderada de álcool, 0,09 g/dl (Grupo Experimental, GE).

Método: Participaram dos experimentos cinco voluntários do sexo feminino (21-30 anos) com acuidade visual normal ou corrigida. Todos participaram das duas condições, com ingestão e sem ingestão de álcool. A ordem em que cada voluntário passou por cada uma das duas condições foi controlada pela técnica de contrabalanceamento. A quantidade de bebida alcoólica ingerida por cada voluntário foi determinada pela fórmula: $C_{max} (g/l) = 0,02 \times dose (g \text{ por } 70 \text{ kg de peso corporal})$. A administração do álcool foi realizada sob a forma de Vodka “*Stolichinaya*” com concentração de 40% diluída em suco de laranja natural com beterraba e sem açúcar na proporção de 1/1. Na condição placebo, a Vodka foi substituída por suco de limão. Um etilômetro (AL6000) foi utilizado para estimar o teor de álcool no sangue. A sessão experimental iniciou quando a concentração de álcool alcançou a marca de 0,09 g/dl. Usou-se um delineamento experimental com medidas repetidas e o método psicofísico da escolha forçada. O procedimento para medir a sensibilidade consistiu na apresentação sucessiva de pares de estímulos e os voluntários foram orientados a escolher sempre dentre eles qual continha a frequência angular. O outro estímulo neutro foi sempre um padrão homogêneo com a luminância média de 0,9 cd/m².

Resultados: A análise de variância para medidas repetidas mostrou efeito significativo de grupo [$F_{1, 118} = 4,55$, ($p < 0,05$)]; efeito significativo de frequência [$F_{2, 236} = 14,68$, ($p < 0,05$)] e efeito de interação de frequência*grupo [$F_{2, 236} = 4,34$, ($p < 0,05$)]. As análises com teste post-hoc Tukey HSD mostraram diferenças significantes entre o GC e o GE na frequência de 3 ciclos/360° ($p = 0,005$).

Conclusões: Os resultados preliminares indicam que a ingestão moderada de álcool altera a FSCa na frequência angular 3 ciclos/360°. Entretanto, pesquisas desta natureza, utilizando frequências angulares e ingestão de álcool, são inéditas na literatura.

Laboratório de Percepção Visual- LabVis

Laboratório de Percepção, Neurociências e Comportamento

Palavras-Chave: Álcool, sensibilidade ao contraste, frequência angular

Apoio Financeiro: CNPq e FACEPE

D.03.019

“O estudo das cenas visuais complexas e sua relação com a esquizofrenia.”

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Objetivo: O presente trabalho teve por objetivo investigar alterações na percepção visual de forma em pacientes com esquizofrenia, utilizando fotografias de alguns quadros do pintor surrealista Salvador Dalí. **Metodologia:** Participaram deste estudo 60 adultos, 30 isentos de patologia neuropsiquiátrica (Grupo controle - GC) e 30 portadores de esquizofrenia medicados e em fase remissiva (Grupo Experimental – GE). Todos voluntários apresentaram acuidade visual normal ou corrigida. Foram utilizados vinte e quatro quadros fotografados na dimensão 20 x 30 cm, que apresentavam figuras de tamanhos variados e com cenas visuais complexas. As fotografias dos quadros foram apresentadas aos voluntários, sucessivamente, uma após a outra, sem limite de tempo para observação. Após a apresentação de cada quadro, o participante indicou a gravura neles que mais se destacou. Em seguida, cada uma delas foi medida em centímetro e utilizada para calcular o ângulo visual pela função matemática $\tan \alpha - 1$. **Resultados:** Os resultados mostraram diferença significativa entre os dois grupos ($F_{1, 58} = 550, 62, p < 0,001$). Isto é, a maioria dos participantes do GE preferiu figuras com tamanho médio maior (entre 10-15°) do que o GC que preferiu figuras com tamanho médio menor (4-5°). No geral, as figuras indicadas pelo GE foram em média 3 vezes maiores que as indicadas pelo GC. Os resultados das medidas foram obtidas pela ANOVA. Um novo estudo está sendo realizado com a mesma metodologia a fim de comparar e ampliar a amostra destes resultados. **Conclusão:** Estes dados indicaram que os portadores de esquizofrenia apresentaram alteração na percepção visual através de cenas visuais complexas e que os mesmos podem ser utilizados para estudar esta patologia. **Palavras-Chave:** Esquizofrenia, Percepção-Visual, Salvador Dalí

Apoio Financeiro: CAPES - CNPq

D.03.020

“CARACTERIZAÇÃO DO FENÔMENO MUITAS-FACES EM PORTADORES DE ESQUIZOFRENIA”

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Objetivo: O fenômeno Muitas-Faces (MF) (Perception 29:1393,2000) caracteriza-se pela percepção de movimento ou mudança de expressão facial; e surgimento de características diferentes ou outras faces. O objetivo deste trabalho foi verificar a ocorrência do fenômeno de Muitas-Faces em indivíduos portadores de esquizofrenia.

Métodos: Participaram dos experimentos quarenta voluntários não ingênuos (que já observaram o fenômeno) de ambos os sexos (18-55 anos) com acuidade visual normal ou corrigida por óculos, divididos em 2 grupos: um formado por portadores de Esquizofrenia (grupo experimental- GE) e outro com pessoas sem o diagnóstico (grupo controle- GC). Antes do experimento foi realizado um pré-teste que consistia em apresentar uma face feminina com um círculo preto no nariz e um ponto de fixação à direita ou à esquerda da face. O participante deveria relatar qualquer alteração percebida. Só as pessoas que conseguiam ver o fenômeno participavam do experimento em que era apresentada a uma distância de 20 cm, em uma folha de papel branco A4, uma face feminina de 21 graus de ângulo visual com um círculo preto no nariz e um ponto de fixação à direita ou à esquerda da face. O participante deveria fixar o olhar no ponto indicado (cada olho separadamente) e relatar quando estivesse percebendo qualquer alteração na imagem. O período de observação para cada olho foi de 90s.

Resultados: Foi realizada uma ANOVA para mensurações repetidas que mostrou diferença significativa entre os voluntários dos GC e GE na categoria Muitas-Faces em MF com o Olho Esquerdo ($p=0,0140$). O teste de proporção entre e intra grupos revelou que mais voluntárias do GE relataram eventos relacionados à categoria MF com OE do que os homens do mesmo grupo (GE), $p=0,0298$, e das mulheres do GC, $p=0,0088$.

Conclusões: Os resultados demonstram predominância da observação do fenômeno com o olho esquerdo, o que sugere uma preferência pelo hemisfério cerebral direito no processamento do Muitas-Faces nos pacientes com esquizofrenia. Por outro lado, novas pesquisas relacionando o Muitas-Faces à esquizofrenia precisam ser conduzidas para discutir de maneira mais aprofundada os aspectos observados.

Palavras-Chave: esquizofrenia, percepção de faces, fenômeno de muitas-faces.

Apoio Financeiro: CNPq

D.03.021

“O FENÔMENO DE MUITAS FACES EM FUNÇÃO DO CONTRASTE”

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Objetivos: Quantificar o efeito da diferença no nível de contraste na percepção do fenômeno de Muitas-Faces (Perception 29:1393,2000) mantendo parâmetros básicos cujos efeitos já foram estudados que apontam para as melhores condições de visualização do fenômeno.

Metodologia: Participaram do estudo 8 voluntários, distribuídos uniformemente entre homens e mulheres. Cada voluntário rodou o experimento 8 vezes, variando o nível de contraste (25% e 75%) e o gênero do estímulo (masculino e feminino). A ordem das variantes foi totalmente aleatória para todos os sujeitos, bem como qual olho seria escolhido para iniciar o experimento. Ao final da coleta de dados, foram realizados 64 experimentos com os 8 sujeitos. O procedimento consistiu na apresentação de um estímulo contendo uma imagem masculina para o olho direito e esquerdo com dois níveis de contraste aleatórios. Posteriormente o mesmo experimento com uma imagem feminina nas mesmas condições, as apresentações seguiram os requisitos científicos de aleatoriedade. Em seguida foi feita a impressão dos resultados de cada ponto e seu agrupamento em curvas com o intuito de formar a planilha de dados com os resultados a serem colocados nos gráficos.

Resultados: Os resultados demonstram que existe uma diferença significativa na ocorrência do fenômeno de Muitas Faces para cada olho e entre as categorias 1 (movimento e expressão facial) e categoria 2 (muitas-faces) mesmo com um universo de oito participantes, o programa utilizado Statistica 6.0 ANOVA apresentou significância de $p = 75775$. Indicam também que há uma equivalência entre os sexos em relação aos níveis de contrastes, de forma a não apresentar diferenças estatisticamente significativas quando submetidos aos testes estatísticos. Posteriormente será avaliado se este resultado se confirma, por se tratar de um estudo piloto com uma população de participantes restrita. **Palavras-Chave:** fenômeno de muitas faces, percepção visual, percepção de faces; ponto-cego; percepção de tamanho

Apoio Financeiro: CNPq

D.03.022

“O FENÔMENO DE MUITAS FACES EM FUNÇÃO DO TAMANHO DO ESTÍMULO”

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Objetivos: Nos estudos sobre a percepção do Fenômeno de Múltiplas Faces (Perception 29:1393,2000) verificamos uma maior ocorrência quando determinadas variáveis eram manipuladas. Dentre as variáveis que merecem atenção, a questão do tamanho das imagens se destaca. O objetivo desta pesquisa foi estudar o Fenômeno de Muitas-Faces em função do tamanho do estímulo.

Metodologia: Na região do ponto cego, colaboraram com o estudo 120 sujeitos, entre 18 e 51 anos, de ambos os sexos, que apresentavam visão normal ou corrigida. Utilizamos quatro tamanhos de estímulos: 11,7; 15,6; 20,6 e 27,0 graus de ângulo visual, impressos em papel cartão. Aos voluntários foi pedido que registrassem o número de vezes das ocorrências de mudanças de expressão ou o fenômeno de muitas-faces, usando dois contadores mecânicos. No experimento na região contralateral ao ponto cego os estímulos foram apresentados, na tela de um computador, a 80 adultos de ambos os sexos, entre 19 e 25 anos, não-ingênuos quanto às Múltiplas-Faces. Nesta contagem os sujeitos utilizaram um teclado de computador onde deveriam pressionar uma tecla para a categoria EFM e outra para a categoria MF. Ao término dos dois experimentos os sujeitos foram instruídos a relatar o que presenciaram e seus relatos foram gravados para estudos posteriores.

Resultados: A análise estatística dos experimentos mostrou que estímulos entre 15 e 20 graus de ângulo visual induzem maiores frequências do fenômeno. Bem como a indicação da existência de lateralidade na percepção do fenômeno. No experimento realizado na região do ponto cego o fenômeno foi mais frequentemente relatado para o olho direito e tamanho 2, i. e. 15,6 graus de ângulo visual.

Conclusão: A percepção de faces de diferentes identidades ocorre com maior frequência para o tamanho 15,6 graus de ângulo visual no lado contralateral ao ponto cego e no próprio ponto cego. Portanto tal evento parece ser independente da interpolação que ocorre nessa região. **Palavras-Chave:** fenômeno de muitas faces, percepção visual, percepção de faces; ponto-cego; percepção de tamanho

Apoio Financeiro: CNPq

**GENE AND PROTEIN EXPRESSION IN CHICKEN EMBRYONIC RETINAL CELLS:
EFFECTS OF LIGHT AND GLUTAMATE.**

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OBJECTIVES: The vertebrate retina is a part of the Central Nervous System (CNS) that shows many rhythmic physiological events, which are coordinated to predict environmental changes in light conditions during the day, optimizing retina function. In order to investigate this coordination in birds, we studied the distribution of Opn4x expressing cells in chicken retina in vivo, as well as the expression of *Opn4x*, *Opn4m*, *Clock*, *N-Acetyltransferase* and *Tyrosine Hydroxylase* genes in chick embryo cultured retinal cells in constant dark, in 12L:12D photoperiod, or in constant dark, in the presence of glutamate.

METHODS: We analyzed the expression of melanopsin in animals kept under 18L:6D and 6L:18D regimens, by RT-PCR and qPCR. Primary cultures of chicken retina from 8-day-old embryos were kept in constant dark, 12L:12D, or in constant dark treated with 100uM glutamate for 12h. Total RNA was extracted every 3h starting at ZT0 of the sixth day along 24h. The samples were submitted to RT-PCR followed by qPCR. To analyze OPN4x expression in these cells, and in the whole retina from one day old chicks, we performed immunocytochemistry with antibodies anti-chicken melanopsin (1:2000) developed in rabbit. **RESULTS:** No rhythm of transcription was seen for any gene in cells kept in constant dark. Under 12L:12D, all genes expressed phase variations. COSINOR analysis confirmed the rhythm for *Opn4m*, *Clock*, *N-Acetyltransferase*, and *Tyrosine Hydroxylase*. In constant dark, glutamate was not sufficient to restore the pattern seen in 12L:12D. However, it reduced the expression of *Opn4x*, *Opn4m* and *Clock*, drastically decreased *Tyrosine Hydroxylase* levels, and triggered a new rhythm of expression. In chicken retina in vivo, the majority of OPN4x expressing cells was seen in the inner nuclear layer, and very few ganglion cells express OPN4x. The presence of immunopositive cells for *G. gallus* OPN4x in the culture was also demonstrated.

CONCLUSION: mRNA transcription in chicken embryonic retinal cells under 12L:12D show circadian variations. In constant dark, these oscillations were damped. We demonstrated that these cells are able to govern its physiology in culture conditions, and that glutamate may regulate retinal circadian events.

Palavras-Chave: Opn4x, Opn4m, Clock, N-acetyltransferase and Tyrosine Hydroxylase

Apoio Financeiro: FAPESP and CNPq (Brazil).

D.03.024

Análise morfométrica de neurônios da camada I do córtex visual do rato: Um estudo com injeção intracelular

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Objetivo: Fazer o estudo morfométrico de neurônios da camada I do córtex visual do rato no plano anatômico coronal. **Métodos:** 50 ratos da raça *Wistar* foram anestesiados e decapitados, sendo o encéfalo rapidamente imerso em líquido cérebro-espinhal artificial (LCEA) oxigenado com 95% de O₂ e 5% de CO₂. Foram feitas secções coronais da área 17 com espessura de 250 µm, marcação intracelular com biocitina (SIGMA) e revelação com o kit ABC (Vectastain Standart ABC kit, VECTOR LABORATORIES). As células foram desenhadas, fotomicrografadas e analisadas morfometricamente com o software Scion Image (SCION CORPORATION). Aplicamos a análise de variância seguida pelo teste Tukey ($\alpha=0,05$). Os valores foram expressos como média $\pm$ erro padrão. **Resultados:** Identificamos três tipos celulares: neurônios superficiais (NS), neurogliaformes (NG) e os neurônios da borda inferior da camada I (NB). Os NS possuem ramificação dendrítica paralela à superfície pial, os NG apresentam ramificação axonal restrita a camada I e os NB apresentam dendritos que se projetam para as camadas II/III. As médias $\pm$ erro padrão das variáveis morfométricas foram: área do corpo celular em μm^2 (NS= 0.62 ± 0.13 ; NG= 0.32 ± 0.07 ; NB= 0.19 ± 0.03); área do campo dendrítico em μm^2 (NS= 51.13 ± 22 ; NG= 87.54 ± 10.7 ; NB= 90.68 ± 6.3); n° de espinhas dendríticas (NS= $56,33 \pm 12.33$; NG= não possui; NB= $122,17 \pm 11.84$); n° de ramificações (NS= $7,33 \pm 1.7$; NG= $22,56 \pm 1.36$; NB= $14,17 \pm 1.53$). O teste estatístico apontou diferenças morfométricas significativas entre os 3 tipos celulares estudados ($p<0,05$). **Conclusão:** A camada I do córtex visual do rato é rica em tipos morfológicos. Isto indica a presença de circuitos neurais complexos, com papel importante no processamento das informações visuais. A localização distinta dos 3 tipos morfológicos estudados elucida diferenças migratórias ao longo do desenvolvimento desta camada.

Palavras-Chave: Córtex visual, camada I, biocitina.

Apoio Financeiro: Universidade Federal do Pará, Instituto Evandro Chagas.

PROCESSAMENTO VISUAL DE ESTÍMULOS RADIAIS E GRADES SENOIDAIS EM CONDIÇÕES FOTÓPICAS DE LUMINÂNCIA EM ADULTOS

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Objetivo: Alguns estudos psicofísicos discutem que o sistema visual humano (SVH) é mais sensível para grade senoidal do que para frequência radial. Neste sentido, este estudo teve como objetivo determinar e comparar a função de sensibilidade ao contraste de adultos jovens para estímulos radiais (FSCr) e grades senoidais (FSC) em condições fotópicas.

Método: Participaram deste estudo oito voluntários, de ambos os sexos, com idade de 19 a 25 anos e acuidade visual normal ou corrigida. Foram mensuradas oito curvas de sensibilidade para grade senoidal (FSC) e oito para frequência radial (FSCr), em condições fotópicas de luminância. Os estímulos circulares, com diâmetro de 7,25 graus de ângulo visual, foram gerados e apresentados, binocularmente, a 150 cm de distância da tela de um monitor LG de 19 polegadas, com resolução de 1024 x 768 pixels, conectado a um hardware Bits ++ (Cambridge Research Systems). As medidas foram realizadas com método psicofísico da escolha forçada, utilizando um delineamento experimental com medidas repetidas. Durante o experimento, eram apresentados pares de estímulos e o participante tinha que escolher aquele que continha uma das frequências espaciais ou radiais de 0,25; 0,5; 1; 2; 4; 8 e 12 ciclos por grau de ângulo visual (cpg), em detrimento de um padrão homogêneo com a luminância média de 42 cd/m². A ordem de apresentação dos estímulos era aleatória e controlada pelo software.

Resultados: A análise de variância para medidas repetidas (ANOVA) mostrou diferença significativa entre a FSC e a FSCr em condições fotópicas [$F(1, 158) = 36,48$; $p < 0,001$]. O teste post-hoc Tukey HSD revelou diferença significativa apenas na frequência de 8 cpg ($p < 0,001$). Os resultados revelam que a sensibilidade de adultos jovens em condições fotópicas foi da ordem de 2,2 vezes maior para grade senoidal do que para frequência radial.

Conclusão: Estes resultados reforçam os achados psicofísicos e neurofisiológicos que sugerem que estes estímulos são processados por áreas visuais diferentes.

Palavras-Chave: Sensibilidade ao Contraste; Percepção Visual; Grade Senoidal; Estímulos Radiais; Fotópica

Apoio Financeiro: CNPq

PROCESSAMENTO VISUAL DE ESTÍMULOS RADIAIS FOTÓPICOS E ESCOTÓPICOS EM ADULTOS

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Objetivo: O Grupo de Pesquisa Percepção, Neurociências e Comportamento tem realizado uma série de experimentos com estímulos elementares de frequências angulares, radiais e grades senoidais. O presente estudo teve como objetivo determinar a função de sensibilidade ao contraste (FSC) para estímulos radiais com frequências espaciais de 0,25; 0,5; 1,0; 2,0; 4,0; 8,0 e 12 ciclos por grau de ângulo visual (cpg) em condições de luminância escotópica (0,04 cd/m²) e fotópica (42 cd/m²). Estudos com estímulos radiais em condições fotópicas são raros e praticamente inexistentes em condições escotópicas.

Método: Participaram da pesquisa quatro voluntários com idade entre 19 e 25 anos, de ambos os sexos e com a acuidade visual normal ou corrigida. Todos foram avaliados nas duas condições e a ordem foi controlada pela técnica de contrabalanceamento. Os estímulos circulares, com diâmetro de 7,2 graus de ângulo visual, eram apresentados à distância de 150 cm da tela, com visão binocular e pupila natural. As frequências foram geradas em um monitor LG de 19 polegadas, com resolução de 1024 x 768 pixels. Usou-se um delineamento experimental com medidas repetidas e o método psicofísico da escolha forçada. O procedimento para medir a sensibilidade consistiu na apresentação sucessiva simples de pares de estímulos e os voluntários foram orientados a escolher sempre dentre eles qual continha a frequência radial em detrimento de um padrão homogêneo com a luminância média de 0,04 ou 42 cd/m².

Resultados: A análise de variância para medidas repetidas revelou diferença significativa entre as duas condições de luminância [$F(1, 158) = 28,54$; $p < 0,001$]. O teste post-hoc Tukey HSD mostrou diferenças significantes entre as condições escotópicas e fotópicas nas frequências radiais de 2,0 e 4,0 cpg ($p < 0,001$).

Conclusão: Estes resultados indicam que o sistema visual humano apresenta sensibilidade ao contraste para estímulos radiais, em média, três vezes maior em condições fotópicas do que em escotópicas, sugerindo níveis de processamento diferentes.

Palavras-Chave: Percepção Visual; Sensibilidade ao Contraste; Estímulos Radiais; Condições de Luminância

Apoio Financeiro: CNPq

D.03.027

Distribution of CB2 receptors in adult rat retina.

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Objectives: Cannabinoid effects are mediated through two receptors, CB1 and CB2, while CB1 is expressed in brain and peripheral tissues, CB2 is expressed in peripheral and immune tissues. However, recent evidences have shown that CB2 is also expressed in central nervous system (CNS)(1). Similarly, in the retina CB1 has been reported in bipolar cells, gabaergic amacrine cells, horizontal cells and inner plexiform layer but CB2 localization seems to be elusive. There is only one report of CB2 receptor mRNA localization by using RT-PCR which has shown the localization of this receptor in photoreceptors, inner nuclear layer and ganglion cell layer (2). The aim of this work was to localize CB2 receptor in the rat retina by using immunocytochemistry.

Methods: Male Sprague Dawley rats were anesthetized, their eyes were removed, fixed by immersion in 4 % paraformaldehyde solution and were cryoprotected in 30 % sucrose solution. Cryostat sections were processed by immunocytochemistry using a CB2 rabbit polyclonal antibody.

Results: Our results showed that CB2 was localized in the inner retina. Immunoreactive horizontal and amacrine cells were observed in inner nuclear layer. Immunolabeling was also observed in inner plexiform layer and strong labeled cells were detected in ganglion cell layer.

Conclusions: These results are in agreement with RT-PCR results that showed CB2 mRNA in the retina and provide further evidence of the presence of CB2 in CNS. Further work is being carried out in our laboratory in order to know the changes of CB2 receptor after light induced degeneration. Future studies will clarify the physiological and pathological roles of this cannabinoid receptor in the retina but certainly this discovery opens a new target for future treatments of retinal diseases.

References: 1- Ann. N.Y. Acad. Sci., 1074:514-536, 2006; 2- Vis. Neurosci., 17:91-5, 2000.

Palavras-Chave: CB2 receptor, rat, retina.

Apoio Financeiro: CONICET PIP 5785

D.03.028

Functional color vision loss as evidence of early neural damage in type 2 diabetes in the absence of diabetic retinopathy

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	Mean $\pm$ SE		
	Groups		
Test parameter	DR	NDR	CTRL
Protan axis*	162.70 $\pm$ 18.17	113.96 $\pm$ 13.08	79.95 $\pm$ 8.07
Deutan axis*	150.19 $\pm$ 8.28	138.74 $\pm$ 22.03	94.80 $\pm$ 16.93
Tritan axis*	302.32 $\pm$ 35.00	203.87 $\pm$ 18.67	132.76 $\pm$ 13.79
Ellipse axes ratio	2.28 $\pm$ 0.23	1.88 $\pm$ 0.13	1.65 $\pm$ 0.10
Ellipse area*	3986.39 $\pm$ 1548.19	253.70 $\pm$ 63.81	113.06 $\pm$ 26.25
Ellipse angle	74.19 $\pm$ 6.69	90.57 $\pm$ 6.07	76.18 $\pm$ 11.15
*thresholds in CIE u'v' units			

Objectives: To evaluate the color vision of type 2 diabetics with and without diabetic retinopathy, correlating performance with clinical status. **Methods:** 89 individuals were examined: 34 without diabetic retinopathy – NDR (56 $\pm$ 10; disease duration: 6 $\pm$ 4 yrs); 27 diabetics with diabetic retinopathy – DR (60 $\pm$ 9 yrs; disease duration: 23 $\pm$ 10 yrs); 28 non-diabetic subjects – CTRL (44 $\pm$ 10 yrs). Color vision was assessed using the Cambridge Color Vision Test[®]. Color discrimination thresholds were measured along protan (P), deutan (D), and tritan (T) color confusion axes. In addition, an 8-vector MacAdam ellipse was determined. Tests were done with monocular fixation of the target at 3 m viewing distance. The examined eye was chosen randomly for each subject. All patients underwent fundus photos and fluorescein angiogram. **Results:** P, D and T thresholds, as well as the ratio of the ellipse axes, were significantly different between DR patients and CTRL. Ellipse areas differed between NDR vs CTRL, and between DR vs CTRL. The angle of the ellipse in the 1976 CIE diagram did not differ between any of the groups. (ANOVA, Tukey test; α = 0.05). Table 1 shows means and standard errors calculated for each group and parameter. None of the functional parameters correlated with the clinical status of the patients as assessed by duration of disease, HbA1c or the glycemic level at the time of the test. **Conclusions:** NDR patients have a broad distribution of chromatic deficits, with some subjects similar to the controls and others performing like DR patients. As reduction in color discrimination in NDR patients occurs even in the absence of diabetic retinopathy, and does not correlate with any tested clinical index, we hypothesize that the color vision losses observed in some NDR patients may be an early sign of diabetic neural damage.

Palavras-Chave: color vision, psychophysics, diabetes mellitus, diabetic retinopathy, neural damage

Apoio Financeiro: FAPESP, Finep

Dysfunctionality of the spatial orientation sense in the Alzheimer's process is in the base of the apparent incontinence

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Objectives: to applied the psychosocial sensory impairment theory of the Alzheimer's disease to understand frequent incontinence in Alzheimer's cases **(1)**. **Methods:** Once the story of life and the facts that have given rise to the process of dementia have been recovered, the diagnose was given on the basis of the criteria of the "National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA Criteria)" revised (1), Minimental State Examination (MMSE) test and also with the evaluation of the workability of the system of identification components of the nine channels (Protocol B, Alzheimer Behavioral and Sensorial Identification Test, ABSIT) **(2,3)** in the nine channels. **Results:** This report includes 38 cases brought to us through consultations done by the family of the patients until August of 2007, and which monitoring was completed by the beginning of 2008. From those cases 21 (55.2%), -16 of which were women-, turned out to be real cases of Alzheimer dementia. Seventeen (44.7%, 13 of which were women) where healthy people between the ages of 52 and 85 who only had an attention deficit disorder or temporary absorption into painful thoughts or translated to a past idyllic scenario affecting her attention or ready to be repeated in the shape of sad thoughts (depression), and due to that, said patients had been diagnosed as suffering Alzheimer disorder according to what was set forth in their medical reports. The most remarkable recoveries have been the ones involving those individuals that apparently suffered an impairment to control their sphincter, and that once recovered their sense of spatial orientation, they started to go again to the bathroom by themselves. **Conclusions:** The simultaneous recovery of spatial orientation sense and sphincters control as a consequence of rehabilitation actions allows understands the causality relations between these variables. This occurs also in sporadic and spontaneous remissions (return to their present reality) or under strong familial stimulation. **References:** 1. Re v. Neurol 2007, 44(4):198; 2. J Am Geriatr Soc. 2007 55(5):800; 3. Lancet Neurol. 2007, 6(8):734;

Palavras-Chave: Alzheimer, incontinence, spatial orientation sense, sphincters control, rehabilitation.

Apoio Financeiro: Universidad Autónoma de Entre Ríos, BBVA Banco Francés, Fundación Alzheimer

D.03.030

Reversal Phi Motion: an effect of Spatial Facilitation

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In a previous work, we showed that a primer concurrent with the moving stimulus increase the flash lag effect, presumably due to a shorter latency in movement perception. A mechanism of pre-activation of neighbouring areas of the cortex by a moving object has been postulated. A consequence of this interpretation would be that the time needed to detect a target should be diminished when its presentation is preceded by a moving object converging to the same spot. Then, with an appropriate combination of the preceding objects we could perturb the perception of an apparent motion.

We check this possibility by asking subjects to indicate the direction of apparent motion of two Gaussian patches that are preceded by two moving Gabor patches. One of them, the primer, arrive just near the location where one of the Gaussian patches would appear and the other moving Gabor patches stops further away from the other Gaussian. To estimate the effect of the primer, the time between both Gaussian was varied systematically.

Results show that task is much easier when the primer affect the Gaussian that appears first. When both Gaussians are presented simultaneously, subjects respond as if the Gaussian affected by the primer was perceived first. Conversely, when the primer affects the second Gaussian, subjects perceive the Gaussians as if they were presented in the inverted order. This is probably due to the fact that the primer facilitates the spot where the Gaussian would appear and, consequently, if the primer affects the first Gaussian the phi motion is more clearly perceived; but if the primer affects the second Gaussian the phi motion is perceived in the opposite order.

This reversal phi motion supports the hypothesis that there is a further facilitation wave leading any moving object, which is consistent with neurophysiologic data concerning long-range horizontal connections. **Palavras-Chave:** Reversal phi motion; Neural processing delays; horizontal connections; Differential latencies; Spatial facilitation

D.03.031

Evidência imunohistoquímica da liberação de serotonina (5-HT) modulada por receptores glutamatérgicos em células da retina de pinto

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OBJETIVO

Evidenciar alterações no padrão de imunorreatividade das células 5-HT-positivas (5-HT⁺) pós-tratamento com diferentes agonistas glutamatérgicos.

MÉTODOS

Foram utilizados pintos pós-eclosão com um dia de idade (P1). Após a enucleação ocular e dissecação das retinas, dois fragmentos centrais foram obtidos e imersos por 30 minutos, respectivamente, em solução de Ringer Locke somente, ou contendo um dos agonistas glutamatérgicos testados: NMDA (100 mM), AMPA (100 mM) e cainato (1 mM, 10 mM e 50 mM). Os fragmentos de retina foram fixados em PA 4% diluído em tampão fosfato 0.1 M (pH 7.4) por 2 horas. Foram realizados cortes transversais de 12-15 µm em um criostato a -21°C, seguindo-se o procedimento imunohistoquímico (5-HT rabbit antibody; 1:5000; Immunostar) para revelação por DAB. A análise morfológica ocorreu pela evidência do padrão de marcação do tecido após a estimulação com os agonistas glutamatérgicos. As alterações da marcação foram quantificadas por análise densitométrica, utilizando o *software* Corel PHOTOPAINT (versão 11). Os dados foram analisados estatisticamente utilizando one-way ANOVA e o pós-teste de Bonferroni, considerando significativo (* $p < 0.05$).

RESULTADOS

As retinas controles imunorreagiram para 5-HT, permitindo a identificação de células amácrinas e bipolares 5-HT⁺ na margem mais interna e mais externa da CNI, respectivamente. As retinas incubadas com os agonistas NMDA e AMPA mostraram imunorreatividade a 5-HT (5-HT-IR), sugerindo que esses moduladores glutamatérgicos não exercem nenhum efeito na liberação de 5-HT, entretanto, as retinas tratadas com o agonista cainato, apresentaram uma evidente redução na 5-HT-IR, resultado confirmado pela análise densitométrica.

CONCLUSÕES

Os resultados permitem inferir que a ativação dos receptores de glutamato do tipo NMDA e AMPA não promove a liberação de 5-HT das células retinianas 5-HT⁺, no entanto, os receptores de glutamato do tipo cainato ativados, induzem essa liberação. O tratamento com cainato, provavelmente, promove uma despolarização das células retinianas 5-HT⁺, e conseqüente liberação de 5-HT, revelada por uma acentuada redução na imunorreatividade dessas células.

Palavras-Chave: Liberação, serotonina, glutamato, retina

D.03.032

A subjective brightness increase reveals spatiotemporal dynamics of luminance comparison for spatially separated asynchronous stimuli

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Objectives: Many illusory phenomena emerge in situations where continuous stimulus attributes are compared (e.g., flash lag effect). The work reported here aims to elucidate mechanisms involved in the comparison of a pair of brief asynchronous luminance stimuli.

Methods: In each trial, two disks were presented against a black background on a CRT. One appeared at the left side of the fixation cross, the other at the right side, in randomized order. The target stimuli, each with a duration of 100 milliseconds (ms), were presented with a stimulus onset asynchrony (SOA) that ranged from 0 to 200 ms. The second disk constituted either a 15% increase or decrease (increment/decrement). Human observers indicated the side of the brighter or dimmer stimulus. Nine observers completed 2640 trials each in four sessions.

Results: Global performance declined slightly as a function of SOA, from 65.8% correct responses for simultaneous presentation, leveling off at 58.5% (binomial standard error of aggregated proportion: 1.1%). More importantly, all observers responded more correctly for increments than for decrements. Mean performance for decrements dropped significantly below chance level for SOA>40 ms. The performance difference attains on average 36%, at a SOA of 120 ms, after which the effect diminishes. A logistic regression (categorical predictors) confirms these observations: all observers showed a significant main effect of the increment/decrement dissociation ($p<0.0001$, likelihood ratio test), while the presence of a main effect of SOA was subject-dependent. SOA strongly modulated the order effect: across the nine observers, the least significant interaction still corresponds to $p<0.002$.

Conclusions: A considerable order effect in luminance comparison exists; for spatially disjoint stimuli, luminance increments are more easily detectable than decrements, in an effect that varies with temporal separation. This is consistent with apparent brightness reduction for the first stimulus, enhancement for the second, or both. This new finding is discussed in the light of attentional dynamics and low-level brightness modulation processes.

Palavras-Chave: psychophysics, luminance, visual perception, attention

Apoio Financeiro: FAPESP, processo 06/50189-9

D.03.033

Determinação do padrão de visão cromática em macacos *Cebus apella*.

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Objetivos: Os primatas são os únicos mamíferos que apresentam visão tricromática, mas a tricromacia não é universal entre os primatas. Humanos e macacos do Velho Mundo têm três classes diferentes de cones, para os comprimentos de onda curto (S), médio (M) e longo (L). Entretanto, entre os macacos do Novo Mundo, o fenótipo dos cones é muito variável. Existem espécies como o *Aotus* na qual todos os indivíduos são monocromatas. Outras espécies são tricromatas como o *Alouatta*. E em outras espécies como o *Cebus* e o *Saimiri* os machos e algumas fêmeas são dicromatas, enquanto outras fêmeas são tricromatas, podendo existir diferentes tipos de dicromacia que surgem de variações individuais nos complementos dos pigmentos dos cones. (1,2). Desta forma, é de grande interesse estudar o processamento do sinal de cor no córtex visual desses primatas e investigar como ele se relaciona com a percepção de cor em primatas di- e tricromatas. Entretanto, para se prosseguir no estudo do processamento do sinal de cor no córtex, necessitamos conhecer o padrão de visão cromática dos animais estudados. Nosso objetivo neste projeto é estabelecer um protocolo simples, rápido e eficiente para se determinar o tipo de visão cromática de cada indivíduo.

Métodos: O estudo do padrão de visão cromática no macaco *Cebus apella*, machos e fêmeas, é realizado através do registro do eletroretinograma (ERG). Para este registro, os animais são anestesiados e posicionados em frente a um monitor onde são apresentados os estímulos visuais. Estes estímulos consistem de ondas quadradas de diferentes cores (azul, verde, vermelho e branco), com diversas luminosidades e apresentados em diversas frequências temporais.

Resultados: Com a análise da amplitude da resposta às diferentes cores do estímulo medida através do ERG e da razão da resposta aos estímulos verde e vermelho, os animais são separados em 3 grupos: tricromatas, dicromatas portadores dos cones S e M e dicromatas portadores dos cones S e L.

Conclusões: Este protocolo mostrou-se bastante eficiente na distinção do tipo de padrão cromático apresentado por estes animais.

Referencias: 1- Vis Res 27:2089, 1987; 2- Vis Res 43:227, 2003.

Palavras-Chave: visão cromática, cones, ERG, primatas

Apoio Financeiro: CNPq, FAPERJ, FINEP, PRONEX.

Stereotypy and diversity in the visual cortex of the agouti (*Dasyprocta agouti*).

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Many studies have compared the morphology of pyramidal cells in the visual areas of a variety of mammalian species. However, only a handful was concerned with non-pyramidal cells. In the present work we investigated the stereotypy and diversity of morphometric features of 210 nitrergic cells throughout the visual cortex of the agouti, a medium-sized Amazonian rodent with a large lissencephalic brain. Eight adult brains were aldehyde fixed, cut and reacted for NADPH-d histochemistry. Type I neurons in both coronal and tangential planes were reconstructed and analysed with the *NeuroLucida* software. Cluster analysis of morphometric features including, number of dendrites by order, areas of both dendritic field and cell body, fractal dimension, and convex hull analysis, followed by a forward stepwise discriminant function analysis revealed the morphological diversity of NADPH-diaphorase type I neurons along the naso-temporal dimension of VI, VII, and VIII visual areas. Smaller soma areas were found in cells located in the medial region, when compared with central and lateral placed cells (medial: 351.47 ± 18.25 sd, intermediate: 396.5 ± 21.0 and lateral: 393.78 ± 23.53 ; two-tail Student's *t*-test $p < 0.05$). Likewise, both the dendritic field area (medial: $47.89 \pm 12.35 \times 10^3$ sd, intermediate: $68.28 \pm 17.41 \times 10^3$ and lateral: $83.30 \pm 18.41 \times 10^3$) and fractal dimension (medial: 1.23 ± 0.04 sd, intermediate: 1.26 ± 0.07 and lateral: 1.27 ± 0.05) became progressively larger towards the lateral margin (two tailed Student's *t*-test $p < 0.05$). Equivalent results were observed across the tangential plane, where all comparisons were highly significant (ANOVA – Newman Keuls *post-hoc* test, ** $p < 0.01$). In addition, cells in VI had fewer ramifications than those in VII. Neurons in VIII were more ramified than VI and VII, conversely. Fractal dimension and convex hull analysis corroborated the tendency reported by the other morphometric variables in both the coronal and tangential planes. Taken together, the results point to a progressive increase of both areal and dendritic arbour complexity in secondary visual areas and may suggest the occurrence of specialized cortical circuits across the agouti's visual cortex, where stereotypy and diversity co-exist.

Palavras-Chave: Visual cortex, Morphometry, rodent

Apoio Financeiro: CNPq, FINEP, UFPA

D.03.035

Vetores elétricos no cortex visual retinotópico a estímulos em movimento no campo visual – análise por EEG

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Objetivo: Através de registros feitos por eletroencefalograma (EEG), buscamos estudar potenciais relacionados a eventos (ERP) correspondentes a um vetor elétrico dinâmico projetado no cortex visual retinotópico em resposta a um objeto em movimento no campo visual com trajetória definida.

Metodologia: Registramos 7 voluntários saudáveis com acuidade visual normal, utilizando para captação da onda 20 canais (sistema 10-20, 600Hz) com referência biauricular e filtro de passagem 0,1 a 35Hz. Os voluntários foram colocados em uma sala escura, em frente a um monitor 60Hz LCD fixando o olhar em um ponto central (0.3 x 0.3°) em torno do qual havia um círculo de baixo contraste (diâmetro 3 graus) em movimento circular por trajetórias isocêntricas (5° do centro), cíclica e ininterruptamente. Realizamos 8 blocos de testes de 200 trials cada, contendo uma trajetória no sentido horário ou anti-horário que começava em 0°, 90°, 180 ou 270°. Utilizamos trajetórias isocêntricas e estímulo de baixo contraste. Para destacarmos a atividade relacionada à trajetória, diminuimos do ERP obtido para cada canal e trajetória a onda média de todas as trajetórias para esse canal. Esta operação intensificou a amplitude do ERP sem alterar as suas características. Implementamos um modelo matemático para predições quanto ao formato das ondas dos vetores, baseado no cosseno médio dos ângulos entre a reta tangente derivada de um segmento da trajetória vetorial estimada e o eixo das derivações do EEG nos planos xy, xz, e yz.

Resultados: Os ERP obtidos condizem com uma atividade relacionada a retinotopia da trajetória. O modelo corrobora esta atividade como sendo vetores elétricos relacionados a retinotopia do cortex visual, correlacionada com a atividade predita nas regiões occipitais mediais (V1/V2/V3) do córtex visual.

Conclusão: De modo análogo ao ECG para os vetores elétricos do coração, obtivemos ondas compatíveis com vetores elétricos dinâmicos produzidos pela atividade cortical retinotópica relativa a um objeto em movimento no campo visual. **Palavras-Chave:** ERP, retinotopia, movimento, vetores eletricos

Apoio Financeiro: CAPES, FAPERJ, CNPQ

D.03.036

Expressão das GTPases Rho e de transducina em fotorreceptores da retina de camundongo após adaptação ao claro e ao escuro

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Objetivos: Os fotorreceptores são neurônios altamente especializados na detecção de luz, que exibem uma polaridade, onde as moléculas importantes para a fototransdução (p.ex. arrestina e transducina) passam bidirecionalmente entre os segmentos interno (SI) e externo (SE), dependendo das condições ambientais. Nosso objetivo foi investigar a localização de algumas GTPases (RhoA, Rac1 e Cdc42), conhecidas reguladoras do citoesqueleto, em fotorreceptores da retina de camundongos e verificar uma possível translocação após exposição a diferentes estímulos adaptativos.

Métodos: Camundongos albinos Balb/c adultos foram divididos em 2 grupos (n=3): adaptados ao escuro por 4hs e depois expostos à luz (600 lux) e sacrificados em diferentes tempos (5, 15, 30, 60 e 240 min) ou expostos ao claro (2000 lux) por 1h e depois ao escuro e sacrificados nos mesmos tempos. As retinas foram processadas para imunofluorescência para RhoA, Rac1, Cdc42 e transducina (1:20-1000, Santa Cruz) e visualizadas em microscopia confocal (Nikon). Marcadores para cones (WGA, wheat germ agglutinin) e bastonetes (PNA, peanut agglutinin) conjugados a fluoróforos também foram utilizados.

Resultados: RhoA, Rac1 e Cdc42 foram expressos em fotorreceptores, principalmente no citoplasma, do SI à terminação sináptica. RhoA mostrou uma marcação mais intensa no SI, próximo ao cílio conector. Rac1 apresentou um padrão puntiforme em toda célula, enquanto Cdc42 evidenciou uma marcação forte na camada plexiforme externa, além de uma pequena marcação no SE dos fotorreceptores. Experimentos de dupla marcação mostraram que RhoA, Rac1 e Cdc42 estão presentes em cones e em bastonetes. Enquanto a transducina sofre uma translocação do SE para o SI já aos 5 min (escuro para claro) e do SI para o SE (claro para escuro) após 240 min, as GTPases não apresentaram nenhuma alteração visível no seu padrão.

Conclusão: A presença dessas GTPases em fotorreceptores sugere que elas podem ter algum papel na manutenção do citoesqueleto. Embora não tenha ocorrido translocação, elas podem estar envolvidas no processo de fototransdução, regulando outras proteínas para terminar a fototransdução e/ou protegendo contra danos induzidos pela luz. **Palavras-Chave:** Fotorreceptor, GTPases e translocação.

Apoio Financeiro: CNPq e Fapesp.

Omega-3 polyunsaturated fatty acids dietary deprivation affects glutamate uptake in rat retina in basal and ischemic conditions.

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Objectives: Omega-3 polyunsaturated fatty acids (w3 PUFAs) are very abundant in retina and it plays an essential role in vision. Glutamate, the main excitatory neurotransmitter in central nervous system, participates of the visual process in a dependent manner. In the present work, we investigated the effect of omega-3 dietary deprivation in retinal glutamate uptake in basal and ischemic conditions.

Methods: We used two different diets: w3-diet (w3 group), with fish oil in its composition that provides w3 PUFAs, and w3 deprived-diet (D group), with peanut oil, which provides all fatty acids except w3 fatty acids. Animals received the diets from birth until 60 days old, when experiments were done. To cause an ischemia by high pressure in retina, anterior chamber of one eye was then cannulated using a 30-gauge needle connected to a reservoir of sterile balanced salt solution and a reperfusion bomb. The pressure of the perfusion was monitored to control the blood flow obstruction (140-180mmHg) After 45 min of ischemia, animals returned to normal conditions for seven days. Retinas from control (basal condition) and ischemic animals were dissected and submitted to [³H]glutamate uptake experiments.

Results: w3 Group showed increased glutamate uptake in basal conditions when compared with D group ($p < 0.05$). Seven days of reperfusion after acute ischemia, D group presented reduced glutamate uptake compared with its control ($0,813 \pm 0,06$; $0,594 \pm 0,03$; $p < 0.01$). D group presented a decrease in glutamate uptake after retinal ischemia ($0,594 \pm 0,03$ to $0,406 \pm 0,02$, $p < 0,001$). However, no decrease in glutamate uptake was observed in w3 group in ischemic conditions.

Conclusion: The modulation of glutamate uptake by DHA could be one important mechanism involved either in the influence of this fatty acid in adequate visual function as in neuroprotection observed in other works.

Palavras-Chave: omega-3 deprivation, retina, glutamatergic system, ischemia, vision

Apoio Financeiro: CNPq/Brazil, CAPES, IBNnet FINEP, FAPERGS

ACUIDADE VISUAL DE CRIANÇAS RECÉM-NASCIDAS COM DESNUTRIÇÃO INTRA-UTERINA MEDIDA PELO POTENCIAL VISUAL EVOCADO DE VARREDURA PARA FREQUÊNCIAS TEMPORAIS DIFERENTES

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Objetivo: Avaliar os efeitos da desnutrição intra-uterina no desenvolvimento da acuidade visual (AV) de resolução de grades em função da frequência temporal pelo Potencial Visual Evocado de Varredura (PVEv).

Métodos: Avaliamos a AV binocular de 13 bebês nascidos bem nutridos (GC, Idade média = $10,8 \pm 5,5$ semanas) e 16 com desnutrição intra-uterina (GE, Idade média = $9,9 \pm 4,4$ semanas). Durante a avaliação visual, os bebês de ambos os grupos apresentavam-se eutróficos, exceto três bebês do GC e três do GE que apresentavam desnutrição leve. Medimos o PVEv para grades senoidais acromáticas de padrão reverso com frequências temporais de 3, 6 e 10 Hz geradas pelo sistema *PowerDiva* (Norcia & Tyler, 1985). Registramos o eletroencefalograma com três eletrodos ativos sobre a área occipital (O1, Oz e O2), cada um estava relacionado ao eletrodo de referência (Cz) no vertex. O eletrodo terra localizava-se sobre a área parietal. Apresentamos os estímulos em um monitor de vídeo monocromático de 21 polegadas com luminância média de $161,13 \text{ cd/m}^2$. Durante cada tentativa com duração de 10 segundos, as frequências espaciais foram varridas linearmente de 0,8 a 8,5 cpg (0-9 semanas de idade); 1,0 a 16 cpg (10-16 semanas de idade) e 2,0 a 30 cpg (³ 17 semanas de idade). Registramos os PVEs somente durante os períodos de fixação do bebê ao estímulo visual. Realizamos, para cada bebê, de cinco a dez medidas por frequência temporal e o PowerDiva calculou a AV baseado na média das respostas. Incluímos para cada condição, pelo menos, três resultados com boa razão sinal-ruído ($>3:1$), fase constante e critério estatístico. Consideramos o eletrodo ativo que registrou a melhor AV como resultado.

Resultados: As análises estatísticas não mostraram diferenças significativas para a AV (LogMAR) entre o GC e GE em 3 Hz [$t = -0,3$; $p = 0,77$], 6 Hz [$t = -1,20$; $p = 0,24$] e 10 Hz [$t = 0,58$; $p = 0,57$]. As análises intra-grupos, GE [$F(2, 34) = 0,22$; $p = 0,81$] e GC [$F(2, 28) = 1,69$; $p = 0,20$], também não mostraram diferenças significativas para a AV em 3, 6 e 10 Hz.

Conclusões: Os resultados iniciais mostraram que a desnutrição intra-uterina não afetou significativamente a acuidade visual dos bebês avaliados. **Palavras-Chave:** Desnutrição intra-uterina; Acuidade visual; Potencial Visual Evocado de Varredura

Apoio Financeiro: FAPESP

Elipses de discriminação de cores de tricromatas medidas com potencial cortical provocado visual transiente e estacionário

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O objetivo do presente trabalho é investigar o uso de diferentes formas de potenciais corticais provocados visuais (VECP – *visual evoked cortical potentials*), captados em Oz, na mensuração de limiares de discriminação de cores e no uso destes para construir elipses de discriminação de cores (MacAdam, 1942). Cinco indivíduos tricromatas normais ($24,5 \pm 2,6$ anos de idade) foram testados monocularmente. Os estímulos consistiram de redes senoidais cromáticas isoluminantes cujos pares de cromaticidades estavam localizados ao longo de quatro diferentes direções de cores radiando de um ponto de referência do diagrama de cromaticidade CIE 1976 ($u' = 0,225$; $v' = 0,415$). A fotometria com *flicker* heterocromático (HFP – *heterochromatic flicker photometry*) foi usada para determinação da condição de isoluminância para cada indivíduo e para todos os pares de cromaticidade. O VECP foi provocado usando redes com frequência espacial de 2 ciclos por grau em três diferentes configurações temporais: transiente, *onset* (300 ms) / *offset* (700 ms), 1 Hz de frequência fundamental; estacionária, *onset* (50 ms) / *offset* (50 ms), 10 Hz de frequência fundamental; e estacionária de padrão reverso de 5 Hz de frequência fundamental (10 Hz de reversão de fase). A amplitude do VECP foi aferida usando o componente N1-P1 para o VECP transiente e o primeiro (10 Hz) e segundo (20 Hz) harmônicos para o VECP estacionário. A amplitude do VECP foi relacionada com a distância cromática no diagrama de cromaticidade CIE 1976 e os pontos de dados foram extrapolados para o nível de amplitude zero para obter os limiares de discriminação cromática. Os resultados foram comparados com medidas psicofísicas com as mesmas configurações de estimulação, assim como com o método pseudoisocromático de Mollon-Reffin (ANOVA – uma via). Para todos os indivíduos e em todas as formas de estimulação, as elipses apresentaram tamanho pequeno, baixa excentricidade e estavam verticalmente orientadas. Apesar de algumas diferenças consistentes nos resultados obtidos com diferentes procedimentos, não houve diferença estatística entre as elipses obtidas eletrofisiologicamente e psicofisicamente. Para o VECP estacionário, as elipses medidas a partir da amplitude do segundo harmônico foram maiores e mais alongadas na direção tritan do que aquelas medidas a partir das amplitudes do primeiro harmônico.

Palavras-Chave: Elipses de MacAdam, Potencial cortical provocado visual, Visão de cores, Método de Mollon-Reffin, Psicofísica visual.

Apoio Financeiro: CNPq, CAPES, CNPq/FUNTEC PRONEX, FINEP IBN-Net.

D.03.040

Comparison between psychophysical and electrophysiological red-green spatial contrast sensitivity function obtained through the steady state visual evoked potential

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Objectives: To estimate chromatic contrast sensitivity function (CSF) using steady state visual evoked potential (ssVEP) by analyzing the second harmonic amplitudes, and to compare ssVEP to psychophysical CSF.

Methods: Five normal trichromats (26.6 ± 1.7 years old) were monocularly tested. A red-green horizontal isoluminant grating (CIE 1976: $u' = 0.288$, $v' = 0.480$ and $u' = 0.15$, $v' = 0.480$) was presented at 5 Hz as a 5° diameter circle against a background of mean luminance (34.3 cd/m²) and mean chromaticity of the grating. Four spatial frequencies (0.4 - 2 cpd), were used. Heterochromatic Flicker Photometry protocol at 20 Hz was used to obtain the isoluminance. Psychophysical thresholds were measured with method of adjustment using the same spatio-temporal configuration as used in ssVEP as well as static presentation. ssVEP data were analyzed with a Fast Fourier Transform protocol. Second harmonic amplitudes were plotted against the log pooled cone contrast and the data were fitted with a regression line. Contrast sensitivity was expressed as the reciprocal of the contrast value corresponding to the extrapolation of the fitted line to zero amplitude.

Results: The results of the dynamic psychophysical CSF agree with the 10 Hz amplitudes from the electrophysiological CSF, while the static psychophysical CSF is differed at all spatial frequencies (ANOVA $p < 0.05$). All CSFs are low-pass functions.

Conclusions: The red-green CSF obtained using ssVEP was better correlated with the dynamic than with the static psychophysical CSF. Static thresholds were elevated in comparison to both dynamic CSFs. The results suggest that the second harmonic for the reversal pattern mode may reflect P pathway activity.

Palavras-Chave: color vision; visual evoked potential, chromatic contrast sensitivity function

Apoio Financeiro: FAPESP, CAPES, CNPq, FUNTEC, and FINEP-IBN Net

DETECÇÃO VISUAL DE GRADE SENOIDAL EM CONDIÇÕES DE LUMINÂNCIA FOTÓPICA E ESCOTÓPICA EM ADULTOS

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Objetivo: O Laboratório de Percepção, Neurociências e Comportamento (LPNeC) tem realizado uma série de pesquisas com estímulos elementares em condições diferentes de luminância. O presente estudo teve como objetivo medir a função de sensibilidade ao contraste (FSC) para grade senoidal com frequências espaciais de 0,25; 0,5; 1,0; 2,0; 4,0; 8,0 e 12 ciclos por grau de ângulo visual (cpg) em condições de luminância fotópica (42 cd/m²) e escotópica (0,04 cd/m²). **Método:** Participaram da pesquisa quatro voluntários com idade entre 19 e 25 anos, de ambos os sexos e com a acuidade visual normal ou corrigida. Todos foram avaliados nas duas condições e a ordem foi controlada pela técnica de contrabalanceamento. Os estímulos circulares, com diâmetro de 7,2 grau de ângulo visual, foram gerados e apresentados em um monitor LG de 19 polegadas, com resolução de 1024 x 768 pixels à distância de 150 cm da tela, com visão binocular e pupila natural. Usou-se um delineamento experimental com medidas repetidas e o método psicofísico da escolha forçada. O procedimento para medir a sensibilidade consistiu na apresentação sucessiva de pares de estímulos e os voluntários foram orientados a escolher sempre dentre eles qual continha a frequência espacial. O outro estímulo neutro foi sempre um padrão homogêneo com a luminância média de 0,04 ou 42 cd/m². **Resultados:** A análise de variância para medidas repetidas revelou diferença significativa entre as duas condições de luminância [$F(1, 158) = 36,65$; $p < 0,001$]. O teste post-hoc Tukey HSD mostrou diferenças significantes entre as condições escotópicas e fotópicas nas frequências espaciais de 2,0; 4,0 e 8,0 cpg ($p < 0,001$). **Conclusão:** Estes resultados indicam que a sensibilidade ao contraste para grades senoidais fotópicas foi, em média, duas vezes maior do que para grades senoidais escotópicas.

Palavras-Chave: Sensibilidade ao Contraste; Grade Senoidal; Fotópica; Escotópica.

Apoio Financeiro: CNPq

Adaptação à cor: influência da adaptação cromática nas Elipses de MacAdam medidas com o método de Mollon-Reffin

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A adaptação a fundos variando em cromaticidade e luminância tem efeito tanto na aparência de uma cor quanto na sua discriminação ao longo de diferentes direções num espaço de cor. Nós determinamos elipses de discriminação de cores em duas diferentes condições de adaptação de fundo, usando o protocolo de Mollon-Reffin (1989) em um sistema de computação para teste psicofísico em plataformas IBM RISC/6000. Foram avaliados 31 tricromatas normais ($22,4 \pm 2,1$ anos de idade) em testes monoculares com estímulos-teste em forma de “C”, sendo este parte de um mosaico formado por círculos de diferentes tamanhos e luminância. O alvo diferia em cromaticidade do restante do mosaico - C2 (CIE 1976: $u' = 0,219$; $v' = 0,480$) e C3 (CIE 1976: $u' = 0,225$; $v' = 0,415$). Os limiares de discriminação foram avaliados ao longo de 20 eixos de cromaticidade irradiando de C2 e C3. Aos sujeitos foi perguntado indicar as orientações da abertura do C a medida que o contraste cromático entre estímulo-teste e o mosaico de fundo foi variada até o limiar de discriminação ter sido determinado por um método de estimativa em escada (*staircase*). Os limiares foram avaliados usando duas condições: (i) o mosaico inserido em um fundo preto; (ii) o mosaico inserido em um fundo com cromaticidade igual a do ponto de referência e luminância igual a luminância média do mosaico. Os limiares de discriminação foram localizados no espaço de excitação dos cones e sobre eles ajustadas elipses de MacAdam. Os valores de contrastes dos cones foram obtidos para as duas condições e comparados. Para as duas condições, as elipses C3 tiveram diâmetro maior (círculo de área equivalente) do que as elipses C2. Ambas as elipses C2 e C3 foram menores, e as elipses C2 tiveram menor excentricidade quando o mosaico foi apresentado na condição (ii) (ANOVA de uma via; $p < 0.05$). Uma melhora na discriminação cromática quando usada a condição (ii) ocorreu em todas as direções de cor exceto próximo da linha de confusão tritan para as elipses C2 e C3, próximo da linha de confusão protan para as elipses C2, e próximo da linha de confusão deutan para as elipses C3. Os resultados indicaram que apresentando o mosaico em um fundo com igual luminância média e cromaticidade igual a cromaticidade dos pontos de referência, houve um aumento da performance dos sujeitos ao longo das direções do espaço de cor fora das direções protan, deutan e tritan. Isso sugere um possível efeito da luminância e adaptação cromática nos limiares de discriminação de cores.

Palavras-Chave: Elipses de MacAdam, Método de Mollon-Reffin, Adaptação cromática, Tricromacia, Psicofísica visual.

Apoio Financeiro: CNPq, CAPES, CNPq/FUNTEC PRONEX, FINEP IBN-Net, NIH 3T37MD001378-07.

Avaliação das Funções Visuais em Pacientes Portadores de Vírus Linfotrófico Humano Tipo 1 Assintomáticos e com Mielopatia Associada ao HTLV-1 / Paraparesia Espástica Tropical

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Objetivo: Investigar através de métodos psicofísicos a função visual em pacientes portadores do Vírus Linfotrófico Humano Tipo 1 (HTLV-1) assintomáticos e com Mielopatia Associada ao HTLV-1 / Paraparesia Espástica Tropical (HAM / TSP).

Métodos: Foram avaliados 8 sujeitos (44 ± 12 , 23-59 anos de idade), 5 assintomáticos e 3 sintomáticos. Todos os testes foram monoculares. Foram estimadas a função de sensibilidade ao contraste espacial de luminância (FSCE) em 11 frequências espaciais de 0,2 a 30 cpg ($n = 7$), a função da sensibilidade ao contraste temporal de luminância (FSCT) em 7 frequências temporais de 0,5 a 32 Hz ($n = 7$), a discriminação de cores pelo teste de ordenamento de 100 matizes de Farnsworth-Munsell (FM100) ($n = 8$), e a sensibilidade absoluta pela perimetria estática de Humphrey (PEH) (modelo HFA-745 CZM; estratégia *Threshold*; protocolos 60-4 e 30-2) ($n = 5$). Os resultados foram comparados com sujeitos controles (FSCE: 16-30 anos de idade, $n=30$); (FSCT: 16-30 anos de idade, $n=33$); (FM100: 44 ± 12 , 23-59 anos de idade, $n=8$); (PEH: banco de dados do PEH).

Resultados: A FSCE dos sujeitos infectados apresentou menor sensibilidade ao contraste do que a dos controles nas frequências espaciais médias (2-6 cpg) ($p < 0,05$; ANOVA), sendo que dois sujeitos sintomáticos e um sujeito assintomático ficaram fora dos limites de tolerância nas frequências espaciais médias. Não houve na FSCT entre sujeitos infectados e controles ($p > 0,05$; ANOVA), sendo que os indivíduos infectados ficaram dentro dos limites de tolerância. No FM100, os sujeitos infectados tiveram erros maiores que os controles ($p < 0,05$; T de Student). No protocolo 60-4 da PEH, os pacientes infectados apresentaram defeito de profundidade médio de $1,6 \pm 3,57$ no campo temporal superior, $0,8 \pm 0,44$ no campo nasal superior, $3,2 \pm 2,16$ no campo nasal inferior e $2,6 \pm 2,70$ no campo temporal inferior. No protocolo 30-2 da PEH, os defeitos de profundidade médios foram de $2 \pm 2,73$ no campo temporal superior, $2,2 \pm 2,68$ no campo nasal superior e $1 \pm 1,22$ no campo nasal inferior e $1 \pm 0,70$ temporal inferior.

Conclusão: Os sujeitos infectados apresentaram diminuição de sensibilidade ao contraste espacial, diminuição da discriminação de cores e comprometimento regional do campo visual central e periférico.

Palavras-Chave: HTLV-1, HAM / TSP, Funções Visuais

Apoio Financeiro: CAPES, CNPq, CNPq / FUNTEC PRONEX, FINEP-IBN Net

AVALIAÇÃO DA SENSIBILIDADE AO CONTRASTE ESPACIAL CROMÁTICO EM HUMANOS

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OBJETIVO: obter dados normativos para a função de sensibilidade ao contraste (FSC) espacial cromático em diferentes faixas etárias. **MÉTODOS:** Utilizou-se redes senoidais cromáticas estacionárias de 6,5° por 5°, luminância média de 15 cd/m², as quais foram construídas ao longo de três eixos cromáticos: vermelho-azul, vermelho-verde e azul-verde (vermelho: $x=0,576$, $y=0,382$; verde: $x=0,243$, $y=0,626$; azul: $x=0,141$, $y=0,067$). No eixo vermelho-verde avaliaram-se 3 grupos: 16 a 30 anos, $n=50$; 31 a 45 anos, $n=40$; 46 a 60 anos, $n=31$. No eixo azul-verde avaliaram-se 3 grupos: 16 a 30 anos, $n=41$; 31 a 45 anos, $n=21$; 46 a 60 anos, $n=25$. No eixo vermelho-azul avaliou-se o grupo de 16 a 30 anos, $n=20$. Foi realizado teste monocular em 4 frequências espaciais de 0,1, 0,2, 0,5 e 1 cpg em diferentes proporções cromáticas. As diferenças estatísticas foram avaliadas com o teste ANOVA de uma via ($p<0,05$), e análise post-hoc (Tukey test). **RESULTADOS:** A FSC cromática apresentou característica passa-baixa em todos os grupos testados. A sensibilidade ao contraste diminuiu com a idade, principalmente para o eixo azul-verde. **FSC vermelho-verde:** o grupo mais jovem apresentou maior SC que os demais em 0,1, 0,2 e 1 cpg. Em 0,5 cpg, não houve diferença entre os grupos. **FSC azul-verde:** o grupo mais jovem apresentou maior SC que os demais em 0,1 cpg. Em 0,2 e 0,5 cpg, o grupo mais jovem obteve SC superior ao grupo mais velho. Em 1 cpg, o grupo mais jovem e o intermediário diferiram do grupo mais idoso. **FSC vermelho-azul:** na faixa etária jovem, essa condição cromática apresentou em 0,1 e 0,2 cpg, SC inferior à do eixo azul-verde, porém em 0,5 e 1 cpg, a média da SC foi superior à do eixo vermelho-verde. No grupo intermediário, para 0,1 e 0,5 cpg, a SC vermelho-verde foi maior que a SC do eixo azul-verde. Em 0,2 e 1 cpg, a SC foi igual para ambos os mecanismos. No grupo mais velho, a SC vermelho-verde foi maior que a SC do eixo azul-verde nas frequências espaciais testadas. **CONCLUSÕES:** O envelhecimento leva a uma diminuição da SC de cor, especialmente no eixo azul-verde. Fatores neurais e ópticos podem contribuir para esse fenômeno.

Palavras-Chave: FSC, Visão de Cores, Envelhecimento.

Apoio Financeiro: CNPq, CAPES, CNPq/FUNTEC PRONEX, e FINEP IBN-Net

SENSIBILIDADE AO CONTRASTE ESPACIAL DE LUMINÂNCIA DOS COMPONENTES DO POTENCIAL CORTICAL PROVOCADO VISUAL

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Objetivo: Comparar a sensibilidade ao contraste obtida com diferentes componentes do potencial cortical provocado visual (VEP) gerado por estimulação acromática sob apresentação reversa de fase espacial e padrão onset-offset.

Métodos: A amostra foi constituída por doze sujeitos adultos saudáveis (6 homens, $22,9 \pm 2,6$ anos de idade). Os estímulos visuais foram redes senoidais acromáticas com frequência espacial de 2 cpg apresentadas em um campo circular de 5° de diâmetro. Os testes foram monoculares a 1 m do monitor. Duas configurações temporais foram utilizadas: apresentação reversa de fase espacial com frequência temporal de 1 Hz; e apresentação do padrão em onset (300 ms) – offset (700 ms). Os contrastes limiares foram estimados ajustando-se funções lineares aos dados de amplitude do VEP em função do logaritmo dos diferentes contrastes testados e extrapolando-se as retas para o nível de amplitude zero.

Resultados: Para a apresentação em reversão de fase espacial, uma função de dupla tendência linear descreveu com boa correlação a função amplitude do componente P100 do VEP versus contraste do estímulo, com uma inclinação em baixos e médios contrastes e outra em altos contrastes. A amplitude do componente N135 do VEP em função do contraste decaiu linearmente com a diminuição do contraste, com saturação em altos contrastes. Para a apresentação onset-offset, a amplitude dos componentes C1 e C2 aumentaram monotonicamente com o aumento do contraste. O componente C2 apresentou saturação em altos contrastes. Os valores maiores de sensibilidade ao contraste foram obtidos usando dados da tendência de baixos e médios contrastes do componente P100 e dados dos componentes N135 e C2, com nenhuma diferença estatística entre essas três medidas (ANOVA, Tukey test, $p < 0.05$), enquanto os dados do componente C1 e da tendência de altos contrastes do componente P100 produziram valores menores de sensibilidade ao contraste, também sem diferença estatística entre si.

Conclusões: Para frequências espaciais medidas (2 cpg) um mecanismo de alta sensibilidade ao contraste possivelmente dependente da via M contribui para o componente P100 em baixos e médios contrastes e para os componentes N135 e C2 em todos os níveis de contrastes. **Palavras-Chave:** Eletrofisiologia humana, Potencial cortical provocado visual, Visão espacial, Sensibilidade ao contraste, Vias visuais

Apoio Financeiro: CAPES, CNPq, CNPq / FUNTEC PRONEX, and FINEP-IBN Net.

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OFF-response of the photopic electroretinogram in *Octodon Degus*

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Objectives. The retina carry visual signal in two parallel streams, from cone photoreceptor to the ganglion cells, through ON / OFF bipolar cells pathways. A diurnal South american rodent, *Octodon degus*, has two classes of cones with (i) an ultraviolet –sensitive photopigment (lmax 360 nm) and (ii) medium sensitive (lmax 510 nm). In previous work, the spectral sensitivity of the b-wave ON response was studied by the electroretinogram (ERG). However the spectral sensitivity of the d-wave OFF response still unknown.

Methods. Adult, *Octodon degus*, from both sexes were examined. The electroretinogram was use and the b-wave and d-wave determinated in function of monochromatic flashes. Additionally a yellow light adaptation was use to improve the detection of the UV-cone contribution.

Results. The spectral sensitivity of the b-wave and d-wave response are compatible with the input of two principal cones of *Octodon degus* retina.

Conclusions. In *Octodon degus* the b-wave and d-wave shows a spectral dependence that reflect the input of two classes of cones **Palavras-Chave:** Retina, b-wave, d-wave and Electroretinogram.

Apoio Financeiro: CS as a Graduate Fellowship from MECESUP. CONICYT PBCT-ACT45 to AGP.

AVALIAÇÃO PSICOFÍSICA DE PESSOAS EXPOSTAS DE MODO CRÔNICO OCUPACIONAL A SOLVENTES ORGÂNICOS: SENSIBILIDADE AO CONTRASTE ESPACIAL E TEMPORAL DE LUMINÂNCIA, DISCRIMINAÇÃO DE CORES E PERIMETRIA ESTÁTICA DE HUMPHREY

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Objetivo: Investigar as repercussões visuais causadas pela exposição ocupacional crônica a solventes orgânicos em frentistas (28±11,8 anos de idade) de postos de distribuição de combustível (54,6±74,5 meses de trabalho). **Métodos:** Foram estudadas a função de sensibilidade ao contraste espacial (FSCE) e temporal (FSCT) de luminância (n=10), a discriminação de matizes pelo teste de Farnsworth-Munsell 100 (FM-100) (n=11) e a perimetria estática de Humphrey (PEH) (n=4). Os resultados de cada frentista foram comparados com intervalos de tolerância de sujeitos controles (FSCE, FSCT, FM-100) ou à análise Statpac do PEH. O teste-t ($\alpha=0,05$) foi realizado para comparar o grupo de frentistas e o grupo controle e o índice de correlação linear de Pearson para tempo de exposição ou idade no desempenho dos testes.

Resultados: FSCE: o grupo de frentistas teve sensibilidade ao contraste espacial de luminância diminuída em 30 cpg. Cinco sujeitos tiveram sensibilidade ao contraste diminuída em pelo menos uma frequência espacial. FSCT: o grupo de frentistas teve diminuição sensibilidade ao contraste temporal de luminância diminuída em 2 Hz. Quatro sujeitos apresentaram sensibilidade ao contraste diminuída em pelo menos uma frequência temporal. FM-100: o grupo de frentistas apresentou valores de erro maiores que sujeitos normais. Oito sujeitos obtiveram valores de erros acima dos de referência. Nenhuma correlação foi observada entre tempo de exposição ou idade com o desempenho nos testes ($p > 0,05$). Não houve alteração nos resultados do protocolo 10-2 da PEH. No protocolo 30-2 da PEH, os defeitos de profundidade médios foram de 1,32±1,21 no campo temporal superior, 1,82±1,17 no campo temporal inferior, 0,84±0,75 no campo nasal superior e 1,17±0,75 no campo nasal inferior. No protocolo 60-4 da PEH, os defeitos de profundidade médios foram de 0,6±1,34 no campo temporal superior, 1,2±0,84 no campo temporal inferior, 3±4,51 no campo nasal superior e 5±4,36 no campo nasal inferior. **Conclusão:** O grupo de frentistas apresentou comprometimento na discriminação de matizes e pouco comprometimento da visão de contraste de luminância. Os campos visuais periférico nasal e central temporal foram os mais comprometidos.

Palavras-Chave: Intoxicação por solventes, psicofísica visual, contraste espacial, contraste temporal, visão de cores, perimetria

Apoio Financeiro: CAPES, CNPq, CNPq / FUNTEC PRONEX, FINEP-IBN Net

INFLUÊNCIA DO TAMANHO DO ESTÍMULO NA SENSIBILIDADE AO CONTRASTE DE LUMINÂNCIA EM DIFERENTES EXCENTRICIDADE DO CAMPO VISUAL CENTRAL

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OBJETIVO: Estimar a sensibilidade ao contraste de luminância em diferentes excentricidades do campo visual central.

MÉTODOS: Foram testados 3 sujeitos ($26,3 \pm 1,1$ anos de idade) tricromatas normais monocularmente a 1 m de distância do monitor. Foram avaliados a partir de métodos psicofísicos criados a partir de um aplicativo desenvolvido sob a linguagem C++ a partir de uma IDE de Desenvolvimento, Borland C++. O estímulo foi gerado a partir de um monitor colorido de 19" cujos canhões foram linearizados a partir de proposições automatizadas computacionais no próprio aplicativo. O teste consistiu de um estímulo quadrado de $0,016^\circ$ e 1° . Os estímulos foram mostrados na fóvea (0°) e em 10 diferentes excentricidades (até 5°) no meridiano horizontal (campo nasal, temporal) e no meridiano vertical (superior e inferior). A luminância inicial do estímulo foi de 20 cd/m^2 e do fundo foi de 5 cd/m^2 (contraste de Michelson de 60%). A cromaticidade do estímulo e do fundo foi a mesma (CIE 1931: $x=0,333$; $y=0,333$). As coordenadas de cromaticidade e luminância utilizadas nos testes foram medidas através de um colorímetro. A sensibilidade ao contraste foi estimada através do método do ajuste em cada uma das excentricidades.

RESULTADOS: Para o estímulo de $0,016^\circ$, o pico de sensibilidade ao contraste ocorreu na fóvea, decaindo com o aumento da excentricidade. A sensibilidade ao contraste do campo superior foi significativamente inferior à do campo temporal (ANOVA, Tukey test, $p<0,01$). Para o estímulo de 1° , o pico de sensibilidade ao contraste ocorreu na fóvea nos campos nasal e inferior e em $0,5^\circ$ nos campos temporal e superior. Não houve diferença de sensibilidade ao contraste entre os diferentes campos para o estímulo de 1° . A sensibilidade ao contraste obtida com o estímulo de 1° foi estatisticamente maior que aquela obtida com estímulo de $0,016^\circ$. O estudo da sensibilidade ao contraste relativa ao pico mostrou uma queda mais acentuada quando utilizou-se o estímulo de $0,016^\circ$.

CONCLUSÃO: O tamanho do estímulo influenciou nos valores de sensibilidade ao contraste absoluta ao longo do campo visual e na diminuição da sensibilidade ao contraste desde o pico até 5° de excentricidade do campo.

Palavras-Chave: Campo visual, sensibilidade ao contraste, vias paralelas visuais

Apoio Financeiro: CAPES, CNPq, CNPq / FUNTEC, FINEP IBN-Net

D.03.049

AVALIAÇÃO ELETRORRETINOGRÁFICA DO EFEITO DA INJEÇÃO INTRAVÍTREA DE ÁCIDO MICOFENÓLICO EM COELHOS

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OBJETIVO

Avaliar o efeito da injeção intravítrea de ácido micofenólico (MPA) na retina de coelhos através de eletrorretinograma (ERG). O MPA é um imunossupressor utilizado em uveítes que provoca muitos efeitos colaterais sistêmicos.

MATERIAIS E MÉTODOS

6 coelhas Califórnia foram incluídas neste projeto piloto. Um olho de cada animal recebeu injeção intravítrea de 1000µg de MPA (5x a dose teórica presente no olho após uso oral) e o outro recebeu injeção de veículo. Foram realizados ERG antes das injeções e 1 dia após. Os procedimentos foram realizados sob anestesia geral (xilasina 6,7mg/kg e cetamina 100mg/kg via intramuscular) e dilatação pupilar (fenilefrina 10%). Para a realização do ERG, os animais eram acomodados no Ganzfeld (LKC 2503B) e pulsos de luz eram gerados por estimulador GRASS (PS33 Plus). As respostas elétricas obtidas foram amplificadas (Grass), digitalizadas e analisadas com o programa Labview. Foi seguido o protocolo da ISCEV para isolamento de respostas de cones (70cd/m²; 0,2Hz; iluminação de fundo: 15cd/m²), bastonetes (0,02cd/m²; 0,5Hz) e resposta escotópica máxima (3cd/m²; 0,5Hz).

RESULTADOS

O MPA não aboliu as respostas do ERG, apenas as reduziu conforme dados da tabela. A onda-a (fotorreceptores) apresentou diminuição de 40% e a onda-b (células de Müller e bipolares) de 24,6%. Não houve diferença estatística entre os resultados de olhos injetados com MPA e veículo.

DISCUSSÃO

A alta dose de MPA utilizada causou diminuição das resposta do ERG, sugerindo toxicidade retiniana da dose de 1000µg. O resultado será útil na determinação de doses terapêuticas a serem testadas em modelos animais de uveíte.

Tabela: Respostas obtidas no ERG.

RESPOSTAS	CONDIÇÃO	Onda-a (µV)	Onda-b (µV)
		Média (DP)	Média (DP)
Bastonetes	Pré-injeção (n=12)		95 (0,14)
	Veículo (n=6)		92,5 (0,17)
	MPA (n=6)		75 (0,1)
Escotópica máxima	Pré-injeção (n=12)		115 (0,38)
	Veículo (n=6)		120 (0,17)
	MPA (n=6)		85 (0,3)
Cones	Pré-injeção (n=12)	5 (0,19)	65 (0,29)
	Veículo (n=6)	5 (0,09)	65 (0,81)

	MPA (n=6)	3 (0,13)	50 (0,16)
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Palavras-Chave: Eletrorretinograma; Injeção intravítrea; Ácido micofenólico; coelhos

Apoio Financeiro: fapesp

D.04.001

Progesterone attenuates neuropathic pain-associated behaviors and induces changes in the expression of NMDA receptor subunits, preprodynorphin and PKC γ in rats subjected to a spinal cord hemisection.

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Central neuropathic pain (CNP) is refractory to conventional treatment and remains a therapeutic challenge. Progesterone (PROG), a neuroprotective steroid, may offer a promising perspective in pain modulation. In this work, we used a model of CNP to study the effects of PROG on the expression of NMDA receptor (NMDAR), preprodynorphin (ppD) and protein kinase C gamma (PKC γ), critical players in nociceptive processing at the spinal level. Male rats subjected to spinal hemisection at T13 level (n=10) received daily subcutaneous injections of PROG (Hx+PROG; 16 mg/kg, n=5) or vehicle (Hx; n=5). Uninjured rats (n=5) were used as control animals (CTL). Mechanical and thermal allodynia were assessed with the von Frey and Choi tests. Real-time PCR was employed to determine the relative mRNA levels of NMDAR subunits (NR1, NR2A and NR2B), ppD, PKC γ and the housekeeping gene cyclophilin A. Thirty days after lesion, Hx rats showed well-established mechanical and thermal allodynic responses. At this time-point, a significant increase in the mRNAs for all NMDAR subunits was observed in the dorsal horn caudal to the injury (results are expressed as fold-increase relative to CTL levels: NR1: 1.303 ± 0.069 ; NR2A: 1.667 ± 0.194 ; NR2B: 2.052 ± 0.200 , $p < 0.05$ vs CTL in all cases). In addition, a 2-fold increase in both ppD ($p < 0.05$ vs CTL) and PKC γ ($p < 0.01$ vs CTL) mRNAs was observed. Hx+PROG animals did not develop mechanical allodynia and showed reduced sensitivity to cold stimulation. In these animals, the levels of NMDAR subunits mRNAs were decreased as compared to Hx, remaining similar to those observed in CTL (NR1: 0.824 ± 0.126 ; NR2A: 1.12 ± 0.11 ; NR2B: 0.987 ± 0.163 , $p < 0.05$ vs Hx, $p > 0.05$ vs CTL in all cases). PROG treatment also resulted in lower levels of PKC γ expression ($p < 0.05$ vs Hx). However, ppD mRNA levels remained up-regulated ($p < 0.05$ vs CTL). Since dynorphin interaction with the NMDAR has been involved in the mechanisms underlying CNP, PROG administration may reduce allodynia by modulating NMDAR expression, thus favoring the analgesic actions of dynorphin. We suggest that PROG, by targeting spinal mechanisms, may be useful in the treatment of chronic pain states (M808-UBACYT).

Palavras-Chave: Neuropathic pain, progesterone, allodynia, spinal cord, NMDA receptor

Apoio Financeiro: M808-UBACYT, PIP5542 - CONICET

D.04.002

Morphology of callosal neurons and axonal arbors in the rat barrel cortex.

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In the primary somatosensory cortex (S1) of small rodents, each large whisker is represented by a discrete cytoarchitectonical module, or barrel. The resulting array of modules, or barrel field, is isomorphic to the set of whiskers on the snout, and hence ideal for anatomo-functional studies of cortical integration processes. Rats scan their surroundings through active, rhythmic bilateral whisking movements. Though the trigeminal pathway is fully crossed, stimulation of a single whisker can exert both excitatory and inhibitory influences onto ipsilateral cortical neurons (Shuler, Krupa, Nicolelis, 2002, *Cereb.Cortex* 12:86-97). Although such effects are presumably mediated by callosal links between S1 areas, the anatomical substrate of interhemispheric integration is largely unknown. Here, we inspect the morphology of callosal cell bodies and axonal arbors, respectively labeled by targeted injections of sensitive retrograde and anterograde tracers in the rat barrel field.

Under ketamine/xylazine anesthesia, multiunit activities were recorded in cortical layer IV using extracellular microelectrodes, and the response to light mechanical stimulations of the whiskers was tested. Biotinylated Dextran Amine (10%, 3 or 10 KDa) was then injected iontophoretically into selected sites. After 2 weeks survival, serial 150mm sections were incubated in ABC complex and processed for Nickel-intensified DAB reaction. Cell bodies and processes were 3D-reconstructed across consecutive sections using the Neurolucida (MBF) system.

Callosal cell bodies were not restricted to septal columns, nor were exclusively located in layers II-III and V, as assumed. Most callosal fibers terminate into homotopically restricted columns. Yet, other axons display more widespread terminal arbors, especially in superficial layers, and could thus convey cross-barrel integration. Finally, callosal neurons also have an ipsilateral collateral, and are therefore capable of driving both hemispheres simultaneously.

Morphological reconstruction of selected neuronal populations reveals key features of the circuits responsible for interhemispheric integration of sensory inputs in S1.

Palavras-Chave: Barrel cortex; Corpus callosum; 3D reconstruction; Morphometry; Anterograde tracing

Apoio Financeiro: CNPq, FAPERJ, PRONEX

PROSTAGLANDIN E₂ AND PERIPHERAL NERVE INJURY INCREASE THE ANTINOCICEPTIVE EFFECT OF CROTALPHINE, A PEPTIDE OBTAINED FROM THE VENOM OF *Crotalus durissus terrificus* SNAKE

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Objetives: Crotalphine (CRP), administrated *p.o.* to rats, induces antinociception mediated by activation of opioid receptors. The peripheral efficacy of drugs endowed with opioid activity is enhanced in the presence of tissue injury. This study aimed to characterize the local antinociceptive effect of CRP and the contribution of tissue injury to this activity. For this purpose, the effect of CRP, administrated in male Wistar rats by intraplantar (i.pl.) route was evaluated on the PGE₂- induced hyperalgesia and neuropathic pain induced by chronic constriction injury (CCI) of sciatic nerve.

Methods: The paw pressure test (to determine hyperalgesia) was applied to both paws before and 3 h after PGE₂ (100 ng/paw) injection. The paw pressure test and von Frey hairs (to determine allodynia) were applied to one paw, before and 14 days after CCI. DAMGO, U-50488 and DPDPE, agonists of MOR, KOR and DOR, respectively, used as controls, or CRP were injected into one paw, 1h prior to nociceptive assessment.

Results: CRP (0.6 ng), DAMGO (5 µg), U-50488 (10 µg) and DPDPE (20 µg) did not modify the pain threshold of naive rats. In contrast, these drugs, in the same doses, blocked PGE₂-induced hyperalgesia and also increased the pain threshold in the injected paw, as compared to baseline (54, 29, 26, and 21%, respectively). These effects were observed only in the paw injected with the drugs, indicating a local effect of these drugs. DAMGO (5 and 15µg), DPDPE (60 µg) and CRP (0.6 ng) blocked CCI- induced nociception, but only CRP increased the pain threshold when compared to baseline (64%). Nor-BNI (50 µg, KOR antagonist) abolished CRP effect in PGE₂-induced hyperalgesia, but partially antagonized its activity in CCI rats. ICI 174,864 (10µg, DOR antagonist), but not CTOP (MOR antagonist), abolished CRP-induced analgesia only in the CCI model.

Conclusion: In conclusion, acute hyperalgesia increased the local antinociceptive efficacy of CRP and selective opioid receptor agonists. Only MOR and DOR agonists and CRP presented local efficacy under neuropathic pain conditions. The type of opioid receptor involved in the antinociceptive effect of CRP was injury-related. **Palavras-Chave:** Crotalphine, opioid receptors, antinociception, neuropathic pain, prostaglandin E₂

D.04.004

NADPH-diaphorase neurons in rat primary somatosensory cortex display different morphology across columnar and radial compartments

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The enzyme NADPH-diaphorase (NADPHd) is responsible for the synthesis of nitric oxide, a gaseous biological messenger able to affect the local vascular tone. It is expressed by a subpopulation of cortical neurons, which might contribute to the dynamic metabolic activation of segregated cortical microdomains. The rodent somatosensory cortex is an ideal model for studies of anatomo-functional parcellation, since it contains easily identifiable modules (barrels) related to the processing of peripheral inputs from distinct parts of the body.

Thus, we analyzed the distribution and morphology of NADPHd-positive neurons in area S1 of adult Wistar rats.

Serial, 200- μ m-thick coronal sections were processed for NADPHd histochemistry and all strongly reactive neurons (type I, n=1360) were plotted and 3D-reconstructed using a 100x oil-objective and the Neurolucida system (MBF). Layers and barrels were delimited after the pattern of diffuse NADPHd labeling.

Nitridergic neurons were randomly distributed throughout S1, and their dendrites could cross the limits of barrels and/or cortical layers. Quantitative analysis indicated that less NADPHd cells were located in supragranular layers than in other layers ($p=10^{-6}$), but there were no differences between columnar compartments (barrels vs. septa: $p=0.95$). Statistical comparison of morphometric parameters indicated that most NADPHd cells had a spherical cell body ($p=0.05$) and that layer IV cells displayed the largest soma area ($p=10^{-6}$). Dendritic length ($p=4.10^{-5}$), number of segments ($p=0.04$), number of nodes ($p=0.0002$) and dendritic field volume ($p=8.10^{-5}$) were significantly larger for dendrites of NADPHd cells located in septal columns as compared to cells located inside barrels, indicating that septal neurons are more ramified. Most dendritic arbors (54%), especially those from granular and infragranular layers ($p=10^{-6}$), were vertically oriented, and most of those were double-tufted.

The results demonstrate that NADPHd neurons from distinct anatomo-functional modules in area S1 have distinct morphological features, and suggest that nitridergic neurons adapt their morphology to the dynamics of each cortical processing compartment.

Palavras-Chave: primary somatosensory cortex, barrel field, nitric oxide, morphometry

Apoio Financeiro: CAPES, FAPERJ, PRONEX

D.04.005

Envolvimento da via HO-CO-cGMP na nocicepção induzida por estímulo térmico nocivo em ratos.

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Dados recentes relatam que o gás composto monóxido de carbono (CO), está envolvido na modulação de diferentes funções orgânicas, tais como a temperatura corporal associada a situações de estresse. A participação do CO nos processos fisiológicos ocorre por meio da atividade da hemeoxigenase (HO) e do CO formando guanilato monofosfato cíclico intracelular (GMPc). Situações de estresse agudo podem ser acompanhadas por redução da nocicepção, sendo que os mecanismos desta analgesia tem sido largamente abordado na literatura. Este trabalho avaliou o envolvimento do CO na nocicepção induzida pelo teste de retirada de cauda em ratos.

Ratos machos ($\pm$ 250grs; Wistar) foram submetidos à cirurgia para o implante unilateral da cânula-guia direcionada para o ventrículo lateral. Após o período de recuperação (5-7 dias), os ratos foram submetidos às medidas da latência basal do teste de retirada da cauda, sendo subdivididos em dois grupos experimentais. Grupo 1 (n=8): os animais receberam administração intracerebroventricular (ICV) de ZnDPBG (inibidor da via HO-CO-GMPc) na concentração de 50nmol/l no volume de 4,0 μ l. No grupo 2 (n=8) os animais receberam administração ICV do heme-lisinato (substrato da via HO-CO-GMPc). Nossos resultados mostram que a administração isolada de ZnDPBG promoveu alteração significativa do índice de analgesia dos animais (0,56 $\pm$ 0,15; 0,69 $\pm$ 0,14; 0,70 $\pm$ 0,08 aos 15, 30 e 45min após o tratamento respectivamente) e também com administração do heme-lisinato (0,76 $\pm$ 0,13; 0,77 $\pm$ 0,09; 0,78 $\pm$ 0,09 aos 45, 60 e 90min respectivamente).

Nossos resultados demonstraram que a administração ICV do ZnDPBG, bem como do heme-lisinato promoveram antinocicepção avaliada pelo teste de retirada de cauda. Algumas evidências corroboram nossos resultados, mostrando efeito antinociceptivo tanto da inibição da via HO-CO-GMPc (ZnDPBG) como pela administração do substrato para a mesma (heme-lisinato). No entanto, novos experimentos serão necessários para avaliar em maiores detalhes a participação do CO na analgesia.

Palavras-Chave: hemeoxigenase, monóxido de carbono, nocicepção

Apoio Financeiro: Capes, CNPq

D.04.006

Comparação morfométrica de fragmentos axonais calosos da área somestésica primária em dois roedores. O tamanho do animal e o seu habitat fazem diferença?

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Objetivo: As características atuais do sistema nervoso dos mamíferos resultam de processos evolutivos de adaptação ao meio ambiente e a complexidade comportamental parece estar correlacionada com o peso relativo do cérebro em relação ao corpo dos mamíferos. Neste trabalho, comparamos as projeções calosas da área somestésica primária (S1) em dois roedores de tamanho e hábitos distintos: a cutia, diurna e de médio porte, e o rato, noturno e pequeno porte. **Métodos:** Fragmentos axonais calosos provenientes de S1 foram analisados quantitativamente através de injeções do neurotraçador dextrana biotinizada (10 Kd Molecular Probes) em animais adultos. A morfologia de fragmentos axonais (rato n=13; cutia n=31) em S1 foi analisada através de reconstrução tridimensional computadorizada com os softwares *Neurolucida* e *Neuroexplorer* (Microbrightfield, Inc.). Para efeito comparativo, foram utilizadas as seguintes variáveis: densidade de botões terminais (DBt) (nº de botões terminais/ μ m) e de passagem (DBp) (nº de botões de passagem/ μ m) (densidade de botões totais: DBT = DBp + DBt), e densidade de segmentos (DS) (nº de segmentos/ μ m). **Resultados:** Os resultados, expressos como média $\pm$ erro padrão, são (cutia/rato): DBt= 0.01 $\pm$ 0.002/0.006 $\pm$ 0.002, DBp= 0.009 $\pm$ 0.001/0.07 $\pm$ 0.01, DBT= 0.02 $\pm$ 0.003/0.08 $\pm$ 0.01, e DS= 0.005 $\pm$ 0.0006/0.01 $\pm$ 0.001. A análise discriminante revelou que os terminais calosos da cutia e do rato constituem populações distintas, com características morfométricas diferentes (p<0.05). Através da análise do coeficiente de variação, a variável que mais favoreceu a distinção entre elas foi a DBt (138,58%). A ANOVA (Tukey *post hoc*) revelou que todas as variáveis analisadas eram maiores no rato (p<0.05). **Conclusão:** Os terminais calosos do rato possuem uma morfologia axonal mais exuberante, em termos de ramificação e densidade de botões, quando comparada com os da cutia, sugerindo que a integração interhemisférica no rato é mais eficiente. **Palavras-Chave:** Morfometria, somatossensório, roedores.

Apoio Financeiro: Ao PIBIC / CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico/Brasil).

D.04.007

Análise Morfométrica de fragmentos axonais cortico-corticais da área somestésica primária (S1) de ratos adultos.

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Objetivo: A informação somestésica é processada através de uma combinação de mecanismos hierárquicos e paralelos no córtex cerebral. Neste trabalho, avaliamos quantitativamente a morfologia de terminais axonais que se projetam da área somestésica primária (S1) para áreas somestésicas de ordem superior no córtex de ratos. **Métodos:** Foram marcados fragmentos axonais de S1 para as áreas somestésicas secundária (S2), parietal ventral (PV) e parieto-rinal (PR) com o neurotraçador dextrana biotinizada (10kD Molecular Probes) injetado iontoforicamente em S1. Realizamos a análise morfométrica de fragmentos axonais através da reconstrução tridimensional computadorizada com os softwares *Neurolucida* e *Neuroexplorer* (Microbrightfield, Inc.). Avaliamos comparativamente os terminais axonais reconstruídos em S2 (n=21); PV (n=14); PR (n=19), através dos seguintes parâmetros: densidade de botões terminais (DBt) (nº de botões terminais/ μ m), de botões de passagem (DBp) (nº de botões de passagem/ μ m) (densidade total de botões: DBT=DBt+DBp) e de segmentos (DS) (nº de segmentos/ μ m). **Resultados:** Os resultados, expressos como média $\pm$ erro padrão, são (S2/PV/PR): DBt=0.01 $\pm$ 0.002/0.01 $\pm$ 0.003/0.01 $\pm$ 0.002; DBp=0.06 $\pm$ 0.009/0.06 $\pm$ 0.013/0.05 $\pm$ 0.008; DBT=0.07 $\pm$ 0.009/0.07 $\pm$ 0.015/0.06 $\pm$ 0.008 e DS=0.01 $\pm$ 0.001/0.01 $\pm$ 0.002/0.01 $\pm$ 0.001. A análise discriminante revelou que os terminais das áreas somestésicas S2, PV e PR constituem populações semelhantes, com características comuns entre si ($p>0.05$); a ANOVA (Tukey *post hoc*) mostra que todas as variáveis são semelhantes nas áreas avaliadas e que DBt é significativamente maior que DBp ($p<0.05$) em todas elas. A análise de conglomerados dos fragmentos, em cada área, revelou que existem dois grupos bem definidos e a variável que mais favoreceu a distinção entre eles foi a DBT, com coeficiente de variação de 64.72%; 119.10% e 70.82% para S2, PV e PR respectivamente. **Conclusão:** As projeções de S1 para S2, PV, PR ocorrem por vias neurais semelhantes morfologicamente e compostas individualmente por duas sub-populações de terminais diferenciadas pelo número de botões sinápticos. **Palavras-Chave:** Morfometria, córtex somestésico, rato.

Apoio Financeiro: Apoio financeiro: Ao PIBIC/ CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico/Brasil).

Análise Morfométrica de Terminais Axonais no Córtex Somestésico da Cutia (*Dasyprocta prymnolopha*)

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Objetivo: Este trabalho descreve as características morfométricas de projeções axonais da área somestésica primária (S1) para áreas de ordem superior no lobo parietal, a área somestésica secundária (S2) e a área parieto-rinal (PR) da cutia (*Dasyprocta prymnolopha*). A partir da estrutura morfológica dos terminais axonais, verificamos a presença de vias neurais paralelas no córtex somestésico deste roedor e papéis funcionais distintos executados pelas áreas S2 e PR. **Métodos:** O neurotraçador anterógrado dextrana biotinilada (10 KD Molecular Probes) foi injetado na área de representação das patas anteriores do córtex somestésico primário de 2 animais. Cortes tangenciais com espessura de 100µm foram processados para revelação do neurotraçador. Foram reconstruídos 31 fragmentos axonais em cada área-alvo, com o auxílio do *software* Neurolucida (Microbrightfield, Inc.). Os resultados foram analisados através do Teste Z ($\alpha=0.05$), com as seguintes variáveis dos fragmentos axonais: número de botões terminais (Bt), número de botões de passagem (Bp), número de ramificações (Ram) e comprimento (Com) em µm. **Resultados:** Todas as variáveis apresentaram distribuição normal nas duas áreas analisadas. Os resultados são expressos como média $\pm$ erro padrão: Bt (S2= 14.73 ± 2.32 , PR= 7.58 ± 1.16), Bp (S2= 15.65 ± 3.99 , PR= 6.74 ± 1.35), Ram (S2= 6.06 ± 1.07 , PR= 1.94 ± 0.36) e Com (S2= 981.14 ± 106.16 , PR= 1161.25 ± 150.74). O teste Z revelou diferenças morfométricas significativas ($p<0,05$) entre os terminais das duas áreas, sendo todas maiores na área S2, exceto o comprimento.

Conclusão: As áreas S2 e PR recebem informação de S1 através de vias neurais segregadas e especializadas. O maior número de botões sinápticos e ramificações em S2 indicam a presença de circuitos neurais distintos daqueles encontrados em PR. Os resultados sugerem que o processamento das informações somestésicas é diferente nas duas áreas analisadas.

Palavras-Chave: Análise morfométrica, Córtex somestésico, *Dasyprocta prymnolopha*

Apoio Financeiro: : Ao PIBIC / CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico/Brasil).

D.04.009

A secção do nervo ciático modifica a reação à NADPH diaforase na medula espinal de rãs

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Objetivos: Os efeitos da injúria nervosa sobre o sistema nervoso central (SNC) de vertebrados não-mamíferos são pouco conhecidos. Uma molécula possivelmente envolvida nesta condição é o óxido nítrico (NO). Para demonstrar a presença do NO, tem sido empregada a reação histoquímica à NADPH diaforase (NADPHd), a qual marca neurônios contendo a enzima óxido nítrico sintase (NOS), e a medida de seus metabólitos. Assim, este estudo demonstrou o padrão de reatividade à NADPHd e determinou a concentração de nitritos e nitratos (NO₂/NO₃) na medula espinal de rãs controle e submetidas a secção do nervo ciático.

Métodos: Rãs *Rana catesbeiana* adultas, machos, foram divididas em grupos: controle (não houve manipulação cirúrgica), sham (nervo ciático isolado) e denervado (nervo ciático seccionado) e sacrificadas nos períodos de 1, 3, 7 e 15 dias da intervenção cirúrgica para remoção da medula espinal lombossacral. Para a histoquímica foram utilizados os grupos controle e denervado, nos períodos de 3, 7 e 15 dias. Resultados expressos pela média de pixels e analisados por ANOVA de uma via, pós-teste de Dunn. Para a quantificação de NO₂/NO₃ empregou-se os grupos controle, sham e denervado nos períodos de 1, 3 e 7 dias. Resultados expressos em mmol/L e analisados por ANOVA de uma via seguida de Tukey.

Resultados: Houve aumento bilateral da reatividade à NADPHd na porção dorsal do funículo lateral e no campo terminal dorsal da medula espinal após 3 ($0,14 \pm 0,006$) e 7 ($0,19 \pm 0,014$) dias da axotomia comparado ao controle ($0,12 \pm 0,005$). Os metabólitos do NO estavam reduzidos aos 3 dias no denervado ($0,02 \pm 0,002$) e aumentados no sham aos 7 dias ($0,11 \pm 0,004$) quando comparados ao controle ($0,04 \pm 0,005$). A reação NADPHd de somas neuronais da banda mediolateral e das porções dorsal e medial do corno ventral, e de prolongamentos presentes nos funículos lateral e ventral, não modificaram sua intensidade. Conclusão: Estes resultados sugerem que a NADPHd está indicando um aumento do metabolismo nos animais denervados e não acréscimo da atividade da NOS. Porém, é preciso cautela na interpretação das medidas de NO₂/NO₃. Estudos complementares são necessários para elucidar esta questão.

Palavras-Chave: Secção nervosa, Nocicepção, Rã, Medula espinal, Óxido Nítrico, NADPH diaforase, Nervo ciático.

Apoio Financeiro: FAPERGS e CNPq

D.04.010

ORGANIZAÇÃO DOS BARRIS EM S1 DA CUTIA (*Dasyprocta prymnolopha*).

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O córtex somestésico primário (S1), localizado no lobo parietal, possui representação completa da metade contralateral do corpo do animal e possui organização laminar semelhante à de outras áreas sensoriais, com as aferências talâmicas terminando principalmente na camada IV. Em roedores, a área S1 apresenta unidades citoarquitetônicas conhecidas como barris, que correspondem à representação das vibrissas. Essas estruturas podem ser facilmente visualizadas através de técnicas histológicas. **Objetivo:** Descrever a organização topográfica da representação das vibrissas em S1 da espécie *Dasyprocta prymnolopha* e verificar a existência de módulos corticais semelhantes aos barris de outros roedores. **Métodos:** 05 cutias foram anestesiadas para a realização de procedimentos cirúrgicos e de registro. Foi realizada uma craniotomia para exposição da superfície cortical. O registro eletrofisiológico multiunitário realizado com microeletrodo de tungstênio (Frederick Haer, Inc). A posição de cada penetração foi marcada em uma fotografia do córtex exposto, utilizando como referência o padrão vascular. Durante o registro, a superfície cutânea foi estimulada com varetas de madeira, deflexão de pêlos da face e do corpo e compressão suave da pele. A localização e as características de resposta foram documentadas em diagramas do corpo do animal. Ao final da sessão de registro, foram feitas lesões eletrolíticas para facilitar a reconstrução das penetrações. Os animais foram perfundidos com fixador, os hemisférios foram aplanados, seccionados tangencialmente e os cortes processados para revelação da mielina pelo método de Gallyas e da atividade enzimática da citocromo oxidase (CO). **Resultados:** A representação cortical das grandes vibrissas da face da cutia apresenta organização topográfica regular, com a deflexão das vibrissas mais ventrais ativando grupos de neurônios localizados mais medialmente, enquanto que a deflexão das fileiras de vibrissas mais dorsais ativa neurônios localizados mais lateralmente em S1. A CO e a distribuição de mielina demonstraram que a cutia apresenta barris organizados em cinco fileiras paralelas com tamanhos e formas variadas.

Palavras-Chave: Somestésico; Roedores; Agouti; Barril

Apoio Financeiro: Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq – Brasil.

**EPIDURAL MOTOR CORTEX STIMULATION REVERSES ALLODYNIA AND
HYPERALGESIA DURING NEUROPATHIC PAIN IN THE PRESENCE OF
PERIAQUEDUCTAL GREY ACTIVATION**

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Background and aims: Electrical motor cortex stimulation (MCS) has been used in clinical treatment of intractable chronic pain, but its mechanism of action remains uncertain. In this study, we investigated the effect of MCS in a neuropathic pain model and the involvement of periaqueductal grey (PAG) in this effect.

Methods: Rats were submitted to chronic constriction injury (CCI) of the sciatic nerve and the nociceptive threshold was evaluated using paw pressure and von Frey tests. One week after CCI, electrodes were placed over the motor cortex in the area corresponding to the right hind paw. One week after electrode implantation, rats were stimulated (1,0V; 60Hz; 210µs) during 15 min and still under stimulation the nociceptive tests were conducted. The brains were collected 1 h after the tests and the c-Fos expression in the PAG was evaluated.

Results: Rats exhibited mechanical hyperalgesia and allodynia 14 days after the peripheral neuropathy. The MCS reversed hyperalgesic and allodynic responses. In animals in which neuropathic pain was reverted by MCS we observed an exacerbated increase of c-Fos expression in both ipsilateral and contralateral PAG to cortical stimulation.

Conclusions: Epidural MCS reverses neuropathic pain in rats, similar to what is observed in humans, and the results suggest the presence of an activation of the descending pain inhibitory system. Further investigation of the mechanisms involved in this effect may contribute to improve the clinic treatment of persistent pain.

Palavras-Chave: Motor cortex; Neuropathic pain; Electrical stimulation; Antinociception; Rat.

Apoio Financeiro: Supported by grants from FAPESP, CNPq, and Teaching and Research Institute, Hospital Sírio-Libanês.

D.04.012

Efeito da secção do nervo ciático sobre a imunorreatividade à proteína c-Fos em medula espinal de rãs

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Objetivos: Em mamíferos, situações que sabidamente ativam nociceptores ocasionam aumento na proteína c-Fos. Todavia, é desconhecido se esta resposta está presente em outros vertebrados. Assim, este estudo avaliou os efeitos da secção do nervo ciático sobre a imunorreatividade da proteína c-Fos em gânglio da raiz dorsal (GRD) e medula espinal de *Rana Catesbeiana*, em condições basais e três dias após a secção. **Métodos e Resultados:** Rãs *R. catesbeiana*, adultas, foram divididos em 3 grupos: controle (animais sem qualquer manipulação); sham (rãs cujo nervo foi isolado, mas não seccionado), e desnervado (animais com secção nervosa). Após perfusão intra-cardíaca com paraformaldeído 4%, os GRDs do nervo ciático e a medula espinal lombossacral foram retirados, seccionados (50 µm) e submetidos à técnica de imunoistoquímica, utilizando anticorpo primário do tipo policlonal, na concentração de 1:700. As alterações na imunorreatividade foram analisadas por técnicas de densitometria óptica e contagem neuronal. Para quantificação da proteína c-Fos na medula espinal utilizou-se técnica de western blot. A manipulação e a secção do nervo provocaram aumento no número de núcleos reativos de células de pequeno diâmetro. A densitometria aumentou apenas nos citoplasmas das células dos GRDs localizados ipsilateralmente à lesão, enquanto a imunorreatividade nuclear e o número de células positivas não modificaram significativamente. Na medula espinal, a manipulação e a secção nervosa resultaram em acréscimo no número de núcleos imunorreativos dos campos terminais dorsal e ventral, e banda mediolateral, sendo este aumento maior no campo terminal dorsal. As demais regiões não alteraram significativamente o padrão de imunorreatividade de c-Fos. A expressão desta proteína também não foi modificada na medula espinal. **Conclusão:** O acréscimo no número de núcleos reativos em neurônios de pequeno diâmetro do GRD e em células do corno dorsal da medula espinal sugere envolvimento desta proteína com o processamento de informações nociceptivas em rãs. Isto demonstra que nestes animais, similar ao que ocorre em mamíferos, a ativação de fibras aferentes primárias modifica a proteína c-Fos. **Palavras-Chave:** Secção nervosa, medula espinal, c-Fos, *Rana catesbeiana*

Apoio Financeiro: CAPES, FAPERGS, CNPq

AVALIAÇÃO DA ATIVIDADE ANTINOCEPTIVA ESPINHAL DA TOXINA TX3-4 ISOLADA DO VENENO DA ARANHA *Phoneutria nigriventer* EM ROEDORES

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Introdução: Os canais de cálcio sensíveis à voltagem (CCSVs) são encontrados em terminais pré-sinápticos e apresentam papel chave na regulação de diversas funções celulares e fisiológicas como a liberação de neurotransmissores em fibras nervosas centrais e periféricas. Toxinas obtidas do veneno da aranha *Phoneutria nigriventer* têm demonstrado grande potencial em bloquear esses canais, sendo a Tx3-4 bloqueadora de CCSVs do tipo P/Q. O objetivo deste estudo foi investigar os mecanismos de ação da toxina Tx3-4 através da medida de cálcio intracelular $[Ca^{2+}]_i$ induzido por KCL em terminações nervosas da medula espinhal (sinaptossomas) de ratos e a atividade antinociceptiva em um modelo de dor tônica administrado por via intratecal em camundongos.

Materiais e métodos: Foram realizados experimentos com sinaptossomas de medula espinhal de ratos Wistar machos (180-250g). O P2 pellet foi resuspenso em tampão Krebs-Ringer, aerado com 95% O₂ / 5% CO₂. Nos sinaptossomas foram colocados 5 mM fura-2 AM e medidas de cálcio livre $[Ca^{2+}]_i$ realizadas fluorimetricamente. O Teste da Formalina foi realizado com camundongos Swiss (25-30g) nos quais a mesma foi injetada intraplantar (20µL) após administração intratecal da Tx3-4 (30pmol/sítio). A latência de lambida, retirada e sacudida da pata foi medida na primeira e segunda fase.

Resultados: Na medida do cálcio intracelular a toxina Tx3-4 (300nM) reduziu o aumento da entrada de cálcio provocada por KCL, com uma inibição máxima de $75,73 \pm 4,89\%$. No teste da formalina mostrou-se efetiva em reduzir a latência na fase inflamatória com pré-tratamento de 30 minutos e 1 hora ($p < 0,05$ e $p < 0,001$) respectivamente, com inibição máxima de $56,96 \pm 6,88\%$.

Discussão: A toxina Tx3-4 bloqueia os CCSVs tipo P/Q após o estímulo despolarizante da membrana com KCL diminuindo o cálcio livre $[Ca^{2+}]_i$, o que poderia justificar sua ação antinociceptiva no teste da formalina. Assim a toxina Tx3-4, poderá ser útil para o tratamento de processos dolorosos.

Palavras-Chave: Canais de cálcio, sinaptossoma, nocicepção, formalina

Apoio Financeiro: FAPEMIG, CNPq.

ANTINOCICEPTIVE EFFECT OF Pha1b, A NEW N-TYPE VOLTAGE-SENSITIVE CALCIUM CHANNEL BLOCKER, ON ACUTE AND PERSISTENT PAIN MODELS IN RODENTS

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Calcium influx through neuronal N-type voltage-sensitive calcium channels (VSCCs) mediates nociceptive information in the spinal dorsal horn. The potential role of N-type VSCCs inhibitors as analgesic drugs has been studied in the last decade. The neurotoxin Pha1b, obtained from the venom of the spider *Phoneutria nigriventer*, acts on N-type (VSCCs) and the aim of this study was to investigate the antinociceptive potential of Pha1b using models of nociception in rodents. Mice were placed into a hot plate test ($55 \pm 0.1^\circ\text{C}$), and the time between placement and the licking of the paws, or jumping, was recorded as index of response latency. Mice were administered with PBS or Pha1b (30-300 pmol/site) from 0.5 to 48 hours before the hot-plate test. In model of persistent pain, the analgesic potential of Pha1b, was evaluated in rats using the formalin test. PBS or Pha1b (30-200 pmol/site) were administered to assess the preventive or the curative antinociceptive action, drugs were administered 0.16 to 24 h before or 0.16 h (10 min) after formalin injection. Pha1b (200 pmol/site) in mice produced a delayed, but long-lasting antinociceptive action in the hot-plate test, from 3 to 24 hours after its injection, the maximum antinociceptive effect ($I_{\max}$) was $85 \pm 7\%$ and the effective dose to produce 50% effect (ED_{50}) was 85 (24-300) pmol/site, 5 hours after Pha1b administration. Next, we assessed the effect of Pha1b in a model of persistent inflammatory pain in rats. The pre-treatment with Pha1b (200 pmol/site) produced antinociception in both, neurogenic and inflammatory phases of the formalin test. In the inflammatory phase, the toxin induced antinociception, showing $I_{\max}$ of 67 ± 9 and the ID_{50} values were 48 (25- 92). Of note, the post-treatment with Pha1b was more effective than its pre-treatment and so, as potent as, with $I_{\max}$ of $87 \pm 5\%$ and ID_{50} of 50 (25- 75) pmol/site. Pha1b was more effective to reverse an established nociceptive process, than to prevent it. The present study demonstrates that Pha1b have analgesic effect in rodent models of pain, suggesting that it may have potential use as a drug in the control of persistent pathological pain.

Palavras-Chave: Antinociception, Channel calcium, Toxin

Apoio Financeiro: FAPEMIG, CNPq

D.04.015

Interleukin-1 β produced in the Dorsal Root Ganglion is important for the development of inflammatory hyperalgesia in peripheral tissue

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Introduction - It is now accepted that prostaglandins production is essential to the development of the inflammatory hyperalgesia, and the non-steroidal anti-inflammatory drugs (i.e., indomethacin) that inhibit the synthesis of prostaglandin is usually used to control inflammatory pain in humans. The synthesis of prostaglandins depends on cytokines, especially Interleukin-1 β (IL-1 β), which is released in site of inflammation. However, the role that the dorsal root ganglion (DRG) cells play in the inflammatory hyperalgesia is not yet elucidated. The aim of this study was to investigate the role of IL-1 β released in the DRG plays in the development of the inflammatory hyperalgesia. **Methods** - Mechanical hyperalgesia (decrease of nociceptive threshold) was evaluated in rats using an electronic version of the von Frey test. Drugs were locally injected in the DRG or in the plantar paw tissue of rats. The cytokine measurement was evaluated by ELISA. **Results** - Administration of IL-1Ra (IL-1 receptor antagonist, 6 μ g) in the DRG of rats prevented the mechanical hyperalgesia measured 1.5, 3 and 6 h after intraplantar (ipl) administration of Complete Freund's Adjuvant (CFA, 150 μ L/paw, in 44, 43 and 29%, respectively), 1, 3 and 5 h after carragenan ipl (100 μ g/paw, in 30, 53 and 64%, respectively) or 3 h after IL-1 β ipl (0.5 pg/paw in 56%), but not by Prostaglandin E₂ (PGE₂) ipl (100 ng/paw). Locally peripheral ipl administration of CFA or carragenan also increased the IL-1 β concentrations in the DRG (24% and 56%, respectively). In addition, hyperalgesic priming mediated by ipl IL-1 β (0.5 pg/paw) significantly increased the hyperalgesia induced by PGE₂ (10 ng/paw), which was completely inhibited by administration of indomethacin (100 μ g) in the DRG. **Conclusion** - These results suggest that the endogenous release of prostaglandins during the inflammation of the peripheral tissue induces mechanical hyperalgesia that depends on IL-1 β -mediated hyperalgesic priming in primary nociceptive neurons and prostaglandins production by releasing of IL-1 β in the DRG.

Palavras-Chave: Hyperalgesia; Interleukin-1 β ; Prostaglandin E₂; Dorsal Root Ganglion.

Apoio Financeiro: CNPq and FAPESP

Natação diária durante 3 semanas produz analgesia opióide em ratos com dor muscular crônica

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Exercícios físicos podem ser utilizados em terapias adjuvantes de pacientes com dores musculoesqueléticas crônicas. O objetivo desse estudo foi determinar se a natação diária durante 3 semanas pode causar analgesia opióide em modelo de dor crônica muscular em ratos. Vinte ratos Wistar machos foram submetidos a duas injeções de salina ácida na pata posterior esquerda (pH = 4; 100uL cada injeção, sob anestesia de halotano vaporizado 2-4%) administradas com 5 dias de intervalo no músculo gastroncnêmio. Após a segunda injeção, surge hiperalgesia mecânica de longa duração na pata posterior que pode ser mantida com injeções musculares semanais. Esses animais com hiperalgesia mecânica foram divididos em 4 grupos: 1) não treinado (NT), 2) não treinado com injeção sistêmica diária de naloxone (1 mg/Kg, subcutânea, 30 min antes do exercício; NT-NX), 3) treinado (T) e 4) treinado com injeção diária de naloxone (T-NX). A natação foi realizada em água aquecida (36°C; 30 min/dia; 5 dias/semana, por 3 semanas). A hiperalgesia mecânica foi testada utilizando-se filamentos de von Frey (FVF) de forças de curvatura crescentes (15-100g) nas superfícies plantares das patas posteriores esquerdas antes e depois da natação. Os resultados foram analisados utilizando-se ANOVA de medidas repetidas de duas vias e teste post-hoc de Tukey. Os dados são apresentados como média do FVF em g $\pm$ e.p.m. A natação diária em água aquecida aumentou os limiares mecânicos de retirada ipsilateralmente após as sessões de exercício de forma não-cumulativa e durante todo o período experimental (grupo T = 57,9 $\pm$ 1,34 g e grupo NT = 49,53 $\pm$ 1,36 g; p < 0,001). Naloxone, injetado diariamente, atenuou significativamente os efeitos anti-hiperalgésicos produzidos pelo exercício (grupo T-NX = 49,83 $\pm$ 1,34) comparado ao grupo T (p < 0,001). A injeção de naloxone não teve efeito sobre os animais não treinados (grupo NT-NX = 48,76 $\pm$ 1,52). A natação diária por 3 semanas em água aquecida produz analgesia opióide em ratos com dor muscular crônica, efeito esse que foi prevenido pelo antagonista opióide naloxone.

Palavras-Chave: nocicepção; dor; naloxone; natação; analgesia

Apoio Financeiro: CNPq e CAPES

ANTI-INFLAMMATORY AND ANTI-HYPERALGESIC ACTIVITY OF PTFE-2 COMPOUND OBTAINED FROM *Piper tuberculatum* Jacq.

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Objectives: Evaluate the possible anti-inflammatory and anti-hyperalgesic effects of trimethoxy-benzenepropanoic acid (PTFE-2, an aromatic aminoacid) isolated from *Piper tuberculatum* Jacq. and some related mechanisms of action. **Methods:** Animals were pretreated with PTFE-2 (0.1-10 mg/kg) by intraperitoneal (i.p.) route. Pleurisy was induced in Swiss mice (25-35g) by Carrageenan (Cg; 1%/100mL) injection, while thermal (Hargreaves method) and mechanical (Randall-Sellito model) hyperalgesia were induced in Wistar rats (250-350g) after intraplantar (i.pl.) injection (100 µl, i.pl.) of Cg (300 ng), prostaglandin E₂ (PGE₂; 10 nmol) or bradykinin (BK; 3 nmol). **Results:** PTFE-2 (0.1-10 mg/kg, i.p.) inhibited pleural exudation in $48 \pm 7\%$ (Cg: 0.9 ± 0.1 ; PTFE-2 10mg/kg: 0.6 ± 0.04) and significantly reduced leukocyte migration in $75 \pm 7\%$ (Cg: 10 ± 1.5 ; PTFE-2 10 mg/kg: 3.78 ± 0.5), due to a decrease of both neutrophils ($73 \pm 4\%$) and mononuclear ($99 \pm 16\%$) cells. Prior i.p. treatment with PTFE-2 did not modify both thermal and mechanical hyperalgesia induced by PGE₂ and Cg. However, BK-induced thermal and mechanical hyperalgesia were significantly reduced by PTFE-2 treatment (30 min beforehand; Vehicle (V): 13 ± 0.9 ; BK: 8 ± 0.9 ; PTFE-2 1 mg/kg: 14 ± 0.6 for thermal; and V: 179 ± 16 ; BK: 57 ± 10 ; PTFE-2 10mg/kg: 204 ± 11 for mechanical hyperalgesia). PTFE-2 (0.1-10 mg/kg, i.p.) was not able to inhibit the overt nociception induced by i.pl. injection of BK (BK: 266 ± 26 and PTFE-2 10 mg/kg: 246 ± 60) in rats, but inhibited in $67 \pm 11\%$ the nociceptive response induced by intrathecal injection of BK (0.1µg/site) in mice [BK: 283 ± 12.2 ; PTFE-2 10mg/kg: 94 ± 29.8 ; DI50:4.656(1.6-13.7)]. **Conclusion:** This study reveals that PTFE-2 produces anti-inflammatory and anti-hyperalgesic/antinociceptive effects after Cg and BK injection, respectively. Moreover, suggest that inhibition/modulation of signaling mechanisms operated by central BK receptors by PTFE-2 could contribute to this analgesic effect.

Palavras-Chave: PTFE-2, nociception, hyperalgesia

Apoio Financeiro: CAPES, CNPq, UFSC

D.04.018

Chronic administration of serotonin/norepinephrine reuptake inhibitor reduces the phosphorylation of cyclic AMP-responsive element-binding protein in the spinal cord dorsal horn following peripheral nerve injury.

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Objective: Here we investigate the effect of chronic administration of imipramine on CREB (cyclic AMP-responsive element-binding protein) phosphorylation in the spinal cord dorsal horn following peripheral nerve lesion.

Methods and results: Adult Balb/C male mice weighting 20-25g were used. Unilateral nerve lesion was induced by partial ligation of the sciatic nerve. Seven days after nerve injury, imipramine (30mg/kg), or saline (10μL/g) were injected daily for 21 days. The development of tactile mechanical hypersensitivity was monitored by Von Frey filaments once a week, 24 hours after the last drug injection. CREB phosphorylation was investigated by immunohistochemistry in the lumbar (L4-L6) spinal cord dorsal horn 7 and 28 days after nerve injury as well as in lesioned animals treated with imipramine for 21 days. Tactile-induced neuropathic pain hypersensitivity was observed from 7 to 28 days after nerve ligation ($p < 0.05$, non-parametric Mann-Whitney test). CREB phosphorylation increased in the superficial laminae of the spinal cord ipsilateral to the lesion after 7 and 28 days post-injury. However, daily administration of imipramine progressively reduced mechanical hypersensitivity from the first week of treatment, and completely reversed tactile stimulus-induced hypersensitivity after 21 days of therapy ($p < 0.05$, non-parametric Mann-Whitney test). Following chronic SNRI administration, the increased phosphorylation of CREB protein was also reversed in the spinal cord dorsal horn. [Surprisingly](#), the antinociceptive effect of imipramine was still observed 3 weeks after cessation of treatment.

Conclusion: these results indicate that chronic administration of imipramine induces plastic changes in the sensory system that may take time to develop and mature, which might explain in part why the clinical analgesic effect of SNRI develops with a delay after the beginning of the treatment. Our data also provide evidence that prolonged SNRI treatment may induce antinociception in neuropathic pain conditions by reducing CREB-mediated transcription.

Experiment protocols were approved by the local Ethical Commission of Ethics in Animal Research (protocol # 213/2005).

Palavras-Chave: Pain

Apoio Financeiro: Fapesp (06/00479-0)

ANTINOCICEPTIVE ACTIVITY OF FRACTIONS AND COMPOUNDS ISOLATED FROM *Piper tuberculatum* Jacq.

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Objectives: The present study aims to evaluate the possible antinociceptive activity of Crude Extract, fractions and isolated compounds obtained from *Piper tuberculatum* in the acetic acid-induced writhing test, as well to assess the effect of the isolated compound PTFE-2 on the motor performance and locomotor activity of animals. **Methods:** The visceral nociception was induced in Swiss mice by an intraperitoneal (i.p.) injection of acetic acid (AA) 0.6%, and the number of writhes was counted cumulatively over a period of 20 min. The animals were pre-treated via i.p. with the compounds PTFLH-1, PELI and PTFE-2 (0.001-30mg/kg), 30 min before the injection of AA, or via oral route with Crude Extract (CE) and fractions Ethyl Acetate (EA), Trichloroethane (TCE), Methanol (Met) and Hexanic (Hex) (3-300mg/kg), 1 h before the irritant injection. Additionally, PTFE-2 (0.1-10mg/kg, i.p., 30 min beforehand) was assessed on the Rota Rod and Open Field tests. **Results:** CE, fractions and isolated compounds reduced significantly and in a dose-dependent manner the nociception induced by AA. The mean $\pm$ SEM, ID50 and inhibition were, respectively: CE: AA- 40 ± 3.2 ; 300mg/kg- 4.4 ± 2.2 , 46.22 (38.05-56.15) and 89 $\pm$ 5%; EA: AA- 36.7 ± 2.3 ; 100mg/kg- 10.6 ± 2.5 , (35.64-69.72) and 71 $\pm$ 7%; TCE: AA- 35.7 ± 2.3 ; 300mg/kg- 9.2 ± 1.9 , 46.65 (32.60-66.70) and 77 $\pm$ 8%; Met: AA- 42.5 ± 2.2 ; 100mg/kg- 9.7 ± 2.9 , 61.50 (48.05-77.41) and 77 $\pm$ 7%; Hex: AA- 39.2 ± 4.1 ; 300mg/kg- 10 ± 3 , 154.07 (135.33-175.40) and 75 $\pm$ 8%; PTFLH-1: AA- 37.5 ± 4.3 ; 10mg/kg- 6.7 ± 2.8 , 5.4 (4.2-6.9) and 73 $\pm$ 13%; PELI: AA- 35.6 ± 2.2 , 1mg/kg- 14.5 ± 2.2 , 0.07 (0.04-0.1) and 56 $\pm$ 7%; PTFE-2: AA- 36.5 ± 2.4 , 1mg/kg- 6.8 ± 1.7 , 0.22 (0.20-0.26) and 81 $\pm$ 5%. PTFE-2 (the most potent between the treatments) did not affect the motor performance on Rota Rod test (Vehicle (V): 178.5 ± 0.96 and PTFE-2: 180.0 ± 0.0) and locomotor activity in the Open Field test (V: 86.75 ± 4.25 and PTFE-2: 70.25 ± 5.36). **Conclusions:** The results confirm the antinociceptive activity of products obtained from *P. tuberculatum*. Furthermore, PTFE-2 compound did not significantly affect the performance and motor activity at a dose that produced marked suppression of the writhing response.

Palavras-Chave: Piper tuberculatum, acetic acid, antinociception

Apoio Financeiro: CAPES, CNPq, UFSC

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EFEITO DO ACUPONTO ‘SANYINJIAO’ (Bp6) NA IMOBILIDADE INDUZIDA PELO TESTE DE SUSPENSÃO DA CAUDA EM CAMUNDONGOS.

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Objetivo: A depressão é uma doença que afeta cerca de 20% da população mundial, sendo considerada potencialmente ameaçadora à vida, com um índice de suicídio entre deprimidos chegando a 15%. Neste contexto diversos estudos clínicos tem reportado efeito antidepressivo da acupuntura (1), entretanto existem poucas evidências desta prática em modelos animais de depressão. O objetivo deste estudo foi investigar o efeito do acuponto ‘Sanyinjiao’ (Bp6) no teste de suspensão da cauda (TSC). **Método:** Foram usados camundongos Swiss machos (35-45g, N=8-14). Os animais foram mantidos em condições de temperatura controlada (22- 25°C) com livre acesso à água e ração, mantidos em ambientação no laboratório 24 horas antes do teste. O Teste de Suspensão da Cauda (TSC) é um teste preditivo de atividade antidepressiva, no qual é registrado o tempo de imobilidade durante 6 minutos. Os camundongos foram isolados acústica e visualmente e suspensos 50 cm acima do chão com fita adesiva colocada a cerca de 1 cm da ponta da cauda. Os grupos foram tratados com os acupontos Bp6, na pata direita (2), ou com o B60 (Kunlun, um acuponto sem indícios de atividade antidepressiva reportada na literatura) na pata esquerda por 10 minutos. Após a retirada da agulha esperou-se 20 minutos para a avaliação no TSC. O grupo controle positivo foi tratado com fluoxetina (32 mg/kg, i.p.) e 30 minutos após o tratamento foi avaliado no TSC. **Resultado:** O acuponto Bp6 reduziu significativamente o tempo de imobilidade no TSC (Inibição: $27 \pm 3\%$ comparado ao respectivo controle=100%). O acuponto B60 não apresentou efeito no modelo preditivo de atividade antidepressiva proposto. A fluoxetina também reduziu significativamente o tempo de imobilidade no TSC (Inibição: $46 \pm 9\%$ comparado ao respectivo controle=100%). **Conclusões:** Os resultados obtidos indicam que o acuponto Bp6 possui atividade antidepressiva no TSC semelhante ao efeito antidepressivo clássico da fluoxetina, sugerindo que este acuponto possa ser utilizado como tratamento na terapêutica deste distúrbio. **Referências:** 1 JAMA 280: 1569, 1998; Am. J. Psychiatry 158: 289, 2001; 2 Braz. J. Med. Biol. Res. 35:731, 2002

Palavras-Chave: acuponto, depressão, TSC

Apoio Financeiro: CNPq, CAPES, UFSC, FINEP (IBN-Net)

ANTI-INFLAMMATORY AND ANTI-HYPENOCICEPTIVE EFFECTS OF (-)-LINALOOL IN A MODEL OF CHRONIC PAIN IN MICE

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Objective: The aim of this study was to investigate the antinociceptive effects of (-)-linalool, a monoterpene alcohol present in the essential oil of aromatic plants, in complete Freund's adjuvant (CFA)-induced hypernociception and cytokine-induced overt nociception. **Methods:** Adult Swiss mice (25–35 g) received a single or repeated intraperitoneal (i.p.) injections of (-)-linalool (50, 100 and 200 mg/kg). Inflammatory chronic pain was induced by CFA (20 µL, i.p.) and then were evaluated the paw oedema and mechanical (von Frey hairs) and cold (acetone test) hypernociception. Nociceptive biting behavior was induced by intrathecal injection of TNF-α (1 pg) and IL-1β (0,1 pg) and was recorded over the next 15 min. **Results:** CFA-induced paw oedema was reduced by (-)-linalool (peak at 4 h; CFA: 0.257 ± 0.020; Lin50: 0.147 ± 0.022; Lin200: 0.088 ± 0.027 µl). Acute treatment with linalool (50, 100 and 200 mg/kg, 30 min early) reduced mechanical hypernociception (peak at 4 h; CFA: 0.476 ± 0.098; Lin50: 2.462 ± 0.214; Lin100: 2.681 ± 0.193; Lin200: 2.701 ± 0.275). (-)-Linalool (50 and 200 mg/kg, i.p.) also showed anti-hypernociceptive effects to cold stimuli until 4 h after treatment (maximal inhibition of 97 ± 2% at 2h; CFA: 12.667 ± 3.518; Lin50: 0.333 ± 0.211; Lin200: 0.833 ± 0.307). Given chronically (twice a day) during 10 days, (-)-linalool significantly reduced the mechanical hypernociception induced by CFA, starting at day 2 and lasting until day 10 (peak at day 5: CFA: 0.399 ± 0.066; Lin50: 1.134 ± 0.405; Lin100: 1.997 ± 0.296; Lin200: 2.677 ± 0.459). Given 30 min before, (-)-linalool (200 mg/kg, i.p.) also inhibited the biting response induced by TNF-α and IL-1β, with inhibitions of 94% (CFA: 217.20 ± 15.98 s; Lin200: 13.33 ± 5.684 s) and 63% (CFA: 121.20 ± 17.94 s; Lin200: 44.83 ± 14.77 s), respectively. **Conclusions:** These data confirmed previous studies and show that (-)-linalool produces pronounced anti-inflammatory and anti-hypernociceptive effects in a model of chronic pain in mice. Thus, (-)-linalool might be potentially useful in the development of new drugs to alleviate sustained painful symptoms in the clinic.

Palavras-Chave: (-)-linalool, CFA, hypernociception, chronic pain

Apoio Financeiro: CAPES, CNPq, ULBRA

Involvement of glutamatergic system, substance P and pro-inflammatory cytokines in the antinociceptive effect caused by the triterpene 3b, 6b, 16b-trihydroxilup-20(29)-ene obtained from *Combretum leprosum* Mart.

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Objectives: The present study aims to investigate the possible involvement of the glutamatergic system, substance P (SP) as well as pro-inflammatory cytokines in the antinociception caused by triterpene 3b, 6b, 16b-trihydroxilup-20(29)-ene (TTHL) in mice. **Methods:** Swiss mice of both sexes were used (25-35g; N=6-8). Firstly, we investigate the effect of TTHL given by intraperitoneal (i.p.), intraplantar (i.pl.) or intrathecal (i.t.) routes, in the glutamate (Glu)-induced nociception in the mouse paw (10nmol/paw, i.pl.). In order to investigate the participation of glutamatergic system, substance P (SP) and pro-inflammatory cytokines, nociception was induced by i.t. injection of Glu, AMPA, NMDA, kainate, trans-ACPD, SP, TNF- α and IL-1 β . **Results:** TTHL given by i.p. (1-30mg/kg), i.pl. (3-30mg/paw) or i.t. (10-300 mg/site) routes evoked dose-dependent inhibition of the Glu-induced nociception. The mean $\pm$ SEM and ID₅₀ values were, respectively, for each route: i.p.: Glu: 107.5 $\pm$ 7.9, TTHL 30: 18.4 $\pm$ 5.6, 5.5 (3.7-7.9)mg/kg; i.pl.: Glu: 93.3 $\pm$ 4, TTHL 30: 18.4 $\pm$ 5.6, 15.7 (12.2-20.2)mg/paw; and for i.t.: Glu: 98.4 $\pm$ 4, TTHL 300: 17.7 $\pm$ 3.9, 107.1 (65.9-173.8)mg/site; and inhibitions of 78 $\pm$ 6; 82 $\pm$ 4 and 77 $\pm$ 8%, respectively. TTHL injected by i.t. route (10-100 μ g/5ml) also caused significant and dose-dependent reduction of nociception induced by i.t. injection of Glu (Glu: 122 $\pm$ 5.3; TTHL 30: 61 $\pm$ 5.2; ID₅₀: 24.79 (23.3-26.3)mg/site and inhibition of 51 $\pm$ 6%). Moreover, TTHL (30 μ g/i.t., co-injected) caused a marked inhibition of the biting responses induced by i.t. injection of NMDA (NMDA: 150 $\pm$ 2.7/TTHL: 27 $\pm$ 5.8), trans-ACPD (tACPD: 203 $\pm$ 10.5/TTHL: 40.3 $\pm$ 9), SP (SP: 89.4 $\pm$ 5/TTHL: 15.5 $\pm$ 4.8), TNF- α (TNF: 208.8 $\pm$ 12.4/TTHL: 27.6 $\pm$ 7.9) and IL-1 β (IL-1: 116.3 $\pm$ 15.4/TTHL: 23.3 $\pm$ 7.8) with inhibitions of 81 $\pm$ 7; 79 $\pm$ 7; 81 $\pm$ 11; 91 $\pm$ 4 and 80 $\pm$ 11%, respectively, but had no effect on AMPA and kainate-induced biting. **Conclusions:** Together, these results provide an experimental evidence for the involvement of glutamatergic system [namely ionotropic (NMDA) and metabotropic (trans-ACPD) glutamate receptors], SP and pro-inflammatory cytokines on the antinociceptive effects of TTHL.

Palavras-Chave: TTHL, antinociception, glutamate, pro-inflammatory cytokines, SP

Apoio Financeiro: CAPES, CNPq, UFSC

EVIDENCE FOR THE ANTIINFLAMMATORY EFFECT OF *SANYINJIAO* (SP6) ACUPOINT IN A MODEL OF PERITONITIS IN MICE

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Objectives: We sought to investigate the anti-inflammatory effects of *Sanyinjiao* (SP6) acupoint on carrageenan (Cg)-induced peritonitis in mice. **Methods:** Acupuncture was performed in awake Male Swiss mice (25-35g; n=6-8) by inserting a needle in the right limb at the SP6 acupoint (1) and Sham acupuncture was performed by punctured the gluteal muscle in a non-acupoint (NA) (2). Animals were placed in an acrylic box during the acupuncture treatment (10min) and then peritonitis was induced by i.p. injection of 0.5 ml of Cg (300mg/0.2 ml). Animals were killed 4h after Cg injection and peritoneal lavage fluid was collected for total and differential leukocyte counts, myeloperoxidase assay (MPO) and peritoneal exudation through extravasation of Evans blue dye. As control, dexamethasone (DEX: 0.5mg/kg, i.p) was administered 30 min prior to peritonitis induction and control animals (C) received sterile saline. Adrenalectomy (ADX) mice were used to evaluate the effects of the adrenal gland in the anti-inflammatory effect caused by SP6. **Results:** SP6 acupuncture pretreatment significantly reduced Cg-induced leukocyte migration (C: 4.7 ± 0.6 , Cg: 12 ± 1.2 , SP6: 4.4 ± 0.3 , NA: 14.2 ± 1.6 cells $\times 10^6$). This reduction was attributed to inhibition of both neutrophil influx ($82 \pm 6\%$) and MPO activity ($98 \pm 1\%$). Furthermore, SP6 acupuncture also inhibited peritoneal exudation ($84 \pm 6\%$). The needle stimulation into NA did not produce any significant anti-inflammatory effect. Like SP6 acupuncture, dexamethasone pretreatment inhibited leukocyte (DEX: 4.3 ± 1) and neutrophil (C: 0.85 ± 0.1 , Cg: 10.2 ± 2 , DEX: 1.1 ± 0.2) influx as well as peritoneal exudation induced by Cg ($89 \pm 7\%$). ADX did not block the anti-inflammatory effect of SP6 acupuncture after Cg-induced leukocyte migration (C: 3.2 ± 0.5 , Cg: 9.6 ± 1 , DEX: 3.8 ± 0.3 , SP6: 6.8 ± 1 cells $\times 10^6$). **Conclusions:** These results show that endogenous glucocorticoids plays little role in anti-inflammatory effect of SP6 acupuncture. The mechanisms by which SP6 promotes anti-inflammatory activity still remain to be defined and require further studies. **References:** Braz. J. Med. Biol. Res. 35:731, 2002; Braz. J. Med. Biol. Res. 36:1673, 2003.

Palavras-Chave: acupoint, acupuncture, Sanyinjiao, peritonitis

Apoio Financeiro: CAPES, CNPq, UFSC

ACUPUNTURA NO ACUPONTO KUNLUN (B60) ACELERA A RECUPERAÇÃO FUNCIONAL E REDUZ ALODÍNIA MECÂNICA E TÉRMICA APÓS ESMAGAMENTO DO NERVO CIÁTICO EM CAMUNDONGOS

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Objetivo. Avaliar os efeitos do agulhamento no acuponto kunlun (B60) sobre a recuperação da função motora e alodínea mecânica e térmica após lesão experimental do nervo ciático em camundongos.

Métodos: 18 camundongos Wistar fêmeas adultos foram utilizados no estudo, os quais foram divididos em 3 grupos: (1) falso-operado, (2) controle operado (tendo o nervo exposto e esmagado por uma pinça hemostática) e (3) operado e submetido a tratamento com acupuntura. O tratamento iniciou no primeiro dia após o esmagamento, que consistiu do agulhamento no acuponto Kunlun (B60) por 10 minutos em dias alternados por 21 dias. A recuperação funcional motora foi monitorada a cada três dias usando o Índice Estático do Ciático (IEC). Para avaliar a alodínea e a hiperalgesia mecânica utilizou-se o teste do filamento de Von Frey através da frequência de retirada. Para avaliar a alodínea térmica ao frio, usou-se o modelo da acetona, a cada três dias. **Resultados:** Na avaliação da recuperação motora, através do IEC, o agulhamento no acuponto B60 reduziu os valores no 6º dia em 53%±6 (p<0,001), no 9º dia em 62%±8 (p<0,001), no 12º dia em 66%±6 (p<0,001). Na avaliação da alodínea mecânica observou-se diminuição na porcentagem de retirada da pata do animal no 7º dia em 61±15% (p<0,01), no 14º em 62±14% (p<0,001) e no 21º dia em 72±8% (p<0,001) em relação ao controle. Já na avaliação da hiperalgesia mecânica observou-se diminuição no 7º dia em 58±8% (p<0,05), no 14º em 45±7% (p<0,05) e no 21º dia em 73±5% (p<0,001), também em relação ao controle. Quanto a alodínea térmica ao frio observou-se uma redução significativa nas médias do tempo de resposta a partir do 9º dia, com redução de 69±9% (p<0,001) no 9º dia e redução de 73±7 (p<0,001) no 21º dia, com relação ao controle. **Conclusão:** Esses dados sugerem que o tratamento crônico com a acupuntura no acuponto B60 iniciado no 1º dia após a lesão do nervo ciático favorece o processo de regeneração nervosa periférica, além de diminuir tanto a alodínea e hiperalgesia mecânica quanto a alodínea térmica ao frio. **Palavras-Chave:** dor crônica, acupuntura, nervo ciático

Apoio Financeiro: UFSC/CNPq

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evidência do efeito antinociceptivo e ANTIINFLAMATÓRIO do acuponto kunlUN (B60)

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Objetivo: Avaliar a atividade antinociceptiva e antiinflamatória da acupuntura realizada no acuponto B60 em camundongos. **Métodos:** Foram utilizados camundongos Swiss machos (25-35g N= 8-10). A agulha de acupuntura foi inserida no acuponto B60 por 10 min e 30 min após foi avaliado parâmetros nociceptivos e inflamatórios. Primeiramente foi utilizado o modelo de nocicepção induzida pela injeção intraplantar (i.pl.) de 20µl de formalina (2,5%) e analisado o tempo de resposta. Outro grupo de animais tratados com B60 foi avaliado contra a ação pró-nociceptiva de citocinas inflamatórias: fator de necrose tumoral alfa (TNF- α – 0,1pg/sítio) e interleucina 1-beta (IL-1 β – 1pg/sítio) e analisado o tempo de resposta. Além disso, o B60 foi avaliado no modelo de peritonite aguda induzida pela carragenina (Cg), onde os animais receberam 30 min antes Azul de Evans (25 mg/kg e.v.) e em seguida foram tratados com o acuponto B60 ou com dexametasona (DEX 0,5 mg/kg, i.p.) ou salina (0.9% NaCl i.p.) e após 30 min receberam Cg (300 µg/0.2 ml, vol de 0,5 ml i.p.). Após 4 horas da indução da peritonite foi coletado o fluido da cavidade peritoneal e avaliado o total e o diferencial de leucócitos, a atividade da enzima mieloperoxidase (MPO), uma medida indireta da migração de neutrófilos e o extravasamento plasmático. **Resultados:** O B60 reduziu a nocicepção induzida pela formalina na fase neurogênica em 29±10% (p<0,05) e na fase inflamatória em 44±9% (p<0,01); também reduziu a nocicepção induzida pelo TNF- α (In:76±10%; p<0,001) e IL-1 β (In:73±10%; p<0,001). O pré-tratamento com o B60 diminuiu significativamente a migração de leucócitos após a injeção de Cg em 71±7% (p<0,001). Bem como à redução de neutrófilos (89±2%; p<0,001) que foi confirmada pela avaliação da atividade da MPO (76±2%; p<0,001). O B60 não alterou leucócitos mononucleares ou extravasamento plasmático. O pré-tratamento com DEX inibiu a migração de leucócitos totais e neutrófilos, contudo foi capaz de inibir também o extravasamento. **Conclusão:** Nossos resultados demonstram que a estimulação do acuponto B60 exibe importante efeito antinociceptivo. Além disso, demonstrou ser capaz de reduzir a migração de células inflamatórias.

Palavras-Chave: acupuntura, inflamação, dor

Apoio Financeiro: UFSC/CNPq

Modelo Fisiológico da Dor Referida de Origem Dentária

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Objetivos: Temos observado que a maioria dos pacientes que têm dor de origem dentária pulpar ou periapical reporta também dor referida na região de cabeça e pescoço. A intensidade da dor favorece o aparecimento da dor referida e interfere em sua distribuição topográfica. Um maior número de áreas de dor referida parece estar associado à presença de polpa viva (PV) em dentes algógenos. Notamos, também, um padrão de dispersão horizontal da dor referida nos casos de PV ou quando mais intensa. Nosso objetivo foi conceber um modelo fisiológico, baseado em mecanismos de integração neural (convergência, divergência, facilitações espacial e temporal), capaz de explicar características da dor referida mensuradas empiricamente.

Métodos: Analisamos uma amostra de 60 pacientes com manifestações dolorosas de origem endodôntica, por meio do preenchimento de uma ficha clínica com dados referentes às características da manifestação dolorosa, como: intensidade, áreas de referência de dor e diagnóstico provável.

Resultados: Pacientes reportando dor intensa apresentaram dor referida em maior número de áreas de dor ($r=0,60$; $P<0,01$) e de ramos trigeminais ($\chi^2=12,42$; $P<0,01$) e com um padrão de dispersão horizontal da dor referida ($\chi^2=4,86$; $P<0,05$). Nosso modelo fisiológico explica os dados obtidos por meio da convergência e facilitação temporal, onde o aumento da frequência de disparos das fibras aferentes contribui para o surgimento e dispersão da dor referida. O diagnóstico de PV apresentou um maior número de áreas de dor referida ($\chi^2=8,44$; $P<0,01$) e um padrão de dispersão horizontal da dor ($\chi^2=12,47$; $P<0,01$), que pode ser explicado pelo mecanismo de facilitação espacial, com a permanência de nociceptores e um ambiente neuroquímico nos tecidos vivos da polpa favorecendo a ampliação da dor.

Conclusões: A organização hodológica do núcleo espinal do trigêmeo (em “casca de cebola”) e mecanismos fisiológicos básicos de integração neural, como a facilitação espacial e temporal, podem explicar de maneira satisfatória várias características da dor referida de origem dentária.

Palavras-Chave: Neurofisiologia, dor, dor dentária, percepção de dor, psicofísica da dor,

Apoio Financeiro: CNPq

MOBILIZAÇÃO NEURODINÂMICA ACELERA RECUPERAÇÃO FUNCIONAL E REDUZ ALODÍNIA MECÂNICA APÓS ESMAGAMENTO DO NERVO CIÁTICO EM RATOS

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Objetivo. Avaliar os efeitos da Mobilização Neurodinâmica (MN) sobre a recuperação da função motora, hipernocicepção e regeneração do nervo ciático após lesão experimental em ratos. **Métodos:** 28 ratos Wistar machos adultos foram utilizados no estudo, os quais foram divididos em 3 grupos: (1) falso-operado (n = 9), (2) controle operado (tendo o nervo exposto e esmagado por uma pinça hemostática) (n = 10) e (3) operado e submetido a MN (n = 9). O tratamento iniciou no décimo dia após o esmagamento, que consistiu da MN do nervo ciático por 32 dias. A recuperação funcional motora foi monitorada semanalmente usando o Índice Funcional do Ciático (IFC) e o Ângulo do Tornozelo no Apoio Intermediário (ATAI). Avaliações histológicas foram realizadas no final do tratamento para avaliar a regeneração nervosa. Para avaliar a hipernocicepção mecânica utilizou-se o teste do filamento de Von Frey. **Resultados:** Na avaliação da recuperação motora, o grupo controle apresentou o IFC±EPM (-16.25±3.25), enquanto o grupo tratado com MN reduziu 69% (-5.64±1.59) o seu valor (p<0,01). Além disso, na avaliação do ATAI, observou-se no 14º dia nos grupos falso-operado, controle e MN os seguintes valores (53.90±5.17, 51.97±3.38, 35.77±3.58), respectivamente. Desta forma o tratamento com MN apresentou um aumento do ATAI em 90% (p<0,01) em relação ao grupo controle. Na avaliação histológica observamos no grupo controle que o segmento nervoso correspondente a lesão sofreu diminuição das fibras de grande diâmetro, afetando algumas células de Schwann. No grupo tratado com MN observou-se nas fibras nervosas aspectos morfológicos tipicamente normal, com presença de fibras mielinizadas de grande e pequeno diâmetro e bainha de mielina de espessura proporcional ao diâmetro axonal. Na avaliação da alodínia mecânica observamos uma diminuição nas médias referentes à porcentagem de resposta de retirada no 20º(0.22±0.14), 37º(1.55±0.44), 42º(1.50±0.42) dia em relação ao controle (1.60±0.47, 3.90±0.45 e 3.70±0.42), nos respectivos dias. **Conclusão:** Esses resultados sugerem que o tratamento com a MN iniciado no 10º após a lesão do nervo ciático favorece o processo de regeneração, sendo capaz de acelerar a recuperação funcional do nervo ciático em ratos.

Palavras-Chave: nervo ciático, mobilização neurodinâmica, alodínia, regeneração de nervo

Apoio Financeiro: UFSC, CAPES, CNPq

OS EFEITOS DA MOBILIZAÇÃO ARTICULAR SOBRE A REGENERAÇÃO DO NERVO CIÁTICO DE RATOS SUBMETIDOS A LESÃO TRAUMÁTICA EXPERIMENTAL

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Objetivo. Avaliar os efeitos da Mobilização Articular (MA) sobre a recuperação da função motora, hipernocicepção e regeneração do nervo ciático após lesão experimental em ratos. **Métodos:** 28 ratos Wistar machos adultos foram utilizados no estudo, os quais foram divididos em 3 grupos: (1) falso-operado (n=9), (2) controle operado (tendo o nervo exposto e esmagado por uma pinça hemostática) (n=10) e (3) operado e submetido a MA (n=9). O tratamento teve início no décimo dia após o esmagamento, que consistiu da MA do tornozelo no membro inferior direito por 32 dias. A recuperação funcional motora foi monitorada semanalmente usando o Índice Estático do nervo Ciático (IEC) e o Ângulo do Artelho Externo (AAE). Avaliações histológicas foram realizadas no final do tratamento para avaliar a regeneração nervosa periférica. Para avaliar a alodínia mecânica e térmica utilizou-se o teste do filamento de Von Frey e o teste da acetona, respectivamente. **Resultados:** Na avaliação da recuperação motora, o grupo controle apresentou o $IEC \pm EPM$ (-35.85 ± 5.96), enquanto o grupo tratado reduziu em 60% (-14.20 ± 5.63) o seu valor ($p < 0,01$). Além disso, na avaliação do AAE, observou-se no 14º, 21º e 28º dia no grupo falso-operado os seguintes valores (3.19 ± 1.70 , 4.59 ± 3.11 , 2.80 ± 1.53), enquanto o grupo controle apresentou (14.03 ± 2.71 , 16.40 ± 1.82 e 16.35 ± 2.51) nos respectivos dias. A MA reduziu os valores no 14º em 45%, (9.28 ± 1.97 ; $p < 0,05$), no 21º em 46% (10.84 ± 1.22 ; $p < 0,01$) e no 28º 29%; (12.33 ± 1.30 $p < 0,05$). Na avaliação da alodínia mecânica observou-se diminuição nas médias referentes à porcentagem de resposta de retirada no 14º (0.66 ± 0.33), 20º (0.33 ± 0.23), 37º (1.44 ± 0.37) e 42º (1.77 ± 0.40) dia em relação ao controle (2.00 ± 0.49 , 1.60 ± 0.47 , 3.90 ± 0.45 e 3.70 ± 0.42), nos respectivos dias. Entretanto na avaliação da hipernocicepção ao frio observou-se uma diminuição significativa ($p < 0,05$) das médias somente no 32º (3.44 ± 0.85) e no 38º (2.44 ± 0.58) dia comparando com as médias do controle 32º (6.70 ± 0.70) e 38º (6.20 ± 1.02) dia. **Conclusão:** Esses dados sugerem que o tratamento com a MA iniciado no 10º após a lesão do nervo ciático favorece o processo de regeneração nervosa periférica, sendo capaz de acelerar o processo recuperação funcional do nervo ciático em ratos.

Palavras-Chave: nervo ciático, mobilização articular, lesão traumática, regeneração de nervo

Chronic intrathecal administration of glial inhibitors induces antinociception in monoarthritic rats but prevents the antinociceptive effect of acute D-serine.

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Objectives: During chronic pain, there is a glial reaction characterized by hypertrophy, proliferation, and up-regulation of molecules such as cytokines, excitatory aminoacids and neuromodulators such as D-serine. The contribution of glia-neuron interactions in the spinal mechanisms underlying hyperalgesia/allodynia in arthritis remains to be clarified. We studied the functional contribution of glial cells in mechanical nociceptive transmission in the spinal cord of monoarthritic rats by using chronic intrathecal administration of the glial inhibitor propentofylline or minocycline and then submitting the rats to Randall-Selitto testing. The effect of the glial mediator D-serine on rat vocalization threshold was also studied.

Methods: Monoarthritis was induced by intra-articular injection of complete Freund's adjuvant into the tibio-tarsal joint. Four weeks after, rats were treated with the glial cell inhibitor propentofylline (100 µg/10 µL i.t.) or minocycline (150 µg/10 µL i.t.) for ten days. On day eleven, rats were injected with the glial mediator D-serine (100 µg i.t.) and then submitted to the Randall-Selitto test.

Results: Data showed that propentofylline produced a significant increase ($12.6 \pm 2.4\%$) on the vocalization threshold to nociceptive mechanical stimulation. Minocycline produced an even greater antinociceptive effect ($45.0 \pm 3.8\%$). D-serine administered to glial inhibitor-nontreated animals induced a significant antinociceptive effect ($38.3 \pm 7.0\%$). This effect of D-serine was not observed in propentofylline- and minocycline-treated rats.

Conclusions: (i) Glial inhibitors are able to suppress mechanical hyperalgesia on a monoarthritic rat model. (ii) D-serine produces antinociception in monoarthritic rats. (iii) The effect of D-serine requires glial intactness, since it was prevented by glial inhibitors. (iv) The present preclinical study suggests glial inhibitors may be useful for treating arthritic/inflammatory pain.

Palavras-Chave: Arthritic pain, Glial inhibition, Antinociception, D-serine

Apoio Financeiro: Grant from FONDECYT 1070115

EFEITO ANTI-NOCICEPTIVO DE UMA FRAÇÃO DA *MIRABILIS JALAPA* NO MODELO DE ARTRITE INDUZIDA POR ADJUVANTE EM CAMUNDONGOS

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Objetivo: *Mirabilis jalapa* (Nyctaginaceae) é uma planta nativa da América Tropical, conhecida popularmente como maravilha ou bonina. As folhas desta planta são utilizadas na medicina popular para o tratamento de dor e inflamação. O objetivo deste trabalho foi de caracterizar o possível efeito anti-nociceptivo e anti-edematogênico da fração acetato de etila (Eta) das folhas de *M. jalapa* no modelo de artrite induzida pela injeção intraplantar do Adjuvante Completo Freund (CFA). **Materiais e Métodos:** Camundongos Swiss machos (30-40 g, N= 9-10) foram usados em todos os experimentos. Os animais foram anestesiados com halotano e posteriormente foi injetado na região dorsal da pata traseira direita 20 µl de CFA. Após 72 h, verificou-se o desenvolvimento de hipersensibilidade dolorosa (alodínia mecânica, avaliada pelo limiar de retirada com filamentos de von Frey 0,02-3,7 g) e edema de pata (caracterizado como um aumento na espessura da pata medida com um paquímetro). O limiar dos animais e o edema de pata foram verificados antes, após a injeção de CFA e também após a administração do extrato (10 mg / kg, v.o.) ou veículo. Novas medidas foram realizadas de 0,5 a 24 horas para avaliação do efeito anti-alodínico ou anti-edematogênico agudos. Após a verificação dos efeitos agudos do extrato, o tratamento foi repetido diariamente durante sete dias para a avaliação dos efeitos subcrônicos. **Resultados:** O tratamento com a Eta de *M. jalapa* produziu ação anti-alodínica até 4h após sua administração, com efeito máximo observado 1h após a administração (inibição de 65±12 %). A Eta produziu efeitos até 6 dias após o tratamento repetido. Entretanto, *M. jalapa* não apresentou atividade anti-edematogênica no tratamento agudo ou repetido. Conclusões: O Eta de *M. jalapa* produz efeito anti-alodínico, mas não reduz o edema, em modelo de dor aguda e subcrônica inflamatória causada por CFA em camundongos. Assim, o presente estudo esta de acordo com a utilização desta planta na medicina popular tradicional como analgésico.

Palavras-Chave: *Mirabilis jalapa*, anti-nociceptivo, anti-edematogênico

Apoio Financeiro: Capes, Cnpq

D.04.031

AValiação DO EFEITO ANALGÉSICO DO NOVO PIRAZOL M9P02 EM UM MODELO DE DOR CRÔNICA, EM CAMUNDONGOS.

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Objetivos: O objetivo deste estudo é avaliar o efeito antinociceptivo do derivado pirazol (M9P02) após administração aguda e subcrônica, no modelo de artrite induzida por adjuvante completo de Freund (CFA). **Métodos:** Camundongos machos e fêmeas Swiss (25-35 g, n=7-8) foram usados em todos os experimentos. Para produzir a inflamação crônica os animais eram anestesiados, e após foram injetados 20 µl de CFA, na região dorsal da pata direita. Foram avaliados a nocicepção (pela alodínia através da frequência de retirada da pata induzida por filamento de von Frey 0,05 g) e o edema de pata (aumento da espessura da pata com o auxílio de um paquímetro). No tratamento agudo, o composto M9P02 foi injetado por via oral (0,6-20 µmol/kg, v.o.). 48 após o CFA, a alodínia e o edema foram observados em vários intervalos de tempo após administração do composto (0,5-24 h). No tratamento subcrônico, o M9P02 foi injetado durante seis dias (20 µmol/kg, v.o.) e foram avaliados os efeitos de curta duração (2 h após tratamento) e de longa duração (após 24 h). **Resultados:** O composto administrado agudamente (0,6-20 µmol/kg) apresentou efeito antinociceptivo, com melhor eficácia 2 h após administração da dose de 20 µmol/kg (83±8%). No tratamento subcrônico com M9P02 (20 µmol/kg, v.o.), o efeito antinociceptivo de curta duração foi observado do primeiro ao quinto dia (com melhor eficácia no segundo dia, 77±11%), mas não sexto e sétimo dia. No efeito de longa duração foi observado que o composto produziu efeito antinociceptivo somente no terceiro e quarto dia (inibição de 37±8%). Em todos os tempos testados o composto não foi capaz de reduzir o edema de pata provocado por CFA tanto no tratamento agudo ou subcrônico. **Conclusões:** Verificamos que o M9P02 foi capaz de diminuir a nocicepção, mas não o edema causado por CFA. A redução do efeito antinociceptivo do composto durante o tratamento repetido sugere que o M9P02 causa tolerância e concorda com dados prévios demonstrando a participação do sistema opióide nas ações farmacológicas deste composto.

Palavras-Chave: Pirazol, antinocicepção, tolerância, opióide

Apoio Financeiro: Capes, CNPq

D.04.032

Spinal effect of a new calcium channel blocker Tx3.3 on inflammatory and neuropathic pain model in mice

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Voltage-dependent calcium channels (VDCC) have an important role in pain sensitization produced by inflammatory and neuropathic states. A vast number of toxins from venoms of spiders and cone snails that presents affinity for VDCC have represented a natural resource of peptides with pain-relieving potential effect. The aim of the present study was evaluate the intrathecal effect of a novel VDCC blocker Tx3.3, a peptide toxin extracted from the Brazilian armed spider *Phoneutria nigriventer*, on inflammatory and neuropathic pain models in mice. Male mice were administered with Tx3.3 (30 pmol/site) by intrathecal (i.t.) route. To evaluate inflammatory nociception, mice were submitted to formalin test or inflammatory pain model induced by complete Freund's adjuvant (CFA). To diabetic neuropathy induction, mice were administered with 200 mg/kg of streptozotocin (STZ) by intraperitoneal route. The mechanical sensitivity was measured through application of Von Frey filaments on mice hind paw plantar surface several times after i.t. injection of Tx3.3. Five minutes pre-administration of Tx3.3 affected neither the first nor the second phase of formalin test. However, when Tx3.3 was administered ten minutes after formalin it inhibited by $47.5 \pm 15.6\%$ the second phase. Spinal administration of Tx3.3 was not able to change mice natural mechanical sensitivity or prevent the allodynic state induced by CFA paw injection. Tx3.3 inhibited neuropathy-induced tactile allodynia. This effect was evident 30 minutes after Tx3.3 i.t. delivery ($19.7 \pm 8.8\%$ of inhibition), had a peak effect at 60 minutes ($69.1 \pm 15.5\%$ of inhibition) and lasting for about 120 minutes, ending after 240 minutes of its administration. These results showed that Tx3.3 alleviated the allodynia resulted from neuropathic state, without, however, changed natural mechanical sensitivity in mice. On the other hand, although effective in reduce the nociception behavior at formalin second phase, administration of Tx3.3 was not able to prevent adjuvant-induced inflammatory pain.

Palavras-Chave: Phoneutria toxin, nociception, neuropathy, inflammation

Apoio Financeiro: Instituto do Milenio MCT/CNPq, Capes, Pronex, Fapemig

D.04.033

Central effects of peptide toxin calcium channel blockers on acute nociception in mice

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Voltage-dependent calcium channel (VDCC) have key role in pain processing at central nervous system. Peptide toxins isolated from mollusk and arthropod venoms target calcium channels and could, therefore, represent a potential analgesic resource. In this study, we investigated the central effect of a novel spider peptide toxin calcium channel blocker, a *Phoneutria nigriventer* toxin, Tx3.3, and compare with those elicited by the *Conus magus* toxin, MVIIC, on acute nociception in mice. Mice were administered with different doses of toxins by intrathecal (it) or intracerebroventricular (icv) route. Thermal acute nociception and abnormal behavior that predict central side-effects was carried out through tail-flick test and motor behavior assessment, respectively. It and icv administration of Tx3.3 (1-100 pmol/site) produced antinociception with peak response occurring 5 minutes post-dosing. The ED₅₀ and E_{max} for Tx3.3 it administration was 8.8(4.1–18.8) pmol/site and 51.1±13.8%, respectively. The ED₅₀ and E_{max} for Tx3.3 icv administration was 3.7(1.6–8.4) pmol/site and 83.9±16.3%, respectively. Tx3.3 (1–300 pmol/site) did not change mice motor performance when compared to control group in rotarod test, neither promote other abnormal behaviors. The icv injection of MVIIC (0.3–3.0 pmol/site) also resulted in antinociception with a calculated DE₅₀ and E_{max} of 0.6 (0.1–2.8) pmol/site and 62.0±13.2, respectively. However, higher doses of MVIIC (10–100 pmol/site) impaired motor performance on rotarod test (7.3±3.0 and 71.8 ±13.8 s for first fall latency to 100 pmol/site of MVIIC and PBS, respectively; p<0.001). When injected by it route, 300 pmol/site of MVIIC produced a high increase in tail-flick latency (203.8±31.0 and 2.4±3.6%, for MVIIC and PBS, respectively; p<0.001), but elicited toxic effects visualized by a delayed on righting reflex (2.78±0.67 and 0.12±0.03 s to twisting the body for MVIIC and PBS, respectively). The minor subsequently dose (100 pmol/site) not changed tail flick latency. These results showed that Tx3.3 had antinociceptive effect with a better pharmacological window when compared to MVIIC.

Palavras-Chave: Phoneutria toxin, nociception, MVIIC, Tx3.3

Apoio Financeiro: Instituto do Milênio MCT/CNPq, Capes, Pronex, Fapemig

Antinociceptive properties of the xanthine oxidase inhibitor allopurinol in mice.

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Objectives: Allopurinol (ALLO) is a potent inhibitor of the enzyme xanthine oxidase used primarily in the treatment of hyperuricemia and gout. It is well known that purines exert multiple effects on pain transmission. We hypothesized that the inhibition of xanthine oxidase by ALLO, reducing purine degradation, could be a valid strategy to enhance purinergic activity. The aim of this study was to investigate the antinociceptive profile of ALLO on chemical and thermal pain models in mice. **Methods:** Mice received an i.p. injection of vehicle (Tween 10%) or ALLO (10 to 400 mg/kg) and were submitted to intraplantar (i.pl.) capsaicin, i.pl. glutamate, tail-flick and hot-plate pain models, hole-board, rotarod tests and high-performance liquid chromatography was performed with aliquots obtained from the cerebrospinal fluid (CSF). To investigate the mechanism of action of ALLO, some animals were also pre-treated with an i.p. injection of the non-selective (A₁ and A_{2a}) adenosine-receptor antagonist caffeine (30 mg/kg), the selective A₁ adenosine-receptor antagonist DPCPX (1 mg/kg), the selective A_{2a} adenosine-receptor antagonist SCH58261 (1 mg/kg), or the non-selective opioid-receptor antagonist naloxone (1 mg/kg). Adenosine (100 mg/kg) and morphine sulphate (6 mg/kg) were used as positive controls for those experiments. **Results:** ALLO presented dose-dependent antinociceptive effects against all pain models ($P < 0.05$ compared to vehicle). The opioid antagonist naloxone did not affect ALLO antinociceptive effects ($P < 0.05$ compared to vehicle). Caffeine and DPCPX prevented ALLO-induced antinociception. No obvious motor deficits were produced by ALLO at doses up to 200 mg/kg. ALLO caused an increase in CSF levels of several purine metabolites, including the nucleosides adenosine and guanosine, and decreased CSF concentration of uric acid ($P < 0.05$ compared to vehicle). **Conclusions:** ALLO-induced antinociception may be related to an adenosine, and perhaps guanosine accumulation. **Palavras-Chave:** Allopurinol, Purines, Pain

INVOLVEMENT OF BOTH KININ B₂ AND B₁ RECEPTORS ON SCRATCHING BEHAVIOUR INDUCED BY ACTIVATORS OF PROTEINASE-ACTIVATED RECEPTOR-2 (PAR-2) IN MICE

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Objectives: Pruritus (or itching), like all other skin sensation, is a product of peripheral- and CNS-neurons activities. It has been proposed that PAR-2 activation on neuronal cells can contribute to itching in both humans and animals, in a process involving neurogenic inflammation components. Herein, we sought to investigate the involvement of both kinin B₂ and B₁ receptors on the scratching behaviour induced by PAR-2 activators in mice. **Methods:** Male adult Swiss mice (35 g, n = 6-8) received a dorsal intradermal injection of saline (50 µl) containing trypsin (200 µg/site) or the selective PAR-2 activating peptide SLIGRL-NH₂ (100 µg/site). After treatment, animals were observed for 40 minutes and their scratching behavior was evaluated. Results are expressed as the number of scratches bouts in 40 minutes. **Results:** The intradermal injection of trypsin (200 µg/site) or SLIGRL-NH₂ (100 µg/site) elicited a marked scratching behaviour in mice (p<0,01). Trypsin or SLIGRL-NH₂ responses were greatly reduced by treatment with the selective peptide PAR-2 receptor antagonist FSLLRY-NH₂ (100 µg/site; 91 ± 7 % [p<0,05] and 83 ± 10 % [p<0,05], respectively). Furthermore, trypsin-induced pruritus was prevented by the selective peptide or non-peptide kinin B₂ receptor antagonists HOE 140 (50 nmol/kg, s.c.; 67 ± 13 % [p<0,05]) and FR173657 (30 mg/kg, i.p.; 79 ± 16 % [p<0,05]), respectively. In a similar manner, the treatment with the selective peptide or non-peptide kinin B₁ receptor antagonists, correspondingly, des-Arg⁹-[Leu⁸]-BK (DALBK; 150 nmol/kg, i.p.) and SSR240612 (1 mg/kg, i.p.) significantly inhibited trypsin-induced scratching (57 ± 22 % [p<0,05] and 75 ± 13 % [p<0,05], respectively). In addition, the non-peptide kinin B₂ and B₁ receptor antagonists FR173657 (30 mg/kg, i.p.) and SSR240612 (1 mg/kg, i.p.), respectively, potently diminished SLIGRL-NH₂-induced scratching behaviour in mice (50 ± 23 % [p<0,05] and 71 ± 14 % [p<0,05], respectively). **Conclusions:** These findings provide evidence that kinins acting at both B₂ and B₁ receptors exert a critical role in controlling the pruritogenic responses elicited by PAR-2 activators in mice. Therefore, we might speculate that selective kinin antagonists against both receptors can become potential drugs to treat some pruriceptive states mediated by PAR-2 activation. However, additional experimental studies are necessary to better clarify the mechanisms involved in this process. **Palavras-Chave:** Pruritus, PAR-2, Scratching behaviour

Apoio Financeiro: CAPES/CNPq/ FAPESC/FINEP/PRONEX

EFEITO DA DOR INCISIONAL EM RATOS JUVENIS

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Objetivos: Evidências sugerem que a exposição à dor aguda no primeiro ano de vida pode provocar alterações na resposta nociceptiva. Existem poucos estudos em modelo de dor pós-operatória em ratos juvenis. Os objetivos foram avaliar os efeitos da exposição à cirurgia em ratos no P21, e de uma nova cirurgia no P45, sobre a latência para retirada da cauda (TFL) e a locomoção em campo aberto. **Métodos:** ratos Wistar divididos em três grupos: CONTROLE (n=20); ANESTESIA (n=20) e CIRURGIA (n=20). A cirurgia consistiu de incisão plantar de 0,5cm no membro posterior após anestesia com halotano 2%. As medidas de TFL foram feitas antes e 30 min após a cirurgia. Após, avaliou-se os números de cruzamentos em campo aberto por 5 min. Para a segunda cirurgia os grupos CONTROLE e CIRURGIA foram divididos em CON21, CON21/CIR45, CIR21 e CIR21/45. A cirurgia, as medidas de TFL e a exposição ao campo aberto foram realizadas conforme descrito acima. Foram utilizadas ANOVA de medidas repetidas para o TFL e ANOVA de uma via para o campo aberto. Os dados foram expressos como média±EPM, considerando diferença significativa $P<0,05$. **Resultados:** 30 min após a cirurgia o grupo CIRURGIA (7.8 ± 0.71) apresentou aumento de TFL em relação aos grupos CONTROLE (4.1 ± 0.44) e ANESTESIA (4.7 ± 0.39). O grupo CIRURGIA (43 ± 4.15) realizou menos cruzamentos que os outros grupos (CONTROLE: 69.0 ± 6.47 ; ANESTESIA: 60.5 ± 6.47). Na segunda cirurgia houve aumento de TFL após 30 min no grupo CON21/CIR45 (9.9 ± 0.051) em relação aos outros grupos (CON21: 7.0 ± 1.37 ; CIR21: 5.8 ± 0.84 ; CIR21/45: 4.6 ± 0.43). Os grupos CON21/CIR45 (40.2 ± 7.55) e CIR21/45 (51.7 ± 6.72) realizaram menos cruzamentos que os grupos CON21 (90.5 ± 18.04) e CIR21 (74.6 ± 10.31). **Conclusões:** Ratos no P21, submetidos à cirurgia, apresentam aumento de TFL, confirmando resultados anteriores, e diminuição na atividade locomotora, que pode ser correlacionada com hiperalgesia mecânica. Na segunda exposição, no P45, os animais não apresentam aumento de TFL, o que pode ser um efeito de longa duração da primeira exposição. Houve diminuição na atividade locomotora, porém não foi possível verificar se existe diferença entre a primeira e a segunda exposição à cirurgia. **Palavras-Chave:** campo aberto; cirurgia; locomoção; tail flick; young rats

Apoio Financeiro: CNPq e PROPESQ/UFRGS

ANÁLISIS DE PATRONES TEMPORALES RELACIONADOS CON EL CÓDIGO DE TEXTURAS EN EL SISTEMA DE LAS VIBRISAS

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Objetivos: Estudios conductuales en discriminación táctil coinciden en que las ratas pueden distinguir superficies de diferente rugosidad moviendo activamente sus vibrisas. Se ha demostrado, recientemente, que existiría un código de texturas en los aferentes periféricos de las vibrisas (1) y que la tasa de disparos sería el mecanismo de codificación que subyace a la discriminación de texturas en la corteza somatosensorial primaria (2). En este trabajo proponemos un método de análisis para estudiar el código neural relacionado con la textura de las superficies en registros de los aferentes primarios de las vibrisas.

Métodos: Se utilizaron ratas Wistar adultas, en preparaciones agudas, a las que se disecó el nervio vibrisal profundo de la vibrisa delta y las ramas motoras del facial. El barrido de las vibrisas se indujo por medio de la estimulación con pulsos rectangulares (5Hz) de las ramas motoras. Se tomaron registros multifibra de la actividad aferente del nervio vibrisal mientras la vibrisa delta rozaba diferentes superficies: 4 lijas (Li) de diferente granulometría (#1000, #600, #400 y #220). Las situaciones de control consistieron en registros de la actividad aferente durante barridos de las vibrisas al aire. Estos registros se intercalaron con los registros experimentales. Se realizó un análisis tiempo-frecuencia para observar la variación del contenido en frecuencia de las señales a lo largo del tiempo. Los Tiempos Entre Eventos Detectados (TEED) en el registro multifibra fueron obtenidos mediante un algoritmo de detección basado en la Transformada Wavelet Continua (TWC). Por otra parte, se obtuvieron parámetros de rugosidad de las superficies (Ra y Rz) y se calcularon las Distancias Entre Picos Locales (DEPL). Este último procedimiento se realizó con el fin de buscar la relación entre los TEED y las diferentes características físicas de las superficies.

Resultados: El método propuesto para el análisis tiempo-frecuencia mostró diferencias significativas ($p < 0.01$, Prueba de Friedman) en diferentes etapas del barrido (tanto en la protracción como en la retracción). Estas diferencias estarían relacionadas con patrones de descarga (eventos) provocados por el barrido de las vibrisas sobre las diferentes superficies. Los TEED obtenidos para cada situación fueron: Li#1000: 2.49 ± 1.42 ; Li#600: 6.06 ± 1.02 ; Li#400: 8.17 ± 1.23 ; Li#220: 9.05 ± 0.63 (eventos de 2.0 ms duración). Los TEED mostraron un comportamiento incremental directamente relacionado con el aumento de los parámetros Ra, Rz y DEPL. Mediante un ajuste sigmoide se determinó que el DEPL sería parámetro físico mejor descrito por los TEED (coeficiente de determinación, $R = 0.83$)

Conclusiones: Los análisis tiempo-frecuencia y los TEED sugieren que existiría una estrecha relación entre las características físicas de las superficies y los patrones temporales de descarga en los aferentes vibrisales.

Referencias: 1- BMC Neurosci 7:42, 2006; 2- PLoS Biol 5:e305, 2007

Palabras-Chave: vibrisas, discriminación, patrones temporales

Apoio Financeiro: Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT), Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT)

EFFECTO DE LA LONGITUD DE LAS VIBRISAS EN LA DISCRIMINACION TACTIL Y EN EL PROCESO SENSORIAL ACTIVO

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Objetivos: Durante la conducta exploratoria, las ratas realizan barridos rítmicos y coordinados con sus vibrisas y, de este modo, adquieren información sobre la distancia, forma y textura de los objetos (1). Cada vibrisa esta asentada en una compleja estructura denominada Complejo Seno-Folículo (CS-F), lugar en donde los mecanorreceptores transducen estímulos mecánicos aplicados a las vibrisas en una señal eléctrica. En este trabajo se presentan estudios conducentes a determinar el efecto de la longitud del bigote en la información táctil que viaja en las fibras aferentes primarias de una vibrisa durante el tacto activo.

Métodos: Los estudios se llevaron a cabo en ratas Wistar bajo anestesia general. Se estimularon las ramas motoras del facial para inducir el barrido rítmico de las vibrisas y, simultáneamente, se tomaron registros multifibra del nervio aferente de la vibrisa DELTA. La vibrisa DELTA fue seccionada en forma progresiva (en tramos de a 1cm) hasta seccionarla totalmente. Se obtuvieron registros sucesivos para las diferentes longitudes y para dos condiciones experimentales: barridos de la vibrisa al aire y barridos sobre una superficie rugosa (lija # 1000). Las amplitudes de las descargas aferentes se cuantificaron empleando el valor RMS. Se realizó el análisis del contenido en frecuencia de las señales utilizando técnicas paramétricas (Método auto regresivo de Burg). Se utilizó el análisis estadístico de Friedman y el método de Dunn (post hoc) para realizar las comparaciones.

Resultados: El análisis con los valores RMS mostró una importante variación en la amplitud de las descargas tanto para los barridos al aire como para los barridos sobre la lija. En el primer caso, se observó una importante caída de los valores a partir de los 2cm de sección (de 26.57 ± 1.63 mV a 23.39 ± 1.05 mV). Para los barridos sobre la lija #1000, los valores RMS mostraron variaciones importantes durante la protracción (vibrisa sin seccionar: 29.71 ± 1.92 mV; sección de 1 cm: 43.29 ± 3.67 ; sección de 2cm: 35.74 ± 1.70 mV; sección de 3 cm: 45.94 ± 3.14 mV). El análisis estadístico mostró cambios significativos en las señales aferentes a partir de la sección de 2cm de la vibrisa ($F = 81.81$, $P < 0.01$). El análisis del contenido espectral de las señales mostró un comportamiento no relacionado con la sección de la vibrisa durante los barridos al aire. Sin embargo, para barridos sobre la superficie rugosa, el contenido en frecuencia de máxima energía (f_{max}) disminuyó con la reducción en longitud de la vibrisa. Mas aún, se observó que a partir de la sección de 2cm, las f_{max} son significativamente diferentes ($P < 0.01$).

Conclusiones: La longitud del pelo podría representar un parámetro de considerable importancia en la activación de los mecanorreceptores del CS-F, tanto durante la conducta exploratoria como durante la discriminación de superficies.

Referencias: 1- PLoS Biol 5:e15, 2007

Palabras-Chave: vibrisas, tacto activo, mecanorreceptores

Apoio Financeiro: Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT), Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT)

D.05.001

Morfometria de terminais axonais intrínsecos e da via perforante do córtex entorrinal medial do camundongo suíço albino adulto

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O córtex entorrinal é uma estrutura cerebral que contribui de forma importante para o aprendizado e memória. As aferências mais densas do hipocampo provêm do córtex entorrinal e a propagação da informação entorrinal aferente no interior do hipocampo se dá através de uma sequência de sinapses excitatórias que de uma forma simplificada é referida como circuito trisináptico. O presente estudo teve como objetivo identificar e reconstruir os terminais axonais (fragmentos de axônios) de conexões intrínsecas e extrínsecas (via perforante) do córtex entorrinal medial de 4 camundongos suíços albinos adultos utilizando como neurotraçador anterógrado a amino dextrana biotinilada (ADB). A análise quantitativa tri-dimensional dos terminais axonais intrínsecos e extrínsecos do córtex entorrinal medial foi realizada com auxílio de microscopia automática tridimensional e Programa especializado (Neurolucida 5.0.1 Microbrightfield). O conjunto de parâmetros morfométricos de 55 terminais intrínsecos e 39 extrínsecos do córtex entorrinal medial foi submetido à análise de grupos e a análise discriminante permitindo a distinção de apenas dois tipos básicos de terminais denominados I e II, sendo a densidade de botões de passagem a variável decisiva na análise discriminante dos terminais. Para os terminais intrínsecos, o grupo II apresentou maior densidade de botões de passagem (média = 90,36 botões/mm; erro padrão = 6,79) do que o grupo I (12,26 botões/mm; erro padrão = 1,88), ANOVA, Tukey p = 0,0001. Nos terminais da via perforante, o grupo II também apresentou maior densidade de botões de passagem (média = 150,07 botões/mm; erro padrão = 12,60) do que o grupo I (17,19 botões/mm; erro padrão = 3,45), ANOVA, Tukey p = 0,0001. Para os terminais do córtex entorrinal medial, o grupo II apresentou maior densidade de botões de passagem do que o grupo I. Os achados morfométricos sugerem que co-existem nas projeções intrínsecas e na via perforante do córtex entorrinal estereotipia e diversidade, antecipando a possibilidade de que as redes neurais dessas regiões possam ser construídas a partir de unidades simples que se replicam. **Palavras-Chave:** córtex entorrinal, amino dextrana biotinilada, terminais axonais

Apoio Financeiro: Cnpq, Capes, Finep e CWPD financiado por IBNnet

Morfometria de terminais axonais intrínsecos da camada granular do giro denteado do camundongo suíço albino adulto

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A formação hipocampal é uma peça chave nos processos de consolidação de memória e aprendizagem. Permanece, entretanto por ser estudada a anatomia quantitativa tridimensional das conexões intrínsecas e extrínsecas que dá suporte estrutural a esse processo de consolidação. Uma vez que o giro denteado (GD) é estação sináptica obrigatória da maioria das aferências provenientes do córtex entorrinal para o hipocampo, o presente estudo objetivou identificar e reconstruir terminais axonais (fragmentos de axônio) intrínsecos da camada granular, provendo a primeira descrição quantitativa em modelo murino de elementos dos circuitos locais do GD. Foram utilizados 4 camundongos suíços albinos adultos, injetados iontoforéticamente, com o neurotraçador anterógrado amino dextrana biotinilada (ADB) na camada granular e molecular do giro denteado. A análise quantitativa tri-dimensional dos terminais axonais intrínsecos do giro denteado foi realizada com auxílio de microscopia automática tridimensional e Programa especializado (Neurolucida 5.0.1 Microbrightfield). Os conjuntos de parâmetros morfométricos de 30 terminais com ramificações múltiplas e de 53 com um único ramo, contidos integralmente na camada granular do giro denteado, foram submetidos à análise de grupos e discriminante, tendo sido distinguidos apenas dois tipos básicos de terminais denominados I e II. O grupo I apresentou maior ângulo planar (média = 38,73; erro padrão = 4,60) do que o grupo II (média = 25,20; erro padrão = 2,91), ANOVA, Tukey, $p = 0,022$. Para os terminais múltiplos, grupo II apresentou maior densidade de botões de passagem (média = 62,46 botões/mm; erro padrão = 4,11) do que o grupo I (média = 17,13 botões/mm; erro padrão = 3,33), ANOVA, Tukey, $p = 0,0001$. Para os terminais únicos, apenas a variável densidade de botões de passagem foi discriminante, com o grupo II apresentando maior densidade de botões de passagem (média = 103,61 botões/mm; erro padrão = 3,66;) do que o grupo I (média = 39,39 botões/mm; erro padrão = 3,67), ANOVA, Tukey $p = 0,0001$. Os dados indicam a presença de estereotipia e diversidade nas terminações axonais intrínsecas da camada granular do giro denteado.

Palavras-Chave: Giro Denteado, amino dextrana biotinilada, terminais axonais

Apoio Financeiro: Cnpq, Capes, Finep, CWPD financiado por IBNnet

MULTISENSORY EVALUATION OF FERRET VISUAL CORTEX

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Recent studies suggest that multisensory processing is a phenomenon found across the mammalian cortex. In some cases, overtly bimodal (e.g., respond both to visual and to auditory stimuli presented alone) were reported, while others suggested that feedback modulation carried non-visual influences (e.g., cross-modal attention) into visual cortex. However, except for our recent report (Allman et al., 2008), the multisensory nature of ferret visual cortical neurons has not been tested. In that experiment, the responses of visual Area 21 (V4 homologue) to visual, auditory and combined visual-auditory stimulation were examined using standard, ketamine-anesthetized, extracellular single-unit recording techniques. No bimodal neurons were identified but ~9% revealed significant modulation of visual responses by an otherwise ineffective auditory stimulus. The present experiment used the identical methods (5 adult ferrets) to study the suprasylvian visual area (SSY, homologue of MT). A total of 281 neurons were quantitatively analyzed for their responses to separate and combined-modality stimulation. Again, no bimodal neurons were identified and a similar small proportion (8%) had visual responses significantly influenced (paired 't'-test) by auditory stimulation. These SSY subthreshold effects (3% facilitated, 5% suppressed) were similar in proportion and in sign as those previously identified in Area 21 (3% facilitated, 6% suppressed) whose effects at the population level appear to cancel-out each other. Given the overwhelming presence of unaffected visual responses, as well as the small proportions of facilitation/suppression that essentially cancel one another indicate that, for anesthetized ferrets, these regions appear to be fundamentally unisensory. It remains to be determined if cross-modal non-visual influences manifest themselves more robustly in these visual areas in awake, behaving preparations where top-down effects of attention and behavior may play a role.

Palavras-Chave: multisensory integration, cross-modal, visual cortex, ferret

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Estereotipia e Diversidade nas Projeções Ipsilaterais do Córtex Somatosensorial da Cutia (*Dasyprocta prymnolopha*): análise morfométrica de fragmentos axonais que se projetam de S1 para S2.

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Introdução Trabalhos realizados em humanos mostram que S2 é uma área de associação multimodal envolvida na integração de *aférentes* cutâneos, proprioceptivos e motores necessários à coordenação dos movimentos manuais durante a manipulação e reconhecimento de objetos (J.Neurophysiol, 97(2):1288, 2007). A complexidade morfológica da arborização axonal tem papel importante na transformação de sinais, mas parece se replicar nas diferentes escalas de análise com alto grau de estereotipia (Cereb. Córtex, 15:152, 2005). Estudos recentes demonstraram que os terminais axonais calosos eferentes homotópicos que se projetam do sítio de representação das patas anteriores e posteriores em S1 para o hemisfério contralateral da cutia, apresentam propriedades morfométricas que se replicam em dois grandes grupos. Os fragmentos classificados como do tipo II são mais arborizados e representam a maior proporção dos fragmentos axonais da pata anterior, enquanto os do tipo I constituem a maior proporção dos fragmentos eferentes do sítio de representação da pata posterior (J. Comp. Neurol, 500:255, 2007). Não há, entretanto estudos detalhados da representação homotópica ipsilateral nesse roedor que permitam verificar se os padrões de representação interhemisférica se replicam nas projeções entre áreas de hierarquia distintas no mesmo hemisfério. **Objetivos:** O objetivo deste trabalho é descrever a morfologia dos terminais axonais ipsilaterais que se projetam de S1 para S2, nas áreas de representação da pata anterior da cutia.. **Material e método** Foram utilizados 4 animais que foram anestesiados e injetados com dextrana biotinilada (BDA, 10 kD) em sítios de S1 identificados eletrofisiologicamente. Após sobrevida realizou-se a perfusão, craniotomia, microtomia e histologia das secções. Cada fragmento axonal foi reconstruído com objetiva a óleo de 60x em um microscópio (Zeiss Standard 25), acoplado a um computador e analisados pelo programa Neurolucida (MicroBrightField inc.). As medidas realizadas em 31 fragmentos incluíram: comprimento médio dos fragmentos axonais, área de superfície média, volume médio, densidade de nós, densidade de botões e densidade de segmentos. Para o cálculo das densidades, o número total de cada variável foi dividido pelo comprimento total do fragmento axonal. Os terminais axonais reconstruídos foram classificados aplicando-se análise de grupos (Statistic 6.0). **Resultados:** A morfometria dos terminais ipsilaterais da representação da pata anterior em S2 revelou dois grupos de terminais classificados como tipo I e tipo II de acordo com a descrição em Rocha et al. (2007) mas os valores percentuais de ocorrência foram diferentes do das projeções interhemisféricas. A análise discriminante demonstrou que a área de campo axonal foi a principal variável para a formação dos grupos. **Conclusão:** A análise qualitativa mostrou que os terminais axônicos ipsilaterais projetando de S1 para S2 na região de representação de pata anterior da cutia apresentam propriedades morfométricas distintas daqueles de projeção homotópica interhemisférica. As características morfométricas dos terminais axonais ipsilaterais observadas no presente trabalho confirmam achados anteriores da presença de grande estereotipia e pequena diversidade na morfologia de terminais axonais no córtex somatosensorial da cutia e corroboram descrições anteriores em outros mamíferos.

Palavras-Chave: somatosensorial, roedor, morfometria, dextrana-biotinilada

Apoio Financeiro: CNPq-Ufpa.Pronex

Plural autobiographical sensory stimulation and return to at present reality, as two of the keys for the rehabilitation of Alzheimer cases in the early stages.

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Objetives: In showing the role that in the process of rehabilitation in reconfirmed cases had the use of massive plural autobiographical sensory stimulation, and action to return to a their at present reality. Informed knowledge, family cooperation, the fact that the person was not suffering a deadly diagnosis disease, and was being properly treated, among others, were requisites of inclusion in patients younger than 77 years old. **Methods:** A video with family pictures previously chosen as appropriate by the most involved family members was used, esthetically combined with the patient's favorite songs, so as stimulation of the olfactory, gustatory and somatosensory channels with the appropriate stimuli. Previous to that, a Protocol is carried out, in which their life's story is compiled with the help of the family members, that allows us to identify the trigger of the process (commonly a painful loss with a strong difficulty to overcome, and which had also caused the loss of the patient's identity), and that had started the alteration in the normal neurophysiology caused by the fixation of the patient in the desire of dying, or causing by the transferring of the denied/rejected present reality into the so yearned past, which generally goes back to the childhood with her family members (1). The process includes overcoming the unsolved mourning up to finished it. **Results:** In 56 cases (75% women) with up to at least two years of being diagnosed and under pharmacological treatment, and after being continuously monitored, we could rehabilitate 46 cases (82%), of which 39 (85% of the total and 93% of all of them) were women, and the effectiveness was way smaller in men (7/14), even though 3 women and 2 men were taken away from the process by their family members before it ended and was produced 5 remissions. The process of rehabilitation has been completed in a period of 6 to 108 days. **Conclusions:** The procedure seems to be effective, and it demands approximately 23 hours of activities for each case in an individual way, with a team of not less than 3 trained professionals and the cooperation of family members. The effectiveness has been greater in women. 1. Rev Neurol 44(4), 198, 2007.

Palavras-Chave: Alzheimer, autobiographical stimulation, rehabilitation, recognition sensory components, trigger identification

Apoio Financeiro: Universidad Autónoma de Entre Ríos, BBVA Banco Francés, Fundación Alzheimer

Plural autobiographical sensory stimulation and return to at present reality, as two of the keys for the rehabilitation of Alzheimer cases in the early stages.

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Objetives: In showing the role that in the process of rehabilitation in reconfirmed cases had the use of massive plural autobiographical sensory stimulation, and action to return to a their at present reality. Informed knowledge, family cooperation, the fact that the person was not suffering a deadly diagnosis disease, and was being properly treated, among others, were requisites of inclusion in patients younger than 77 years old. **Methods:** A video with family pictures previously chosen as appropriate by the most involved family members was used, esthetically combined with the patient's favorite songs, so as stimulation of the olfactory, gustatory and somatosensory channels with the appropriate stimuli. Previous to that, a Protocol is carried out, in which their life's story is compiled with the help of the family members, that allows us to identify the trigger of the process (commonly a painful loss with a strong difficulty to overcome, and which had also caused the loss of the patient's identity), and that had started the alteration in the normal neurophysiology caused by the fixation of the patient in the desire of dying, or causing by the transferring of the denied/rejected present reality into the so yearned past, which generally goes back to the childhood with her family members (1). The process includes overcoming the unsolved mourning up to finished it. **Results:** In 56 cases (75% women) with up to at least two years of being diagnosed and under pharmacological treatment, and after being continuously monitored, we could rehabilitate 46 cases (82%), of which 39 (85% of the total and 93% of all of them) were women, and the effectiveness was way smaller in men (7/14), even though 3 women and 2 men were taken away from the process by their family members before it ended and was produced 5 remissions. The process of rehabilitation has been completed in a period of 6 to 108 days. **Conclusions:** The procedure seems to be effective, and it demands approximately 23 hours of activities for each case in an individual way, with a team of not less than 3 trained professionals and the cooperation of family members. The effectiveness has been greater in women. 1. Rev Neurol 44(4), 198, 2007.

Palavras-Chave: Alzheimer, autobiographical stimulation, rehabilitation, recognition sensory components, trigger identification

Apoio Financeiro: Universidad Autónoma de Entre Ríos, BBVA Banco Francés, Fundación Alzheimer

D.06.001

“Somatostatin effects upon fictive respiration in the isolated CNS of neonatal mice”

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Somatostatin is an inhibitory neurotransmitter found in nerve terminals at different levels of the respiratory ventral group and in somata of neurons belonging to the pre-Bötzinger complex, a nucleus fundamental for generating the respiratory rhythm. The aim of this work was to characterize the inhibitory responses induced by prolonged somatostatin superfusion upon fictive respiration in vitro.

CF1 mice at postnatal days 0 to 2, were anesthetized by cooling and ether inhalation. Their CNS were removed and immediately immersed in artificial cerebrospinal fluid (aCSF) containing (mM): NaCl, 125.0; KCl, 5.0; NaHCO₃, 24.0; KH₂PO₄×H₂O, 1.25; CaCl₂, 0.8; MgSO₄×7H₂O, 1.25 at 20 C. The medium was supplemented with D-glucose (Merck, Darmstadt, Germany) to a final concentration of 30 mM and equilibrated with O₂:CO₂ = 95%: 5%, (pH 7.37-7.40). The CNS was decerebrated through a pontomesencephalic transection, transferred to a recording chamber of 1 ml in volume, and superfused with aCSF at 25 C. Spontaneous spinal cord activity from C4-C6 ventral roots was recorded with suction microelectrodes connected to a differential amplifier, integrated, displayed, and analyzed with AD data acquisition system (Digipack 1320 A, Axon Instruments). Stimulation was done by changing the basal superfusion medium with other medium containing somatostatin 10⁻¹⁴ to 10⁻¹⁰ M.

Basal frequency of fictive respiration ranged between 3 to 12 inspiratory burst per minute. Somatostatin 10⁻¹³ M increased the duration of the cycles up to three fold the basal frequency. The increase in duration was not produced in a continuous and sequential way, but rather than in a jumping way, suggestive of quantal steps. Somatostatin 10⁻¹³ M also decreased the amplitude of the integrated inspiratory burst. Higher doses of somatostatin tend to induce a complete ceasing of fictive respiration.

Our results are suggestive that somatostatin actions are exerted at least upon two different oscillatory networks uncoupling them. **Palavras-Chave:** Somatostatin, pre-Bötzinger, Respiratory Rhythm, Brainstem, Respiration.

Apoio Financeiro: Fondecyt 1060110

D.06.002

Caracterización de las propiedades electrofisiológicas de las células piramidales de la corteza motora primaria de las vibrisas

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Nuestro grupo se propone caracterizar los circuitos involucrados en la generación de comandos motores en la corteza motora primaria de las vibrisas. En este trabajo se caracterizaron las propiedades electrofisiológicas de neuronas piramidales de las láminas II/III y V de la corteza motora primaria de vibrisas en ratas. La caracterización se llevó a cabo en dos situaciones: 1) a través de registro de la actividad poblacional y unitaria espontánea de las células piramidales; y 2) a través de registros de la actividad poblacional y unitaria de las células piramidales en respuesta a estímulos eléctricos de diferente intensidad en el parche de vibrisas contralateral. Para realizar estos registros, los animales experimentales se sometieron a cirugía esterotáxica. Se insertaron electrodos de registro en la corteza motora primaria de las vibrisas en los siguientes puntos (con respecto al punto Bregma): Antero-posterior: 1,5, 2,5 y 3,5 mm; Lateral: 1,5 mm (a la izquierda); Profundidad: 0,3-0,5 mm para las láminas II/III y 0,8-1,2 mm para la lámina V. Electrodos bipolares de estimulación se implantaron en el parche de vibrisas contralateral. Se caracterizaron diferentes tipos neuronales con base en la forma de su potenciales de acción, sus patrones de disparo (histograma de intervalos, histograma de autocorrelación), su correlación con la actividad poblacional y su respuesta a estímulos somatosensoriales (histograma periéstimulo). En posteriores fases de esta investigación se caracterizarán las propiedades circuitales de la corteza motora de las vibrisas evaluando las modificaciones funcionales y estructurales de sus células piramidales en respuesta a lesión de las motoneuronas faciales contralaterales.

Palavras-Chave: Corteza motora primaria, Vibrisas, Neuronas piramidales, Registros Electrofisiológicos

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ASPECTOS ANATÔMICOS E TOPOGRÁFICOS DO NERVO PUDENDO EM HUMANOS

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Introdução: o nervo pudendo distribui ramos motores e sensitivos para a região perineal e órgãos genitais externos, innervando músculos esqueléticos (como os esfíncteres externos do ânus e da uretra) e grande parte da pele desta região, como a do pênis, escroto, lábios maiores e clitóris. Tem importância funcional para a micção, defecação, ereção e o parto. Pesquisas anatômicas sobre este nervo apontam variações em sua topografia, número de ramos e relação com os vasos pudendos internos. Sob o ponto de vista aplicado, é realizado o bloqueio anestésico deste nervo na prática clínica obstétrica, eletroestimulação do mesmo em casos de incontinência fecal ou urinária e procedimentos cirúrgicos para analgesia do períneo, quando o nervo está comprimido. **Objetivos:** fornecer detalhes anatômicos quanto à conformação, biometria e topografia do nervo pudendo humano, aplicáveis a procedimentos clínicos e cirúrgicos que o abordem. **Métodos:** dissecação de 30 regiões glúteas, de 15 cadáveres formolizados, dos Laboratórios de Anatomia da UFSC. Foi observada a conformação do nervo, número de ramos, relação com os vasos pudendos internos e ligamentos sacrotuberal e sacroespinal, e medida sua largura com paquímetro digital. **Resultados:** o nervo apresentou-se como tronco único em 53,3% e dividido em ramos em 46,7% (dois ramos em 36,7%, três em 6,7% e quatro em 3,3%). Quando o nervo estava dividido, em 36,7% os ramos permaneciam separados e em 10% se uniam antes de entrar no forame isquiático menor. Quando tronco único, o nervo teve uma largura média de $4,31 \pm 0,49$ mm. O nervo (único ou dividido) foi medial aos vasos pudendos internos em 70% e lateral a eles em 3,3%. Nos 26,6% restantes o nervo estava dividido em dois ou três ramos, que situavam-se medial e lateralmente aos vasos ou os cruzavam posteriormente. O nervo foi anterior ao ligamento sacrotuberal em 93,3% e em 6,7%, um ou dois ramos do nervo dividido perfuravam o ligamento. Em 100% o nervo (único ou dividido) situou-se posteriormente ao ligamento sacroespinal. **Conclusões:** há importantes variações na conformação e topografia do nervo pudendo que devem ser consideradas durante procedimentos clínicos e cirúrgicos que o abordem.

Palavras-Chave: nervos periféricos; períneo; nervo pudendo

EFEITOS DO ENVELHECIMENTO NA LATERALIDADE DURANTE O MOVIMENTO DE ALCANCE

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Estudos realizados com idosos têm demonstrado que com o avançar da idade, os indivíduos modificam vários aspectos em relação ao controle motor, sugerindo que existem diversas diferenças entre jovens e idosos. **Objetivos:** investigar os efeitos do envelhecimento na coordenação motora durante movimentos de alcance, mais especificamente examinar se estas modificações estão relacionadas a diferenças no controle inter-articular entre os membros (direito e esquerdo) através da análise de variáveis cinemáticas específicas. **Métodos:** dois grupos foram examinados (J) 12 jovens (20 ± 2 anos) e (I) 14 idosos (70 ± 4 anos). Sensores magnéticos de posição (6 graus de liberdade) foram utilizados para avaliação do movimento de alcance de ambos os braços no plano horizontal em 3 direções distintas em relação ao eixo horizontal (alvo-0 orientado a 45° ; alvo-1 orientado a 90° e alvo-2 orientado a 135°). Participantes realizaram 30 tentativas/alvo, apresentadas aleatoriamente e foram orientados a mover a mão até o alvo com um movimento único, rápido e sem correção. Sete variáveis cinemáticas foram calculadas para investigar a diferença entre os membros superiores dos grupos: erro de posição final, desvio de linearidade, aceleração máxima, velocidade máxima, duração da aceleração, duração do movimento e distância percorrida. As variáveis foram analisadas através da ANOVA

(2(grupos)X2(membros)X3(alvos)) **Resultados:** análise preliminar dos dados mostrou diferenças significativas entre os membros nas seguintes variáveis: duração do movimento ($p=0.0037$; $I=0.64 \pm 0.22s$, $J=0.52 \pm 0.16s$), distância percorrida ($p=0.0320$; $I=0.15 \pm 0.02m$, $J=0.17 \pm 0.03m$), aceleração máxima ($p=0.0014$; $I=6.70 \pm 4.59m/s^2$, $J=10.29 \pm 5.83m/s^2$), velocidade máxima ($p=0.0230$; $I=0.64 \pm 0.27m/s$, $J=0.90 \pm 0.30m/s$) e duração da aceleração ($p=0.0040$; $I=0.24 \pm 0.10s$, $J=0.19 \pm 0.07s$). As variáveis erro de posição final ($p=0.0030$; $I=0.02 \pm 0.02m$, $J=0.04 \pm 0.02m$) e desvio de linearidade ($p=0.0028$; $I=0.07 \pm 0.05$, $J=0.08 \pm 0.05$) apresentaram diferenças significativas entre os grupos (mas não entre membros).

Conclusão: esses resultados confirmam o já esperado declínio na coordenação motora de indivíduos idosos, e sugerem maior semelhança entre os membros (direito/esquerdo), ou seja, uma diminuição da lateralidade (menor assimetria entre lados) com o envelhecimento.

Palavras-Chave: Envelhecimento, cinemática, lateralização

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ALTERAÇÕES FUNCIONAIS PÓS-TERAPIA DE USO FORÇADO (FUT) NA REABILITAÇÃO DO MEMBRO INFERIOR DO PACIENTE HEMIPERÉTICO

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Objetivo: Verificar se a Terapia de Uso Forçado (FUT), uma terapia desenvolvida para a reabilitação do membro superior (MS) parético, pode promover ganhos adicionais nos Membros Inferiores (MMII). **Materiais e Métodos:** Participaram deste estudo 13 sujeitos de ambos os sexos, que sofreram Acidente Vascular Encefálico (AVE), não importando sua etiologia (isquêmico ou hemorrágico) e que apresentavam hemiparesia (direita ou esquerda) como seqüela motora. A imobilização do MS não parético foi feita através de uma malha tubular, com o MS posicionado em adução e rotação interna de ombro e flexão de cotovelo maior que 90° por um período de 24 horas, durante cinco dias por semana, por um período total de um mês. Diariamente, a malha era retirada pelos examinadores, feita higienização, mobilização e alongamento muscular do MS em constrição e do MS parético. Foram realizadas duas avaliações: inicial (1AV) e final (5AV). Na admissão foram utilizadas duas escalas: National Institute of Health Stroke Scale (NIHSS) e Stroke Impact Scale (SIS). Para avaliar o membro inferior (MI) foram utilizadas as escalas Berg Balance Scale (BBS), Fugl-Meyer Motor Assessment (FMA) – sinergia flexora e Wolf Motor Function Test (WMFT). Para a análise da marcha foi utilizado o Teste dos 10 metros (T10) e o Timed Up & Go Test (TUGT). Os dados da 1AV e 5AV foram submetidos ao teste em t de Student com valores de p significativos <0,05. **Resultados:** A comparação dos dados na 1AV e 5AV, para as escalas admissionais, mostraram uma diminuição na NIHSS e um aumento na SIS ($t = 2,05$; $P < 0,05$) e ($t = -3,45$; $P < 0,005$), respectivamente. Ambas revelam uma melhora geral da motricidade e da qualidade de vida. Na mesma direção, a comparação da 1AV e 5AV revelou aumentos significativos na performance motora dos sujeitos através das escalas FMA – sinergia flexora ($t = -2,56$; $P < 0,05$), BBS ($t = -4,80$; $P < 0,001$) e WMFT ($t = 2,78$; $P < 0,05$). Ainda, verificamos uma diminuição do tempo necessário para a execução do T10 e no TUGT ($t = 6,73$; $P < 0,001$) e ($t = 4,39$; $P < 0,001$), respectivamente. **Conclusão:** Os resultados sugerem que a FUT é eficaz na reabilitação do MI parético.

Palavras-Chave: Acidente vascular encefálico, hemiparesia, reabilitação neurofuncional, terapia de uso forçado

Apoio Financeiro: FAEPA

CORRELATOS BIOMECÂNICOS E FUNCIONAIS PÓS-TERAPIA DE CONSTRIÇÃO COM INDUÇÃO DE MOVIMENTO (CIMENT) NA REABILITAÇÃO DO MEMBRO INFERIOR DE PACIENTES COM HEMIPARESIA: ANÁLISE DE 30 DIAS DE FOLLOW UP.

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Objetivos: Verificar se a CIMENT, uma terapia utilizada na reabilitação de membros superiores (MS) paréticos, pode proporcionar ganhos funcionais nos membros inferiores (MMII). Ainda, verificar se os ganhos funcionais se mantêm após 30 dias da conclusão do protocolo. **Metodologia:** Participaram deste estudo 7 sujeitos de ambos os sexos, que sofreram Acidente Vascular Encefálico e que apresentavam hemiparesia como seqüela motora. A imobilização do MS não parético foi feita através de uma malha tubular, por um período de 24 horas, durante cinco dias por semana, por um período total de um mês. Diariamente, a malha era retirada para a realização de uma bateria de estimulação motora no MS parético. Foram realizadas três avaliações [inicial (1AV), final (5AV) e 30 dias após o protocolo (FA30)]. Na admissão foram utilizadas duas escalas: *National Institute of Health Stroke Scale* (NIHSS) e *Stroke Impact Scale* (SIS). A funcionalidade dos MMII foi avaliada pelas escalas de *Berg Balance Scale* (BBS), *Fugl-Meyer Motor Assessment* (FMA), *Barthel Index* (BI), *Rivermead Mobility Index* (RMI). Para a análise da marcha foi utilizado o Teste dos 10 metros (T10) e o *Timed Up & Go Test* (TUGT). Os dados foram submetidos a uma análise de variância de uma via (ANOVA) e as diferenças entre os grupos foram evidenciadas através do teste *post hoc* de Dunnett. **Resultados:** A comparação dos dados na 1AV, 5AV e FA30, para as escalas admissionais, mostraram uma diminuição na NIHSS e um aumento na SIS, quando comparados com a 1AV ($F_{2,21} = 8,75$; $P < 0,05$) e ($F_{2,21} = 13,52$; $P < 0,05$), respectivamente. Ambas revelam uma melhora geral da motricidade e da qualidade de vida. Na mesma direção, a comparação da 5AV e FA30 revelou aumentos significativos na performance motora dos sujeitos em relação a 1AV através das escalas BI ($F_{2,21} = 10,60$; $P < 0,05$), FMA ($F_{2,21} = 54,21$; $P < 0,05$), BBS ($F_{2,21} = 47,83$; $P < 0,05$) e RMI ($F_{2,21} = 3,42$; $P < 0,05$). Ainda, verificamos uma diminuição do tempo necessário para a execução do T10 e no TUGT ($F_{2,21} = 10,47$; $P < 0,05$) e ($F_{2,21} = 111,56$; $P < 0,05$), respectivamente. **Conclusão:** Os resultados sugerem que a CIMENT é eficaz na reabilitação do MI parético e que os ganhos funcionais se mantêm após 30 dias do término do protocolo de estimulação. **Palavras-Chave:** Terapia de Constrição com Indução de Movimento, Hemiparesia, Acidente Vascular Encefálico, Membro Inferior Parético.

Apoio Financeiro: FAEPA

ALTERAÇÕES BIOMECÂNICAS E FUNCIONAIS PÓS-TERAPIA DE CONSTRIÇÃO COM INDUÇÃO DE MOVIMENTO (CIMT) NA REABILITAÇÃO DO MEMBRO SUPERIOR DE PACIENTES COM HEMIPARESIA: ANÁLISE DE 30 DIAS DE *FOLLOW UP*.

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Objetivo: Analisar as alterações biomecânicas e funcionais no membro superior (MS) parético de pacientes com quadro de hemiparesia crônica, após 4 semanas de protocolo de estimulação motora pela Terapia de Constrição com Indução de Movimento (CIMT). Ainda, verificar se os ganhos funcionais se mantêm incorporados à organização neural do movimento após 30 dias da conclusão do protocolo.

Metodologia: Participaram deste estudo 8 sujeitos de ambos os sexos, que sofreram Acidente Vascular Encefálico e que apresentavam hemiparesia como seqüela motora. A imobilização do MS não parético foi feita através de uma malha tubular, por um período de 24 horas, durante cinco dias por semana, por um período total de um mês. Diariamente, a malha era retirada para a realização de uma bateria de estimulação motora no MS parético. Foram realizadas três avaliações [inicial (1AV), final (5AV) e 30 dias após o protocolo (FA30)]. Na admissão foram utilizadas duas escalas: *National Institute of Health Stroke Scale* (NIHSS) e *Stroke Impact Scale* (SIS). A funcionalidade do MS foi avaliada pelas escalas de *Barthel Index* (BI), *Wolf Motor Function Test* (WMFT), *Fugl-Meyer Motor Assessment* (FMA) e *Berg Balance Scale* (BBS). Os dados foram submetidos a uma análise de variância de uma via (ANOVA) e as diferenças entre os grupos foram evidenciadas através do teste *post hoc* de Dunnett. **Resultados:** A comparação dos dados na 1AV, 5AV e FA30, para as escalas admissionais, mostraram uma diminuição na NIHSS e aumento na SIS, quando comparados com a 1AV ($F_{2,21} = 7,26$; $P < 0,05$) e ($F_{2,21} = 14,08$; $P < 0,05$), respectivamente. Ambas revelam uma melhora geral da motricidade e da qualidade de vida. Na mesma direção, a comparação da 5AV e FA30 revelou aumentos significativos na performance motora dos sujeitos em relação a 1AV através das escalas BI ($F_{2,21} = 4,54$; $P < 0,05$), FMA – ombro/cotovelo/antebraço ($F_{2,21} = 6,86$; $P < 0,05$), FMA- coordenação/velocidade ($F_{2,21} = 9,34$; $P < 0,05$), BBS ($F_{2,21} = 19,69$; $P < 0,05$) e WMFT ($F_{2,21} = 147,10$; $P < 0,05$). **Conclusão:** Os resultados sugerem que a CIMT é eficaz na reabilitação do MS parético e que os ganhos funcionais se mantêm após 30 dias do término do protocolo de estimulação.

Palavras-Chave: Terapia de Constrição com indução de movimento, Follow Up, Hemiparesia, Acidente Vascular Encefálico, Membro Superior.

Apoio Financeiro: FAEPA

ALTERAÇÕES FUNCIONAIS PÓS-TERAPIA DE USO FORÇADO NA REABILITAÇÃO DO MEMBRO SUPERIOR DO PACIENTE HEMIPARÉTICO: ANÁLISE DE 30 DIAS E DE FOLLOW UP.

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Objetivo: Verificar as modificações funcionais no membro superior (MS) parético pós - Acidente Vascular Encefálico (AVE) através da Terapia de Uso Forçado (TUF). Ainda, verificar se os ganhos funcionais se mantêm incorporados à organização neural do movimento após 30 dias de conclusão do protocolo. **Materiais e Métodos:** Participaram deste estudo 11 indivíduos de ambos os sexos, que sofreram AVE e que apresentem hemiparesia como seqüela motora. A imobilização do MS não parético foi feita através de uma malha tubular, por um período de 24 horas, durante cinco dias por semana, por um período total de um mês. Diariamente, a malha era retirada pelos examinadores, feita higienização, mobilização e alongamento muscular de ambos MMSS. Foram realizadas três avaliações [inicial (1AV), final (5AV) e 30 dias após o protocolo (FA30)]. Na admissão dos sujeitos foram utilizadas as escalas: *Stroke Impact Scale* (SIS) e *Escala de Ashworth* (AS). Para avaliar o MS foram utilizadas a *Barthel Index* (BI), *Wolf Motor Function Test* (WMFT), a *Fugl-Meyer Motor Assessment*, parte 1 (FMA). Os dados foram submetidos a uma análise de variância de uma via (ANOVA) e as diferenças entre os grupos foram evidenciadas através do teste *post hoc* de Dunnett. **Resultados:** A comparação dos dados na 1AV, 5AV e FA30, para as escalas admissionais, mostraram aumento significativo dos dados da SIS e uma diminuição dos valores da escala de AS, quando comparados com a 1AV ($F_{2,32} = 4,08$; $P < 0,05$) e ($F_{2,32} = 6,40$; $P < 0,05$), respectivamente. A modificação da SIS revela uma melhora na qualidade de vida dos sujeitos e a de AS um diminuição da espasticidade. Na mesma direção, a comparação da 5AV e FA30 revelou aumentos significativos na performance motora dos sujeitos em relação a 1AV através da escala de WMFT nos itens tempo e pontos ($F_{2,32} = 10,66$; $P < 0,05$) ($F_{2,32} = 7,57$; $P < 0,05$), respectivamente. As escalas de FMA ($F_{2,32} = 0,42$; $P = 0,65$) e BI ($F_{2,32} = 0,05$; $P = 0,92$) não se modificaram significativamente. **Conclusão:** Os resultados sugerem que a FUT é eficaz na reabilitação do MS parético e que os ganhos funcionais se mantêm após 30 dias do término do protocolo de estimulação.

Palavras-Chave: Acidente vascular encefálico, hemiparesia, reabilitação neurofuncional, terapia de uso forçado

Apoio Financeiro: FAEPA

PARTICIPACION DE LA CORRIENTE ACTIVADA POR NMDA EN LA LATERALIDAD DEL GLOBO PALIDO DE RATA

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Experimentos con animales sugieren que las asimetrías neuroquímicas y anatómicas existen en los ganglios basales particularmente en globo pálido (GP) donde el hemisferio izquierdo contiene mayor concentración de dopamina y acetilcolinesterasa que en el derecho (Kooistra and Heilman, 1988. *Neurology*, 38(3):388-90). Estas evidencias quizá reflejan las bases estructurales de la integridad motora en humanos. Nos interesa investigar si existe alguna relación neuroquímica que contribuya al fenómeno lateralidad cerebral

OBJETIVO: Caracterizar las corrientes iónicas inducidas por N-metil-D-Aspartato (NMDA) en neuronas de GP del hemisferio izquierdo y derecho del cerebro; para determinar su participación de la lateralidad motora

METODOLOGIA

Registro de corrientes NMDA en neuronas disociadas de GP de rata, técnica de célula completa con fijación de voltaje (Bargas et al., 1994. *J. Neurosciense*. 14: 6667 –6686.) el procedimiento ya ha sido descrito (Flores – Hernández *et al.*, 2002. *J Neurophysiol*. 88(6):3010-20.)

RESULTADOS

Se han registrado 19 células, con una capacitancia entre 8 y 22 pF. Las corrientes de NMDA se generaron aplicando NMDA 100 μ M y 20 μ M Glicina y a un potencial de mantenimiento de -70 mV

La diferencia en el tamaño de corriente de las neuronas aisladas de GP izquierdo y derecho es evidente, se analizaron 11 neuronas de GP derecho y 8 neuronas de GP izquierdo, obteniendo la densidad de corriente al pico y estado estacionario.

Densidad de corriente (pA/pF)	Izquierdo	Derecho
Pico	16.45 $\pm$ 2.2	32.5 $\pm$ 4.6
Estado estacionario	7.4 $\pm$ 2.7	13.6 $\pm$ 3.2

Los datos obtenidos se analizaron con una “t” no pareada con una t= 2.99 y una significancia p= 0.008 para la densidad de corriente NMDA al pico; una t= 2.6 y una significancia p= 0.018 para la densidad de corriente en el estado estacionario

Conclusión

La diferencia encontrada entre las neuronas del GP izquierdo y derecho de las corrientes NMDA es evidente en los resultados de los registros analizados, es conveniente aumentar el número de neuronas de ambos lados para poder mostrar el comportamiento de este fenómeno.

Palavras-Chave: lateralidad, NMDA, ganglios basales

Apoio Financeiro: Becario CONACYT, VIEP-BUAP

D.07.008

Estratégias de controle postural nos diferentes planos de movimento

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Diversos estudos têm sugerido que o sistema de controle postural faz uso de diferentes estratégias para a manutenção do equilíbrio nos planos ântero-posterior (AP) e médio-lateral (ML). Foi observado que mudanças na base de suporte (BS) comprometem de modo diferenciado o equilíbrio nos planos AP e ML. Contudo, não se sabe de que modo o centro de pressão dos pés (CoP) e seus componentes *rambling* (RA) e *trembling* (TR) se comportam nos diferentes planos de movimento quando assumimos diferentes BS.

Objetivo: analisar os efeitos de diferentes BS – pés afastados (P1); unidos (P2); ou alinhados (P3) – no deslocamento do CoP e de seus componentes nos planos AP e ML. **Métodos:** oito sujeitos permaneceram por 30 segundos em cada BS, sobre uma plataforma de força. O desvio padrão médio (DP) de oscilação foi obtido a partir da série temporal do CoP e de seus componentes. A análise estatística consistiu de ANOVA de 2 fatores para amostras repetidas, e pós-teste de Tuckey-HSD, para $P \leq 0,05$. Os dados são apresentados como média $\pm$ desvio padrão. **Resultados:** para o CoP, no plano AP, não houve diferença entre P1 e P2 ($2,74 \pm 0,54$ vs $3,31 \pm 0,58$ mm, $P = 0,74$), sendo DP maior em P3 ($4,48 \pm 1,61$ mm, $P < 0,01$). Em ML, o DP aumentou de P1 para P2 ($0,99 \pm 0,29$ vs $3,00 \pm 0,61$ mm, $P < 0,01$), e de P2 para P3 ($4,38 \pm 0,65$ mm, $P = 0,02$). Houve diferenças entre os planos somente em P1 ($P < 0,01$). As mudanças no DP em RA foram semelhantes àquelas observadas para o CoP. Para o TR foi observado um aumento de P1 (AP - $0,59 \pm 0,13$; ML - $0,49 \pm 0,20$ mm) para P2 (AP - $0,80 \pm 0,13$; ML - $1,03 \pm 0,24$ mm, $P < 0,01$), e de P2 para P3 (AP - $1,72 \pm 0,48$; ML - $1,82 \pm 0,25$ mm, $P = 0,02$) em ambos os planos, não havendo diferenças entre eles para nenhuma das BS analisadas. **Conclusão:** as diferenças observadas para o DP entre os planos ML e AP sugerem que o sistema de controle postural utiliza estratégias distintas para o controle do equilíbrio nos diferentes planos de movimento. Nesse estudo, a comparação entre AP e ML nas BS analisadas nos indica que a contribuição da oscilação em cada um dos planos para o deslocamento total do CoP é alterada quando há uma maior exigência de controle do equilíbrio postural. **Palavras-Chave:** estabilometria, equilíbrio postural, controle motor

Apoio Financeiro: CNPq, CNPq-PRONEX, FAPERJ, FINEP

Influência da idade de ocorrência da perda da visão sobre o controle postural

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O controle da postura envolve a integração das informações visual, somatosensorial e vestibular. Estudos têm mostrado que a visão tem um papel importante no desenvolvimento de funções multisensoriais de alta complexidade durante os primeiros anos de vida¹. No entanto, pouco se sabe sobre como a deficiência visual afeta o desenvolvimento do controle postural. **Objetivo:** Avaliar a influência da perda visual precoce (G1) ou tardia (G2) sobre o controle postural de portadores de deficiência visual completamente cegos (B1) e com baixa visão (B2), classificados de acordo com a Associação Internacional de esportes para cegos. **Métodos:** dezesseis deficientes visuais participaram do estudo e foram divididos em quatro grupos. Três B1-G1, seis B2-G1, quatro B1-G2 e três B2-G2. A classificação para cegos precoces teve como critério a perda visual até três anos de idade. Para avaliar o controle postural, os voluntários permaneceram em pé por um minuto sobre uma plataforma de força com pés unidos (U) e pés afastados (A). Os parâmetros área, desvio padrão (DP), velocidade média (v) e frequência média (f) das oscilações posturais foram avaliados nos eixos ântero-posterior (y) e médio-lateral (x). Devido à amostra não apresentar uma distribuição normal, a análise estatística dos resultados foi realizada separadamente para o fator idade da perda visual (G1: n= 7; G2: n=9) e grau de comprometimento (B1: n=10; B2: n=10), utilizando-se o teste não-paramétrico U de Mann-Whitney ($p<0,05$). Os dados são apresentados como mediana (intervalo de confiança de 95%). **Resultados:** Observou-se diferença para o fator G no DP em y para a condição U ($G1=5,0$ mm (4,6 - 5,6) e $G2=6,3$ mm (5,5-7,6), $p=0,011$), em f para a condição A ($G1=0,1$ Hz (0,1-0,2) e $G2=0,2$ Hz (0,2-0,3), $p=0,039$) e em v para a condição A ($G1=8,4$ mm/s (8,1-8,7) e $G2=10,2$ mm/s (8,6-1,5), $p=0,039$). Não foi encontrada diferença estatística para nenhum dos parâmetros analisados no eixo x, bem como para o grau de comprometimento visual. **Conclusão:** A idade em que ocorreu a perda visual parece ter mais consequências para o sistema de controle postural do que o grau de comprometimento visual.

Referência:¹ - Nature neuroscience 10:1243, 2007.

Palavras-Chave: controle postural; cegos; estabilometria

Apoio Financeiro: CAPES; CNPq-PRONEX; FAPERJ; FINEP

D.08.001

VENTRAL TEGMENTAL AREA NEURONS PROJECTING TO THE ACCUMBENS EXPRESS INSULIN RECEPTOR SUBSTRATE-1

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Objectives: There is a strong interaction between reward/motivation and energy homeostasis circuits. In particular, the ventral tegmental area (VTA), a nodal link in brain reward mechanisms, can directly assess the metabolic status of the organism via insulin and leptin receptors. On this basis, Figlewicz and coworkers (Brain Res 964:107, 2003) postulated that these hormones may modulate dopamine release in the striatum and influence approach behavior related to food-intake. To substantiate this hypothesis, we examined: 1) the intra-VTA distribution of insulin receptor substrate-1 (IRS-1), an early protein of the insulin signaling pathway; and 2) the presence of IRS-1 in VTA neurons projecting to the accumbens (Ac).

Methods: Three adult Wistar albino rats were perfused and had coronal midbrain sections immunoreacted for the simultaneous detection of IRS-1 and tyrosine hydroxylase (TH; used as a dopaminergic marker). Three other rats received iontophoretic injections of cholera toxin subunit b (CTb) in the Ac. After 7 days, they were perfused, and coronal sections through the Ac and midbrain were collected. One series of sections was processed for CTb by immunoperoxidase to visualize the injection site and retrograde labeling in VTA. Another series was double-labeled for CTb and IRS-1 by immunofluorescence.

Results: The results show that IRS-1 immunoreactivity has a nuclear location and is widely distributed in the VTA and substantia nigra. Double-labeling experiments for IRS-1 and TH indicate that IRS-1 is highly co-localized in VTA and nigral dopaminergic neurons. Moreover, double-labeling experiments for IRS-1 and CTb reveal that the large majority of VTA neurons projecting to the Ac express IRS-1.

Conclusions: Having in mind that a high proportion of dopaminergic neurons express IRS-1, and that the overwhelming majority of VTA neurons projecting to the Ac are dopaminergic, it seems likely that dopaminergic inputs to the Ac arise in part from IRS-1 positive neurons. Overall, the data confirm that VTA dopamine neurons are a direct target for insulin. They suggest, in addition, that the effects of this hormone on reward-seeking behavior may be mediated by mesolimbic dopaminergic projections to the Ac.

Palavras-Chave: ventral tegmental area, accumbens, ventral striatum, dopamine, insulin, limbic system

Apoio Financeiro: CAPES; FAPESP 05/51607-6

Immunohistochemical characterization of the GABAergic system in the cerebellum of the zebrafish, *Danio rerio*.

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; ²CNV, Facultad de Ciencias - Universidad de Valparaíso

Objectives. Gamma-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the central nervous system. Its role is especially prominent in the cerebellum, where most neuron types are GABAergic. However, little is known about its function in the teleost cerebellum, which is only partly homologous to its mammalian counterpart. Here we investigated the expression and distribution of GABA, the GABA-synthesizing enzyme glutamic acid decarboxylase 65 (GAD65) and the receptor subunits GABA_{Aα1} and GABA_{B1} in the cerebellum of adult zebrafish.

Methods. We used immunohistochemical techniques to characterize the expression of GABA, GAD65 and the receptor subunits GABA_{Aα1} and GABA_{B1} in the cerebellum of adult zebrafish (*Danio rerio*).

Results. GABA and GAD65 presented a similar expression pattern that comprised the molecular layer, Purkinje cells and groups of presumed Golgi cells in the granular layer of cerebellar corpus and valve. GABA_A receptor subunits were principally found on fine radial fibers in the molecular layer, while GABA_B receptor subunits localized prominently to the cell bodies of Purkinje cells in the ganglionic layer, and to their dendrites that span the molecular layer.

Conclusion. This study shows that GABA, GAD65 and the receptor subunits GABA_{Aα1} and GABA_{B1} are widely expressed in the zebrafish cerebellum and present a distribution pattern similar to the mammalian cerebellar cortex. The overall expression pattern of the GABAergic system seems to be conserved among vertebrates from fishes to mammals.

Palavras-Chave: cerebellum, zebrafish, teleost, GABA receptor, GAD65

Apoio Financeiro: PBCT-Conicyt project ACT-45,

D.08.003

Corticomotor excitability in chronic cerebellar infarcts

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Objectives: A previous study reported increased short-interval intracortical inhibition (SICI) and decreased intracortical facilitation (ICF) in cerebellar infarcts in the acute phase. Our goal was to investigate inter-hemispheric differences in corticomotor excitability in patients with unilateral cerebellar infarcts in the chronic phase.

Methods: Seven patients (four males) aged 21-63 years (mean 45.8 years) with chronic (> 4 months) cerebellar unilateral infarcts participated in the study. Transcranial magnetic stimulation (TMS) was administered to the motor cortex in the cerebral hemisphere contralateral (CH) and ipsilateral (IPSH) to the cerebellar infarct. Motor evoked potentials (MEPs) were recorded in the abductor pollicis brevis contralateral to the stimulated cerebral hemisphere. Resting motor thresholds (rMT), MEP/M ratios at intensities of rMT, 130%rMT, and 100% stimulator's output (s.o.), SICI (interstimulus interval, ISI, 2ms) and ICF (ISI, 10ms) were performed. Stimulus intensities for paired-pulse measurements were: conditioning stimulus, 80%rMT; test stimulus, intensity required to elicit MEPs of 0.5-1mV.

Results: There was a significant asymmetry in SICI (paired t-test, $p=0.002$). In all patients, SICI was decreased in CH compared to IPSH. ANOVA with repeated measures with factors "hemisphere" (CH or IPSH) and "TMS intensity" (rMT, 130% rMT or 100% s.o.) showed a significant effect of "TMS intensity" ($F=12.4$; $p=0.009$) but no effects of "hemisphere" or interaction "hemisphere" and "TMS intensity".

Discussion: These results, taken together with previous reports in the acute phase after a unilateral cerebellar infarct, suggest that SICI increases in the CH in an early phase, and decreases in the chronic stage.

Palavras-Chave: cerebellar infarct, corticomotor excitability, intracortical inhibition, intracortical facilitation

D.08.004

Alterações na distribuição de NADPH-diaforase no cerebelo de ratos submetidos à hipóxia-isquemia perinatal

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O cérebro infantil humano submetido a hipóxia apresenta perda de oligodendrócitos, hipomielinização, astrogliose e alterações no desenvolvimento cortical. Robinson e cols. (S. Robinson et al. / *Neurobiology of Disease* 18 (2005) 568–581) usando modelo de hipóxia-isquemia (HI) por clampeamento das artérias uterinas na rata grávida no décimo oitavo dia de gestação, observaram astrogliose, morte de oligodendrócitos e ruptura axonal, alterações similares às observadas em cérebros humanos que passaram por HI perinatal. Em modelos de HI em ratos neonatos a atividade da NOS aumenta após o evento, principalmente nas áreas mais suscetíveis a lesão. Neste trabalho avaliamos a distribuição da NADPH-diaforase (NADPH-d) no cerebelo dos ratos submetidos a este modelo de HI, visto que alterações nesta estrutura também podem estar relacionadas com distúrbios motores observados na HI peri-natal.

Métodos:

Ratas grávidas no 18º dia de gestação são anestesiadas com tribromoetanol intra-peritoneal. Após exposição dos cornos uterinos um grupo de ratas teve as 4 artérias uterinas clampeadas (HI) por 45 minutos, e em outro (SHAM) não há clampeamento. Após a cirurgia a gestação prosseguiu. Somente os animais nascidos a termo são utilizados. Um terceiro grupo de animais não sofreu nenhum tipo de manipulação até o nascimento (NM). Os animais são anestesiados e perfundidos com solução salina 0,9%, paraformaldeído (PF) a 4% e PF 4% com 10% de sacarose. Cortes de 40 a 60µm são recolhidos em tampão Tris e submetidos à histoquímica da NADPH-d, montados em lâminas e observados ao microscópio.

Resultados:

Os grupos experimentais não apresentaram diferenças no peso corporal ou tamanho. Animais P23 do grupo HI apresentaram células NADPH-d+ ectopicamente localizadas na camada molecular do cerebelo. Os animais do grupo Sham e NM não apresentaram células nessa região. Este padrão se mantém até a idade adulta (P90), indicando uma alteração persistente na distribuição das células NADPH-d+ no cerebelo.

Conclusões:

Nossos resultados demonstram que a HI perinatal afeta a distribuição da NADPH-d no cerebelo o que pode indicar uma alteração persistente na citoarquitetura cerebelar. Esta alteração pode estar relacionada a distúrbios motores observados em ratos jovens adultos que foram submetidos à HI.

Palavras-Chave: Cerebelo, Hipóxia-isquemia, NADPH-diaforase, Ratos

Apoio Financeiro: CNPq ,FAPERJ ,SR2/UERJ

D.08.005

NMDA DEPENDENCY OF MEDIUM SPINY NEURON FIRING DURING UP STATES

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Although the role of NMDA receptors in corticostriatal synaptic plasticity is widely acknowledged, little is known about which conditions allow NMDA receptor activation in the striatum *in vivo*. The aim of the present work is to determine the role of NMDA receptors on the firing activity of striatal medium spiny neurons (MSN) *in vivo* during slow waves.

We recorded extracellular multiunit activity (MUA) with 16 channel silicon probes or the membrane potential of MSN in urethane-anaesthetized rats. Simultaneously, aCSF with or without NMDA antagonist were infused through reverse microdialysis close to the recording electrode. All the experiments were performed during cortical slow wave activity, which was assessed from the motor cortex local field potential (LFP).

Striatal MUA was markedly modulated by cortical LFP with most spikes locked to the active phase of slow waves. The intra-striatal infusion of AP-5 (100 μ M) reversibly reduced the discharge frequency to 56% of basal ($p < 0.05$). Similar results were obtained with another NMDA antagonist (CGP39551) but no reversion could be observed. Spike sorting of MUA revealed the presence of phasically and tonically active single units. Infusion of AP-5 reduced specifically the activity of phasically spiking neurons to 22.2% of basal frequency ($p < 0.01$, $n = 14$) without affecting the tonically active neurons ($p > 0.05$, $n = 7$). Intracellular recordings of MSN confirmed that infusion of AP-5 reversibly inhibited their firing ($n = 2$). In addition, analysis of UP states showed a reduction in the amplitude (13.1 ± 1.9 mV to 9.2 ± 1.2 mV, $p < 0.05$, $n = 5$) without affecting their duration.

Our results suggest that NMDA receptors directly contribute to the spontaneous firing of MSNs through an increase in UP state amplitude. The recurrent activation of NMDA receptors during offline processing modes (e.g., during slow wave sleep) could be important in memory consolidation. **Palavras-Chave:** dorsal striatum; *in vivo*; extracellular recordings; intracellular recordings; reverse microdialysis

Apoio Financeiro: FONCYT, CONICET and UBA (Argentina)

D.08.006

CHALCEDONY – DNA INTERACTION IN PURKINJE CELLS

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The cerebellum is an especially appropriate organ for the analysis of the traumatized and aged neurons. In the cerebellum of the old people, Cajal (1911) observed axonic metamorphoses of Purkinje neurons: a) axonic hypertrophy; b) axonal swellings; c) axon ending in a ball and d) also disappearance of numerous Purkinje cells. Chalcedony (a microcrystalline silica, SiO₂) has been observed to be present in the human cerebellum from aged patients by using a DMLP polarized Light microscope (Prado Figueroa *et al.*, 2006; 2008). This paper documents the visualization of chalcedony in the human cerebellum of the old people by using a Leica TSC TC2 Confocal Laser Scanning Microscope. Autofluorescent chalcedony is in the balls of the axon ending, swellings and also within the nucleus of the Purkinje cells, as very small grains. This observation is in relation with the Purkinje cells loss. Chalcedony plays a direct role in cellular transformation and it is the agent that damage DNA.

Palavras-Chave: Neurodegeneration Purkinje cells; silica

Apoio Financeiro: Supported by grants from the SGCyT, UNS – Bahía Blanca, Argentina.

ANÁLISE DOS SISTEMAS NITRÉRGICO E DOPAMINÉRGICO NOS SISTEMAS MESOESTRIATAL E MESOLÍMBICOCORTICAL DE CAMUNDONGOS *GIRK2^{WV}*

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Objetivos: A perda de neurônios dopaminérgicos mesencefálicos no camundongo mutante *GIRK2^{WV}* é similar ao sinal patológico do parkinsonismo. O objetivo é analisar a presença da sintase de óxido nítrico (nNOS) e tirosina hidroxilase (TH) em diferentes fases do desenvolvimento dos mutantes *GIRK2^{WV}*.

Métodos e Resultados: Foram utilizados camundongos selvagens (C57Bl) e *GIRK2^{WV}* com 10 dias, 1 mês e 2 meses de idade (n=5/grupo). Os animais foram anestesiados com 60mg/kg de pentobarbital ip.e então perfundidos com PBS e PFA 4%. Os cérebros foram pós-fixados em PFA a 4°C por duas horas, desidratados, incluídos em parafina e seccionados (5mm). Foi então realizada imunohistoquímica para nNOS e TH no córtex cingular e motor, no estriado dorsolateral (CpuDL) e dorsomedial (CPuDM), *acumbens* (Acb), amígdala, subtalâmico (Sth), área tegmental ventral (VTA) e substância negra compacta (SNc). A análise estatística foi realizada por meio do teste *t* e da análise de variância de dois fatores, seguida de Duncan. Os camundongos *GIRK2^{WV}* apresentaram redução da TH aos 10 dias apenas no STh; com 1 mês no CPuDM e CPuDL, STh, VTA e SNc; e com 2 meses em todos os núcleos analisados: ($t_{(10)}=2,85$ a $145,77$; $p<0,05$). A ANOVA mostrou efeito da idade ($F_{(10)}=126,06$ a $3639,89$; $p<0,05$), linhagem ($F_{(10)}=29,62$ a $5408,16$; $p<0,05$) e interação ($F_{(10)}=21,69$ a $1485,98$; $p<0,05$), com redução da marcação para TH nos animais *GIRK2^{WV}* em todos os núcleos analisados. Os animais *GIRK2^{WV}* apresentaram também redução na marcação para nNOS com 1 mês no STh ($t_{(10)}=2,33$; $p=0,042$) e aumento na SNc ($t_{(10)}=6,53$; $p<0,001$); aos 2 meses foi observado redução no Acb ($t_{(10)}=2,91$; $p=0,012$) e STh ($t_{(10)}=3,08$; $p=0,007$) e aumento na SNc ($t_{(8)}=-14,01$; $p<0,001$). A ANOVA mostrou efeito da idade em todos os núcleos ($F_{(10)}=9,59$ a $61,96$; $p<0,05$), da linhagem no STh ($F_{(10)}=7,14$; $p<0,11$), com redução nos mutantes, e na SNc ($F_{(10)}=157,41$ $p<0,05$), com aumento nos mutantes; interação foi observada apenas na SNc ($F_{(10)}=66,79$; $p<0,05$).

Conclusão: Nosso trabalho sugere um possível papel do NO na degeneração de células dopaminérgicas de camundongos *GIRK2^{WV}* e também a adaptações plásticas compensatórias a perda da inervação dopaminérgica.

Palavras-Chave: neurodegeneração espontânea, Parkinson, óxido nítrico

Apoio Financeiro: CAPES/COFECUB, FAPESP e CNPQ

Organization of ventral tegmental area projections to the ventral tegmental area-nigral complex in the rat.

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Objectives: The ventral tegmental area (VTA) is a nodal link in reward circuitry. Based on its striatal output, it has been subdivided in a caudomedial part that targets the ventromedial striatum, and a lateral part that targets the ventrolateral striatum (1). Whether these two VTA parts are interconnected and to what extent the VTA innervates the substantia nigra compacta (SNc) and retrorubral nucleus (RR) are critical issues for understanding information processing in the basal ganglia.

Methods: VTA projections to the VTA-nigral complex were examined in rats by using the *Phaseolus vulgaris* leucoagglutinin (PHA-L) anterograde tract tracing technique.

Results: The results show that the dorsolateral VTA projects to itself, as well as to the dorsal tier of the SNc and RR, but it largely avoids the caudomedial VTA. The ventrolateral VTA innervates mainly the interfascicular nucleus. The components of the caudomedial VTA (the interfascicular, paranigral and caudal linear nuclei) are connected with each other. In addition, the caudomedial VTA (especially the paranigral and caudal linear nuclei) innervates the lateral VTA, and, to lesser degree, the SNc and RR. The caudal pole of the VTA sends robust, bilateral projections to virtually all the VTA-nigral complex, which terminate in the dorsal and ventral tiers. Modest inputs from the medial supramammillary nucleus to ventromedial parts of the VTA-nigral complex were also identified. In double-immunostained sections, PHA-L labeled varicosities were sometimes found closely apposed to tyrosine hydroxylase-positive neurons in the ventral mesencephalon.

Conclusions: The results underscore that VTA projections to the VTA-nigral complex are substantial and topically organized. In general, these projections, like the open, spiralated striato-nigro-striatal loops display a medial to lateral organization. This anatomical arrangement conceivably permits the ventromedial striatum to influence the activity of the lateral striatum. The caudal pole of the VTA appears to be a critical site for a global recruitment of the mesotelencephalic system.

References: 1-Ikemoto, Brain Res Rev 56:27-78, 2007

Palavras-Chave: basal ganglia, dopamine, reward, substantia nigra, retrorubral nucleus, supramammillary nucleus

Apoio Financeiro: CAPES, FAPESP

D.08.009

Striatal neuronal nitric oxide synthase mRNA and protein expression is associated with L-DOPA-induced dyskinesia in a rat model of Parkinson's disease

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Introduction: The dopamine precursor L-DOPA is the most effective drug for the treatment of Parkinson's disease (PD). However, chronic administration of L-DOPA to Parkinsonian patients can induce the development of uncontrollable movements known as dyskinesia. Nitric oxide (NO) can be considered one of the most innovative discoveries of the last decade in cell signalling. In several medical disorders NO has been implicated in the disease pathology due to persistent activation or overproduction of neuronal NO synthase isoform (NOS1). Little information is available about the relationship between the NOS inhibitors and dyskinesia.

Objectives: Study the role and the mechanism of NO in dyskinesia.

Methods: Male Wistar rats received a single stereotaxic injection of 6-OHDA or saline into the right medial forebrain bundle. L-DOPA treatments started 3 weeks after surgery. Control (n=8) and Parkinsonian (n=25) rats were treated with acute (n=5-11) or chronic (16 doses once a day; n=5-14) L-DOPA 100 mg/kg. The non-specific NOS inhibitor L-NOARG (50 mg/kg, i.p.) was administered 30 min prior the acute and the 16th L-DOPA dose. We used the rat scale for abnormal involuntary movements (AIMs) to monitor the dyskinesia which affects axial, limb and orofacial muscles at 1 and 2 hours after L-DOPA intake. Twenty four hours after the last treatment, the rats' striatum were processed for western blot and real time-PCR.

Results: Control rats did not exhibit any behavior following L-DOPA treatments. L-NOARG blocked 33 to 55% of AIMs development when administered 30 min before acute ($t_{(5)}=2.86$; $P=0.035$) or chronic L-DOPA ($t_{(8)}=3.80$; $P=0.005$). NOS1 mRNA expression slightly increased in the lesioned but not in the unlesioned striatum after 6-OHDA-lesion ($F_{(2,16)}=14.91$, $P<0.001$). L-DOPA-chronic treatment further increased NOS1 mRNA expression in both lesioned and unlesioned striatum ($F_{(2,17)}=5.06$, $P=0.001$). The enhancement of NOS1 mRNA after L-DOPA treatment in the striatum of 6-OHDA-lesioned rats were accompanied by an increase in the protein levels ($F_{(2,6)}=82.56$, $P<0.001$).

Conclusion: The NO system can interfere with plastic mechanisms related to dyskinesias. This could be a possible new therapeutic approach to PD treatment side-effects.

Palavras-Chave: Basal Ganglia, Parkinson, Nitric Oxide, Striatum, Dyskinesia, L-DOPA

Apoio Financeiro: FAPESP, CAPES, CNPq

D.08.010

AVALIAÇÃO NEUROQUÍMICA DO MODELO ANIMAL DE PARKINSONISMO INDUZIDO POR INFUSÃO BILATERAL DE ROTENONA NA SUBSTÂNCIA NEGRA DE RATOS

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Objetivos: Avaliar níveis nigroestriatais de alguns neurotransmissores (NT) e metabólitos no modelo animal de rotenona na substância negra (SN) de ratos Wistar machos.

Métodos e resultados: Ratos Wistar machos foram infundidos bilateralmente com rotenona 12µg/µl (ROT12) ou com PEG (veículo). Os grupos foram divididos em: Controle (N24hs=6, N30d=8), PEG (N24hs=8, N30d=11) e ROT12 (N24hs=7, N30d=5). Esses animais foram eutanasiados em dois tempos diferentes: 24hs e 30 dias após a cirurgia. Seus cérebros foram dissecados e os tecidos cerebrais (SN e estriado) foram congelados a -70°C para posterior análise por HPLC com detecção eletroquímica. Os NT e metabólitos quantificados foram: dopamina (DA), ácido 3,4-dihidroxifenilacético (DOPAC), ácido homovanílico (HVA), noradrenalina (NA), serotonina (5-HT) e ácido 5-hidroxitriptofano (5-HIAA). Resultados analisados por ANOVA de duas vias seguida do teste de Newman-Keuls; P<0,05 foi considerado significativo; dados expressos pela média ± EPM em ng/g de tecido. Os resultados da SN mostraram que houve um aumento significativo de 5-HIAA para o grupo ROT12 (4052,6±603,1) quando comparado ao grupo controle nas 24hs; e uma diminuição nos níveis de HVA (200,1±25,0) e um aumento da concentração de NA (352,2±68,6) no grupo ROT12 no dia 30 quando comparado aos grupos controle e veículo. Já na análise do estriado desses grupos (controle, veículo e ROT12), no dia 30 observamos uma diminuição de DA no grupo ROT12 (3263,9±360,1), bem como de seus metabólitos DOPAC (860,6±60,7) e HVA (454,3±15,3) (todos comparados ao controle e veículo). Porém, na análise do HVA, o grupo ROT12 24hs mostrou um aumento na concentração (1084,1±174,4) quando comparado aos grupos controle e veículo.

Conclusão: Nossos dados mostraram que o modelo animal de parkinsonismo induzido por rotenona foi capaz de mimetizar alguns eventos bioquímicos da doença de Parkinson, mais especificamente uma diminuição progressiva de níveis estriatais de DA e seus metabólitos.

Palavras-Chave: Doença de Parkinson; Rotenona; Dopamina; HPLC

Apoio Financeiro: CNPq

EFEITO DE INIBIDOR DA SÍNTESE DE ÓXIDO NÍTRICO E DA AMANTADINA SOBRE OS MOVIMENTOS INVOLUNTÁRIOS ANORMAIS INDUZIDOS POR L-DOPA

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Introdução: A perda progressiva dos neurônios dopaminérgicos nigroestriatais é uma das características da doença de Parkinson (DP). O tratamento clínico utiliza a L-DOPA, porém está associado com surgimento de complicações motoras (discinesias). Sabe-se que existe uma interação entre o óxido nítrico e dopamina no SNC, entretanto pouco é conhecido a respeito do NO nas discinesias. **Objetivos:** Investigar os efeitos do inibidor seletivo para a NOS neuronal 7-Nitroindazol (7NI) e da amantadina (utilizada no tratamento clínico) sobre as complicações motoras induzidas pela administração crônica de L-DOPA em ratos parkinsonianos. **Métodos:** Ratos machos *Wistar* (n=6/grupo; ±250 g) foram submetidos à cirurgia esterotáxica e receberam microinjeção de 6-OHDA ou salina no feixe prosencefálico medial (via nigroestriatal). Vinte e um dias após cirurgia, os animais com degeneração completa da via nigroestriatal (parkinsonianos) foram tratados com doses diárias de L-DOPA 10mg/kg (2 semanas) depois 30mg/kg (3 semanas). Uma vez estabelecida a discinesia, foram testados os efeitos da administração de amantadina (40mg/kg) e de 7NI (30 e 60mg/kg) previamente à administração de L-DOPA, sobre os movimentos involuntários e o comportamento rotatório. **Resultados:** O tratamento com L-DOPA aumentou escores de AIMS (axial, orofacial, membro anterior e locomoção) e comportamento rotatório contralateral à lesão. A amantadina (40mg/kg) atenuou a discinesia orofacial (-48,61±10,63%) e a discinesia do membro anterior (-44,36±16,34%; P<0,05, paired *t*-test). O 7NI (30 e 60mg/kg) reduziu completamente os escores de AIMS e de comportamento rotatório (P<0,05, paired *t*-test). **Conclusão:** O inibidor seletivo para a NOS neuronal reduziu os AIMS e comportamento rotatório, de forma mais efetiva que a amantadina. Esses achados reforçam a idéia de que o óxido nítrico esteja envolvido com patologias neurodegenerativas e aponta para uma possível utilização de inibidores da NOS para tratamento das discinesias. **Referências Bibliográficas:** 1 - Exp. Neurol. 194:66, 2005; 2 - Cell. Mol. Neurobiol. 25:371, 2005. **Palavras-Chave:** Parkinson, Discinesia, Óxido Nítrico, Dopamina

Apoio Financeiro: FAPESP, CAPES, CNPq

Pre-synaptic modulation of glutamate release by neurotransmitters in rat striatum

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Objectives: Glutamate plays a major role in basal ganglia circuitry and motor function. In this study we aimed at a systematic description of the modulating roles of neurotransmitters on striatal glutamate release.

Methods: Rat striatal chopped tissue was loaded with ³H-Glutamate and submitted to a standard superfusion protocol with two stimuli with 35 mM KCl. The first stimulation was taken as control and test drugs were added afterwards until the second stimulus.

Results: Glutamate release was calcium-dependent, (P/Q type channels) and TTX had only a small effect. Glutamate agonists NMDA 50mM, AMPA 10mM and Kainate 10mM alone were ineffective, but in the absence of magnesium or paired with AMPA or Kainate, NMDA increased spontaneous and stimulated releases. B-agonist Isoproterenol 1mM enhanced spontaneous release but modestly affected stimulated release. DA antagonists Haloperidol 100mM, Spiperone 10mM and SCH23390 100mM reduced stimulated release. Agonists Bromocriptine and SKF38390 did not produce any effect alone but when tested with antagonists that reduce stimulated release, Bromocriptine 10mM further decreased stimulated release. SKF38390 10mM partially reversed the Spiperone-induced inhibition. Acetylcholine 100mM reduced spontaneous and stimulated release. Nicotine 1mM increased spontaneous while Anatoxin-a 10nM increased both releases. GABA agonist Muscimol 100mM and antagonists Bicuculline 100mM and Picrotoxin 100mM decreased stimulated release. Glycine 300mM increase stimulated release while Adenosine 1mM reduced. Glutamate transporter inhibitors PDC and HAA (transportable) enhanced stimulated release but non-transportable inhibitors DHK and TBOA had no effect at 100mM.

Conclusions: Our results argue a) against the glial release of glutamate; b) positive effects of NMDA when tissue is previously depolarized by AMPA, Kainate or absence of Mg²⁺; c) positive effect of glycine and nicotinic agonists; d) D1 agonists counteract D2 antagonists inhibition while D2 agonists further inhibit D1 antagonist's inhibition; e) GABA agonist and antagonists inhibited KCl-stimulated glutamate release.

Palavras-Chave: Striatum glutamate superfusion neurotransmitters slice

Apoio Financeiro: FAPESP 2003/04408-2 AND 2002/04545-6

INFLUÊNCIA DO TEMPO DE PREPARAÇÃO NO DESEMPENHO EM TAREFAS SIMPLES E COMPLEXA

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Objetivo: Investigamos a influência do tempo de preparação no desempenho de uma tarefa motora simples e uma tarefa motora complexa. **Métodos:** testamos 12 indivíduos jovens destros. Os voluntários realizavam uma tarefa em que deviam despressionar e pressionar novamente uma tecla e outra tarefa em que deviam despressionar e pressionar sequencialmente duas teclas de modo a completar quatro submovimentos. Cada voluntário realizava uma seqüência específica de submovimentos, com o dedo médio esquerdo, direito ou ambos simultaneamente. O estímulo deflagrador da resposta era o piscar de um disco no local da fixação ocular. Este estímulo era apresentado em um monitor posicionado à frente do voluntário e era precedido de 300, 1000 ou 2000 ms pelo piscar de três discos localizados 2° acima, um na mesma vertical, um 2° à esquerda e outro 2° à direita. Sendo que na tarefa de tempo de reação simples o voluntário liberou e pressionou apenas uma vez as chaves, e na tarefa de tempo de reação complexa, foram quatro vezes, nas 30 tentativas que compunham cada um dos três blocos de cada sessão. **Resultados:** nas duas tarefas, o tempo de reação foi menor para o intervalo de 1000 ms (média± e.p.m: 237 ± 15 ms $p < 0,001$ na tarefa complexa e 214 ± 8 ms $p = 0,009$ na tarefa simples) e de 2000 ms (respectivamente 220 ± 13 ms, $p < 0,001$, e 214 ± 8 ms, $p = 0,003$) do que para o intervalo de 300 ms (297 ± 19 ms e 229 ± 13 ms). Na tarefa simples o tempo de reação foi menor na condição unimanual direita (205 ± 5 ms) do que na esquerda (223 ± 9 ms, $p = 0,008$) e na bimanual (esquerda 223 ± 6 ms e direita 225 ± 7 ms; $p = 0,02$ para ambos). **Conclusão:** o aumento do tempo de preparação aproxima o desempenho em uma tarefa complexa ao desempenho em uma tarefa simples. Pelo menos em tarefas simples, o modo de resposta (unimanual ou bimanual) influencia o desempenho.

Palavras-Chave: tempo de reação, tarefa motora simples, tarefa motora complexa

Apoio Financeiro: Fapesp, Pró-Reitoria de Pesquisa da USP

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AFFORDANCE AND HANDEDNESS RECOGNITION

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OBJECTIVES: In handedness recognition tasks, there is an implicit activation of motor circuits involved with the real movement of the hand. It has been shown that these motor circuits can also be implicitly activated by the simple visualization of an object (affordance). We investigated if the affordance of a visual object facilitates handedness recognition of hand drawings. Moreover, we evaluated whether the Manual Reaction Time (MRT) to handedness recognition is faster when the hand drawing is in the best orientation to interact with the visual object.

METHODS: Sixteen right-handed volunteers participated in the study. The visual object was presented at the center of the screen (door handle or kettle) during 1500ms. Then, it disappeared after 1000ms, and the figure of the left or right hand appeared in each of several orientations (0° – palm down / 90°- thumb upward). The task was to press the right key for the right hand drawing and the left key for the left hand drawing. MRTs were used in an ANOVA with the factors: Object (door handle/kettle), Compatibility (compatible/incompatible) and Hand orientation (0°/90°). The compatibility factor was a interaction between affordance of the visual object (to the right or left) and the hand drawing (right or left hand).

RESULTS: The results showed that the compatibility ($F(1,15)=6.01$; $p<0.029$) and hand orientation ($F(1,15)=47.31$; $p<.001$) factors were significant. MRT to compatible conditions (561ms) were 12 ms faster than incompatible conditions (573ms). MRT to the 0° orientation (603ms) was 72ms slower than the 90° orientation (531ms). There was an interaction between hand orientation and object ($F(1,15)=5.94$; $p<0.027$). MRT when a door handle appeared and the hand drawing was in 0° orientation (598ms) was slower than if the hand drawing was in 90° orientation (536ms). MRT when appeared a kettle and the hand drawing was in 0° orientation (607ms) was slower than if the hand drawing was in 90° orientation (525ms).

CONCLUSIONS: These results show that the affordance of the visual object may facilitate the handedness recognition, but, the representation triggered by affordance is not a action in a specific orientation.

Palavras-Chave: Affordance, Handedness recognition, Action representation, Manual Reaction Time

Apoio Financeiro: CNPq, CAPES, FAPERJ, PIBIC-UFF/CNPq, PROPP-UFF

COVERT SPATIAL COMPATIBILITY TASK MODULATES THE SIMON EFFECT

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Objectives. The spatial compatibility task depends on the spatial correspondence between response and stimulus positions and reaction time is faster when they are compatible. In Simon task, even though spatial localization is irrelevant, reaction time is nonetheless faster when there is correspondence between stimulus and response positions. It has been shown that when a Simon task followed a spatially incompatible task, there is a reduction or an inversion of the Simon effect. In this work, we studied the modulation of the Simon effect by a previous spatial compatibility task in which there is no real movement of the hands but just a covert reaching response toward the stimulus (compatible condition) or away from the stimulus (incompatible).

Methods. Sixteen volunteers performed simulated compatibility tasks (eight-compatible and eight-incompatible). After that, they performed a Simon task in which the relevant parameter is shape.

Results. In the compatible group, MRTs were shorter in the correspondent than in the non-correspondent condition, resulting in a Simon effect of 26 ms ($F(1,7) = 19.41226$ $P = 0.0031$). In the incompatible group, on the other hand, there is no significant difference between correspondent and non-correspondent conditions ($F(1,7) = .398849$, $P = 0.5477$) and a reverse Simon effect of -7 ms (not significant) was found.

Conclusions. These results show that the long-term memory modulation elicited by a working memory task is present even when the participants just imagine an action in spatially compatible or incompatible conditions.

Palavras-Chave: Spatial compatibility, Simon effect, short-term memory, long-term memory, Reaction time

Apoio Financeiro: CNPq, FAPERJ, CAPES, PROPP-UFF, PIBIC/UFF-CNPq

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The role of instruction on mental rotation of objects and body parts.

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Objectives. The Manual Reaction Time (MRT) for the mental rotation of inanimate objects is a linear function of angular displacement. Mental rotation of drawings of the human hand, however, is constrained by biomechanical factors. For instance, the 90deg lateral (90L) rotation of the hand takes more time to perform mentally than a 90deg medial (90M) rotation, because, in the former, the actual movement is harder to perform. Methods. In the first part of this work, we presented drawings of five-petaled daisies oriented at 0, 90 (90L), 180 and 270 (90M) deg and measured the MRT for each orientation during a laterality judgment test. Two groups of twelve volunteers were tested. In group 1 (DAISY), they should consider the figure as a FLOWER and press a left or right key according to the relative position between the fifth petal and the stem. In group 2 (HAND), subjects should consider the drawing as a palm view of a HAND and press the left or right key according to its laterality.

Results. Our main results are: (i) for the DAISY group, MRT varies only with the rotation angle ($F(3,11)=9.2262$, $p<.0002$ and there is no lateral/medial MRT difference (894ms and 657ms, respectively); (ii) for the HAND group, MRT varies with the rotation angle ($F(3,11)=13.657$, $p<.0001$) and MRT is longer for the 90L (1770ms) than the 90M (1232ms) rotation ($p<.05$). These results show that the same visual stimulus (a daisy) may be processed as an external object (a flower) or as a body part (a hand) depending on instruction. In the second part, we presented palm view hand drawings oriented at 0, 90 (90L), 180 and 270 (90M) deg and instructed the sixteen participants to rotate the hand drawing to the vertical position before judging the thumb position (left or right). MRT varies with the rotation angle ($F(3,11)=38.3385$, $p<.0001$), but there is no lateral/medial MRT difference (1109ms and 966ms, respectively). These results show that a hand drawing may be processed as an external object.

Conclusions. Since the cortical areas processing external objects and body parts are distinct, our results suggest that it is the instruction and not the visual stimulus per se that triggers the cortical stream involved in a mental rotation task.

Palavras-Chave: Mental rotation, Handedness recognition, Reaction time, Cortical streams

Apoio Financeiro: CNPq, FAPERJ, CAPES, PROPP-UFF, PIBIC/UFF-CNPq

PRIMING ELICITED BY A HAND DRAWING IS TASK-SENSITIVE

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OBJECTIVES. A visual stimulus (letter, word, object) can have a lasting effect (facilitation or inhibition) on the processing of subsequent stimuli. Here, we investigated the effects of presentation of hand drawings (left/right hand, back/palm view) on Manual Reaction Time (MRT) to subsequent hand drawings. These drawings were equal or differed in compatibility (laterality), congruency (view) or color.

METHODS. There were three different tasks. In all tasks, a non-informative black hand appeared for 200ms in the center of a screen. After 1000ms, a second hand appeared at the same location and remained on until the left or right switch was pressed.

RESULTS. In the first task, the response was based on handedness of the second hand, that is, for a left (right) hand drawing, the correct response was to press the left (right) switch. An ANOVA employing compatibility (comp) and congruency (cong) as factors showed that only comp has a significant effect on MRT. When the handedness of both stimuli is equal, MRT (747ms) is shorter ($F(1,7)=9,74$; $p<0,0167$) than when they differ (788ms). In the second task, the response key was selected according to the view (back/palm) of the second stimuli. An ANOVA employing comp and cong as factors showed a main effect of comp ($F(1,7)=10,8841$; $p<0.0131$) and an interaction between comp and cong ($F(1,7)=21.98308$; $p<0.0022$). When handedness is similar, MRT is shorter (436ms) than when they differ (451ms). However, this difference only occurs between the comp/cong condition (same laterality/view - 427ms) and the others, which did not differ among them (446, 455 and 447ms - comp/incong, incong/cong and incong/incong, respectively). The third task was similar to those described above, except that response was selected according to the color of the second hand drawing. We found that handedness or view of the first stimulus has no effect on MRT to the second one in task III (that is, we do not find comp nor cong effects).

CONCLUSIONS. These results show that presentation of a body part influenced MRTs to a second stimulus only in tasks in which its configuration (handedness or view) is used to select the response.

Palavras-Chave: priming, handedness recognition, hand, reaction time, compatibility

Apoio Financeiro: CNPq, FAPERJ, CAPES, PROPP-UFF, PIBIC/UFF-CNPq

DEFICIT OF BIOLOGICAL MOTION PREDICTION IN CEREBELLAR PATIENTS

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Objective: Recent literature proposed the use of internal models in action-observation in order to infer motor commands from kinematics produced by others during perceptual inference (1). However, the cerebellum's specific role in prediction mechanisms triggered by action observation remains unclear. The aim of this study was to investigate the effect of a cerebellar lesion in a prediction task consisting in estimating the final position of a moving point.

Methods: Eleven healthy subjects (5 men and 6 women, 27±8 years old) and three cerebellar patients (3 men, 54±3 years old; lesion of vermis) participated in this experiment. The study consisted in estimating the end point position of the shoulder's trajectories in Sit to Stand (STS) or Back to Sit (BTS) conditions, represented by one dot. For the two first conditions, the dot moved on the screen following STS or BTS kinematics. For the two others, a conflict was introduced between trajectory and its velocity profile: STS motion was displayed with a velocity profile corresponding to BTS movement (STS non biological). Inversely, BTS motion was displayed with the STS velocity profile (BTS non biological). Only 65% of the trajectory was displayed. Subjects gave their responses using the mouse. We measured the constant and variable errors in mm. Control task consisted in estimating the vanishing position of the moving dot in the four above described conditions.

Results: A test of homogeneity between the two populations demonstrated that cerebellar patients were less accurate and more variable than healthy subjects to infer the hidden part of biological motion ($p=0.045$; $p=0.011$ for the STS and BTS condition, respectively). In contrast, no statistical difference was found for non biological STS and BTS conditions ($p=0.12$; $p=0.25$ respectively). Finally, cerebellar and control subjects behaved similarly to estimate the vanishing point position in biological profiles.

Conclusion: This behavioral study demonstrates the cerebellum involvement during a biologically tuned perceptual prediction task. Classically implicated in movement control, the cerebellum appears also be involved into prediction ability during motion observation.

References: 1- Neuroreport. 14:1, 2003

Palavras-Chave: biological motion; cerebellum; prediction ability; internal models; perceptual inference

Apoio Financeiro: CAPES

Prereceptor conditioning and object interaction in electric image generation.

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Objectives: Electroreceptive fish detect nearby objects by processing the information contained in the pattern of electric currents through the skin (the electric image), resulting from its own electric organ discharge. Any object (whose impedance differs from that of the water) perturbs the field generating a signal, the object's perturbing field¹. This object not only generates its own image, its perturbing field influences other objects' images². This fact poses interesting questions regarding the contribution of different objects present in the scene to a given electric image, even the contribution of the fish body, an object itself, as a pre-receptor structure³.

We used a realistic model of *Gnathonemus petersii* for analyzing the pre-receptor conditioning effect of the bodily structures as well as the interaction among objects of different characteristics in the generation of electric images.

Methods: Electric images and electric fields were calculated by a realistic model based on the boundary elements method (BEM)² that allows the study of the influence of different parameters of the scene (water, tissues and objects conductivity; objects geometry, location, etc.) on the generation of the electric image.

Results: The high conductivity of the fish body enhances electric images, by changing the electric field in magnitude and orientation. The high conductivity of the fish skin also amplifies the electric image, this effect being maximum at the mormiromast epithelium, where electroreceptors are embedded. The image generated by two or more objects differs from the sum of the individual image of each object, in a manner depending on objects' shape, impedance and relative position.

Conclusions: The body of the fish is a pre-receptor structure, exerting this effect by the interaction of its own object's perturbing field with the object's perturbing field of other things present in the scene. Two or more objects will generate an electric image that is composed by the effect of each object on the fish's electric field plus the effect each object exerts on the others.

1. *J. Exp. Biol* **35**:486, (1958).

2. *Biosystems* **71**:169, (2003).

3. *PLoS Comp Biol* **1**: e16, (2005).

Palavras-Chave: active electrolocation, image generation, prereceptor conditioning, action/perception cycle

Apoio Financeiro: PEDECIBA, Uruguay. PDT, Uruguay

D.09.008

Virtual hand Illusion induced by visual-proprioceptive and motor correlations

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Objectives: Our body schema gives the subjective impression of being highly stable. However, a number of easily-evoked illusions illustrate its remarkable malleability. The rubber-hand illusion evokes ownership towards a rubber-hand placed by providing synchronous visual and tactile stimulation to the visible rubber and the hidden real arm (Botvinick and Cohen, Nature 391: 756, 1998). We have shown that this illusion also occurs in virtual reality (Slater et al., Frontiers in Human Neuroscience, 2008, in press). Our objective here has been to explore the extent to which illusions of ownership can be induced only by visuo-proprioceptive-motor correlations. With that purpose we have used a data-glove that detects hand and finger positions and transmits them to a virtual hand. **Methods:** Fourteen subjects moved their hidden right hand wearing this data-glove. Visual feed-back was provided by a 3D stereoscopic virtual hand projected out of their shoulder. Subjects were given the task of counting with their fingers. This continued for 3 min, after which the virtual arm slowly drifted towards the left for 20s. Before the 3 min of hand movement a beep signalled that the subject should indicate the position of his right forearm by sticking blue-tack under the table level with his forearm – and repeated after the final 20s. Each subject was exposed to 2 conditions in random order: synchronous and asynchronous visuo-motor correlations. Illusion of ownership was measured by means of questionnaires. Proprioceptive displacement provided an objective measure. **Results:** Questions related to ownership gave significantly larger values for the synchronous than for the asynchronous condition. The median and interquartile ranges of the proprioceptive displacement were 1.2 ± 3.5 cm for the synchronous and -2.0 ± 4.5 cm for the asynchronous, where positive is towards the final position of the virtual hand. The difference is significant ($P = 0.01$, Paired Sign Rank test). The correlation between the feeling of ownership of the virtual arm and the size of the drift was significant (Spearman's $\rho = 0.45$, $P = 0.02$). **Conclusions:** We conclude that synchronicity between visual and proprioceptive information along with motor activity are able to induce an illusion of ownership over a virtual arm.

Palavras-Chave: virtual reality, multisensory correlations, body ownership

Apoio Financeiro: PRESENCCIA, EU FP6-027731.

D.09.009

O EFEITO MODULATÓRIO DA AÇÃO NA ESTIMATIVA DE INTERVALOS TEMPORAIS VISUAIS

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OBJETIVOS: Um crescente corpo de evidências tem demonstrado o efeito modulatório de ações motoras em diversos aspectos da percepção sensorial. Dentre esses efeitos, está a redução da latência perceptiva de estímulos visuais ou auditivos que são causados por atos motores voluntários. Nesse trabalho, investigamos a modulação da ação na percepção de intervalos de tempo entre estímulos visuais.

MÉTODOS: 10 participantes foram submetidos a uma tarefa psicofísica visual na qual deveriam estimar o intervalo de tempo passado entre o apagar de um estímulo na tela de um monitor (ponto de fixação, PF) e o surgimento de um estímulo alvo. Fisicamente, esses intervalos poderiam durar 250, 300, ou 350 milissegundos. Os participantes foram testados em duas condições. Numa delas (PASSIVA), o instante em que o ponto de fixação sumia era controlado pelo computador. Na outra (ATIVA), o participante determinava o desaparecimento do PF por meio de um ato motor (apertando uma alavanca).

RESULTADOS: Nas duas condições, os participantes superestimaram a duração do intervalo entre o apagar do PF e o estímulo alvo. Na condição ATIVA, em que os participantes ativamente causavam o desaparecimento do PF, a superestimativa foi estatisticamente menor do que na condição PASSIVA ($F(1,9)=10,14$; $P=0,01$). Além disso, observamos que a superestimativa diminuiu ao longo dos intervalos em ambas as condições, sendo os intervalos curtos mais superestimados do que os intervalos longos ($F(2,18)=8,08$; $P<0,01$).

CONCLUSÕES: Uma vez que a previsibilidade temporal sobre o instante de ocorrência do alvo era a mesma nas duas condições, concluímos que o planejamento e/ou a execução de um ato motor voluntário foi capaz de modular a estimativa de intervalos temporais, provavelmente reduzindo a latência com que o alvo era percebido.

Palavras-Chave: percepção tempo ação latências

Apoio Financeiro: FAPESP

D.09.010

RELAÇÃO ENTRE AÇÃO E PREVISIBILIDADE NA MODULAÇÃO DE LATÊNCIAS PERCEPTIVAS

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Objetivos: Estudos recentes têm demonstrado o efeito modulatório de ações motoras em diversos aspectos da percepção sensorial. Dentre esses efeitos, está a redução da latência perceptiva de estímulos causados por atos motores voluntários. Porém já foi sugerido que esta modulação pode ser apenas causada por mecanismos atencionais. O objetivo deste trabalho é diferenciar as modulações atencionais e da ação voluntária sobre latências perceptivas.

Métodos: 12 voluntários realizaram quatro sessões experimentais na quais deveriam julgar se um estímulo visual (flash) e um estímulo auditivo (tom) eram apresentados simultaneamente ou não após o desaparecimento de um ponto de fixação (PF). Em uma sessão (Sem Ação-Intervalo Fixo) o instante em que os estímulos eram apresentados era controlado pelo computador, mas o intervalo entre o desaparecimento do PF e aparecimento dos estímulos era fixo. Em outra sessão (Sem Ação-Intervalo Aleatório) o intervalo entre o desaparecimento do PF e aparecimento dos estímulos era aleatorizado. Nas sessões Ação-Intervalo Fixo e Ação-Intervalo Aleatório, o participante determinava o aparecimento do estímulo auditivo (tom) por meio de um ato motor (apertando uma alavanca).

Resultados: A distribuição de respostas simultânea/não-simultânea foi analisada. Os dados sofreram tratamento estatístico e o ponto de simultaneidade subjetiva (PSS) foi encontrado para cada sessão. Uma ANOVA de dois fatores com medidas repetidas (Ação X Sem Ação; Intervalo Fixo X Intervalo Variável) foi realizada, sendo encontrado um efeito significativo para a interação Ação X Intervalo ($F(1,11)=7,04$; $P<0,025$). O PSS e, conseqüentemente, a latência do tom, na condição Ação-Intervalo Fixo era significativamente menor do que nas outras três sessões.

Conclusão: Estes resultados mostram que a presença da ação era fundamental para a diminuição da latência do tom sugerindo que esse efeito não é mero desdobramento de mecanismos atencionais.

Palavras-Chave: ação percepção atenção

Apoio Financeiro: FAPESP

D.09.011

REDUÇÃO DE LATÊNCIAS PERCEPTIVAS VISUAIS POR MEIO DE AÇÕES MOTORAS VOLUNTÁRIAS

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OBJETIVOS: Ações motoras voluntárias são capazes de modular diversos aspectos da percepção sensorial. Neste trabalho, investigamos o efeito modulatório da ação sobre a latência perceptiva de estímulos visuais.

MÉTODOS: Realizamos três experimentos psicofísicos em que os participantes deveriam julgar o alinhamento entre um estímulo móvel e um fixo. Os três experimentos possuíam duas condições: uma PASSIVA, em que o marcador temporal utilizado pelos voluntários para efetuar o julgamento era gerado pelo computador; e uma ATIVA, em que esse marcador era gerado pelo próprio sujeito, por meio de uma ação motora (o movimento do dedo indicador). Em ambos os casos, esse marcador era um estímulo visual de início abrupto. No Experimento 1, o estímulo móvel e o abrupto (marcador temporal) eram apresentados em lados opostos do campo visual, com a mesma excentricidade de 7°. No Experimento 2, a estimulação visual era a mesma do Experimento 1, sendo, porém, injetado um atraso de 250 ms entre a ação e o aparecimento do marcador. No Experimento 3, o marcador temporal foi apresentado centralmente (próximo ao ponto de fixação) e o estímulo móvel a uma excentricidade de 7°.

RESULTADOS: Em todos os experimentos, os participantes perceberam um desalinhamento entre o estímulo móvel e o fixo: quando ambos estavam fisicamente alinhados, o estímulo móvel era percebido como estando à frente do estímulo fixo (ilusão conhecida como *efeito flash-lag*). Esse desalinhamento foi significativamente diminuído nas condições ATIVAs em todos os experimentos, quando comparados com as condições PASSIVAs ($P < 0,05$). O efeito da ação foi amplificado quando o atraso de 250 ms foi injetado (Experimento 2, $P < 0,05$). No Experimento 3, a apresentação central do marcador temporal causou uma inversão significativa do desalinhamento perceptivo (*efeito flash-lead*, $P < 0,05$).

CONCLUSÕES: O planejamento e/ou a execução de um ato motor voluntário é capaz de modular a latência perceptiva de estímulos visuais. Esse efeito modulatório da ação depende de fatores como a existência de atrasos entre a ação motora e a consequência sensorial dessa ação, assim como do local de apresentação dos estímulos no campo visual.

Palavras-Chave: ação, percepção visual, tempo

Apoio Financeiro: FAPESP

DIVISION OF LABOUR BETWEEN THE M AND P VISUAL PATHWAYS: DIFFERENT VISUAL PATHWAYS MINIMIZE JOINT ENTROPY DIFFERENTLY

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Visual perception and action are strongly linked with parallel processing channels extending from the retina through the lateral geniculate nucleus (LGN) to the input layers of the primary visual cortex (V1). Achromatic vision is provided by at least two of such channels formed by the M and P neurones, which connect the retinal inner plexiform layer to the magnocellular and parvocellular layers of the lateral geniculate nucleus (LGN) and to V1 layers 4C α and 4C β , respectively. These cell pathways are similarly organized in primates having different life styles, including species that are diurnal, nocturnal, and exhibit a variety of colour vision phenotypes. We describe the M and P cell properties by 3D Gábor functions and their 3D Fourier transform. The M and P cells occupy different loci in the Gábor information diagram or Fourier Space. This separation allows the M and P pathways to transmit visual signals with distinct 6D joint entropy for space, spatial frequency, time, and temporal frequency. By mixing M and P influences on the cortical neurones placed beyond V1 entrance layers, different cortical pathways can process aspects of visual stimuli with a better precision than it would be possible using the M or P pathway in isolation. This performance fulfills the requirements of different behavioural tasks.

Palavras-Chave: Vision, M and P pathways, Visual parallel processing, Fourier Space, Joint entropy, Information theory

Apoio Financeiro: CAPES, CNPq, CNPq / FUNTEC PRONEX, and FINEP-IBN Net.

D.10.001

Correlation between immediate early-gene expression and chronic multielectrode implants

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Multielectrode arrays have been used with great success to simultaneously record the activity of neuronal populations in behaving, awake animals.

In particular, there is great promise in the use of this technique to allow the control of neuroprosthetic devices by human patients. However, since the implicit rule of any therapy is to first do no harm, it is necessary to fully characterize in animal models, ahead of the human trials, the tissue response to the chronic implants. Immediate-early genes (IEGs) are rapidly and transiently expressed to a variety of stimuli, and are helpful in orchestrating cell response following injury. In the present study we set out to evaluate IEG expression levels in animals subjected to chronic multielectrode recordings.

Fifteen adult male Wistar rats were implanted with 32-channels multielectrode arrays (35 µm tungsten wires coated with Teflon) in the primary motor cortex. The animals were separated in three experimental groups: one, two and three months of implantation. The animals were weekly recorded in order to verify the level of cortical activity during the period of implantation. At the end of the recording period, animals were perfused with phosphate-saline buffer and 4% paraformaldehyde. The tissue was sectioned frontally at 20 µm in a cryostat and submitted to immunohistochemistry for IEGs *egr-1* and *c-fos*. We verified tissue integrity with cresyl-violet staining and immunohistochemistry for neurofilament M (NF-M), a cytoskeleton constituent specifically found in neuronal cells. There was no evidence of pronounced cell loss, and staining for NF-M was equivalent in both the implanted and contralateral hemispheres. Most importantly, chronic multielectrode implants did not significantly alter the expression of both *egr-1* and *c-fos* around implanted sites, in comparison with non-implanted animals.

Our results indicate that chronic multielectrode implants do not substantially alter the functionality of target brain regions. **Palavras-Chave:** Multielectrode; immediate-early genes; electrophysiology; biocompatibility; rat.

Apoio Financeiro: AASDAP; Duke University; FINEP.

D.10.002

Microelectrodes neural recording stability for brain-machine interface

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Objectives: Significant advances in multielectrode design for multichannel neural signal recordings have allowed improving experimental approach to develop human-brain interfaces. There are essential aspects, as electrophysiological and tissue responses, related to chronic recording of neural ensemble that should be clarified.

Methods: All procedures were approved by the local Ethical Committee and performed in accordance with NIH guidelines. Twenty adult Wistar rats, 250-350g, were implanted with a microelectrode array of 4 by 8 Teflon coated tungsten sharp microwires (50mm diameter separated by 300mm) in the left motor cortex (1-3mm rostral to bregma, 2-3mm lateral to bregma, 1.8-2.0mm depth for layer V of M1). Rats were anesthetized with Ketamine (100mg/kg) and Xylazine (5mg/kg) following induction with Halothane. Single-cell and multiunit activity were recorded (Plexon Inc, TX) during the microwire lowering in a stereotaxic for best quality and quantity. Cianoacrilate adhesive was used to fixate firmly the set to the skull bone and to four mini screws. Rats were recorded moving freely, once a week, during 30 minutes. Only cells that had a clearly identified waveform were labeled and considered during the session.

Results: In average 72 (sd 21) different cells were chronically recorded and the recording sessions ended when no waveform was identified (12-20 weeks). The perfused brain was sectioned frontally at 20 µm to evaluate the tissue. Nissl staining showed the electrode tracks with absence of pronounced cell loss. There was an increased glial activation around electrode tracks as revealed by immunohistochemistry for GFAP and ED-1. There was a minimal loss of cells, as indicated by caspase-3 immunohistochemistry. There was normal activation of immediate early genes (IEG) *egr-1* and *c-fos* and expression of calcium-binding proteins parvalbumin, calretinin and calbindin. There was equivalent cytoskeleton (NF-M) in both hemispheres.

Conclusions: Although a progressive level of waveform degradation was found, immuno-hystological data indicated that chronic microelectrode arrays do not substantially alter the functionality of the brain. In this way, more detailed researches need to elucidate the dynamic binding between brain and microelectrodes to allow a durable brain-machine interface. **Palavras-Chave:** brain-machine interface, motor control, chronic microelectrode signal, immunohystology, multiunit recording

Apoio Financeiro: Alberto Santos Dumont for Research Support, Sírio Libanês Hospital, FINEP, CNPq, DUKE

E.01.001

Atividade de sistema ocitocinérgico sob estimulação osmótica e ação estrogênica.

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Objetivos: Há receptores para estrógeno(E₂) nos núcleos paraventricular (PVN) e supraóptico (SON) do hipotálamo e os esteróides ovarianos podem influenciar o balanço hídrico. O objetivo desse trabalho foi analisar atividade de sistema ocitocinérgico sob estimulação osmótica e ação estrogênica.

Métodos: Ratas Wistar ovariectomizadas (OVX) tratadas por três dias com estrógeno (10 µg/0,1 ml/animal) ou veículo (óleo vegetal) foram divididas em três grupos: I - com alimentação *ad libitum*; II- jejum por 48h; III- realimentação por 2h após jejum por 48h. No dia do experimento às 15h, os animais foram anestesiados para coleta de sangue (separação do plasma para determinação da osmolalidade e da concentração de ocitocina-OT por radioimunoensaio), seguida de perfusão para remoção do cérebro para imuno-fluorescência para OT e FOS, no PVN (porções magnocelular anterior-MA e magnocelular medial-MM) e no SON. **Resultados:** A osmolalidade plasmática (mOsm/KgH₂O) dos animais realimentados tratados com veículo (318,1±1,36) ou E₂ (320±3,83) foi significativamente maior do que dos animais em condições *ad libitum* (305,4±1,6 e 309,7±1,98) e jejum (301,6±1,4 e 302±3,22) respectivamente. Não houve diferença significativa na osmolalidade dos animais tratados com veículo ou E₂. A concentração plasmática de OT (pg/ml) foi significativamente menor nos animais realimentados tratados com E₂ (21,6±2,8) quando comparada àquela dos animais realimentados tratados com veículo (50,7±9,0). Houve ativação de neurônios OT em ratas OVX tratadas com veículo ou E₂, na situação de realimentação após jejum de 48h, mas não nas situações de alimentação *ad libitum* ou jejum por 48h. Entretanto, a porcentagem de neurônios que co-expressaram FOS-OT foi significativamente menor nas porções MA (12±5,36) e MM (3±1,89) do PVN e no SON (24,63±6,23) dos animais tratados com E₂ que nas porções MA (43,25±6,8) e MM (45,67±9,13) do PVN e no SON (52,6±7,76) dos animais tratados com veículo. **Conclusões:** O estrógeno exerce ação inibitória sobre a atividade dos neurônios OT e poderia modular a secreção de OT em resposta ao aumento da osmolalidade resultante da realimentação, após o jejum.

Palavras-Chave: Ocitocina, estrógeno

Apoio Financeiro: CAPES, CNPq, FAPESP.

**LA VIMENTINA COMO HERRAMIENTA PARA IDENTIFICAR TANICITOS
EPENDIMARIOS Y SUBEPENDIMARIOS DEL TRONCO ENCEFÁLICO.**

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Los tanicitos, células endimarias especializadas en el transporte de sustancias hacia y desde el líquido cefalorraquídeo, se han descrito en el tercer ventrículo. Pocos trabajos han descrito tanicitos en el cuarto ventrículo. **Objetivo:** En este trabajo se utiliza como herramienta a la vimentina (VIM), un filamento intermedio, para describir la distribución de tanicitos en el tronco encefálico de felinos. **Métodos.** Troncos encefálicos de gatos adultos fijados fueron seccionados mediante criostato para realizar los ensayos de inmunohistoquímica para detectar VIM, Neu-N (marcador neuronal) y GFAP (marcador glial). **Resultados.** Bordeando el acueducto y IV ventrículo, se observaron tanicitos endimarios marcados intensamente con VIM con prolongados procesos basales que se extienden en dirección a sectores ventro-mediales. De forma interesante, se detectaron células VIM+ en sectores subependimarios: un grupo de ubicación central (núcleos pontinos y bulbos del rafe) y dos grupos laterales, así como en el área subcomisural y área postrema. En núcleos del rafe, estas células eran similares a los tanicitos endimarios, con largos procesos basales que parecen relacionarse con procesos de tanicitos endimarios. En cambio, las células VIM+ laterales presentaron una morfología diferente: menor tamaño y cortas prolongaciones extendiéndose desde todo el soma. Observamos que las células VIM+ en el tronco encefálico no se marcaron ni con Neu-N ni con GFAP, mientras que, en un trabajo anterior, demostramos que se marcaron para la hormona concentradora de melanina (MCH). **Conclusiones.** Mediante el empleo de una técnica simple describimos la distribución de tanicitos endimarios y subependimarios en núcleos como el rafe dorsal, importante en regular distintas funciones como el ciclo sueño-vigilia. Estas células conformarían una especie de red conectadas con el ventrículo, lo cual apoyaría la hipótesis de que estas células transportarían sustancias desde el ventrículo regulando así la función de las neuronas del rafe y de sectores más ventrales del tronco encefálico. *Parcialmente financiado por PDT 76/36.*

Palabras-Chave: tanocytes, vimentin, raphe nucleus, MCH, ependyma

Apoio Financeiro: Proyecto de Desarrollo Tecnológico, Dinacyt 76/36. Uruguay

Déficit no desenvolvimento do cerebelo de camundongos mutantes para o receptor tipo beta do hormônio tireoidiano

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O desenvolvimento do cerebelo no período pós-natal caracteriza-se por uma intensa proliferação celular na camada granular externa (EGL) e pela migração das células granulares (CG) na camada molecular (ML), através das fibras da glia de Bergmann (GB), um tipo glial específico do cerebelo, formando então, a camada granular interna. Nestes eventos, o hormônio tireoidiano (HT) tem um papel fundamental. A maior parte dos efeitos biológicos do HT é mediada pela ligação do T3 aos seus receptores nucleares do tipo alfa (TRa) e beta (TRb). Neste trabalho, analisamos o desenvolvimento do cerebelo de animais contendo a mutação D337T para o gene TRb, o que impede a ligação do hormônio a essa isoforma de receptor. Ensaios *in vivo* revelaram drásticas alterações na morfologia cerebelar, com reduções em todas as camadas cerebelares do animal homozigoto (HO) em nove (P9) e vinte e um dias pós-natal (P21), levando a um cerebelo 22% ($p < 0,01$) e 30% ($p < 0,001$) menor, respectivamente, associado a severos prejuízos na sua foliação. Ensaios de proliferação *in vitro* e *in vivo* mostraram uma redução na incorporação do análogo de timidina, BrdU, de 34% ($p < 0,05$) e 23% ($p < 0,05$), respectivamente, pelos precursores granulares nos animais HO. Imunomarcagem para GFAP, marcador de GB, revelou uma redução no número de fibras de GB nos animais mutantes de 26% ($p < 0,001$) e 64% *in vivo* e *in vitro*, respectivamente, associada à drástica alteração na sua morfologia. Estes dados sugerem que tanto a proliferação quanto a migração de precursores granulares seja prejudicada pela mutação. A análise do cerebelo de animais mutantes adultos demonstrou severos déficits na arborização e organização das células de Purkinje, embora sem as alterações morfológicas previamente descritas para idades mais jovens. Em conjunto, nossos dados sugerem que a ação do HT via TRb pode regular eventos importantes da ontogênese cerebelar, como proliferação de precursores na EGL, migração das CG, maturação da GB e foliação cerebelar, contribuindo para o entendimento sobre algumas desordens neuroendócrinas.

Palavras-Chave: Cerebelo, foliação, glia de Bergmann, hormônio tireoidiano

Apoio Financeiro: CNPq, CAPES, FAPERJ e SBEM

E.01.004

Efeitos da expansão isotônica e hipertônica de volume extracelular sobre o conteúdo de catecolaminas e ativação neuronal em estruturas do hipotálamo

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OBJETIVOS: O objetivo principal deste estudo foi avaliar a participação de estruturas hipotalâmicas na modulação de vias simpáticas centrais envolvidas na manutenção da homeostase dos fluidos corporais em resposta a alterações de volume e osmolalidade plasmática.

MÉTODOS: A expansão do volume do líquido extracelular (EVEC) foi realizada em ratos machos conscientes através de injeção intravenosa de NaCl 0.15 M (EVEC-I) ou 0.3 M (EVEC-H) (2 ml/100 g p.c). Os animais (± 240 g p.c) foram decapitados antes e 5 minutos após EVEC-I e EVEC-H, os cérebros foram removidos e os núcleos paraventriculares (PVN) e supra-óptico (SON) e eminência mediana (EM) foram coletados. O conteúdo de noradrenalina (NA) e dopamina (DA) nestas estruturas foi determinado por cromatografia líquida de alto desempenho (HPLC) com detecção eletroquímica. Outro grupo de animais foi submetido à perfusão cardíaca 90 minutos após a EVEC para estudos imunoistoquímicos (Fos/Tirosina hidroxilase – TH). **RESULTADOS:** Nossos resultados mostraram redução no conteúdo de NA no PVN (66% e 51%), no SON (8% e 38%) e na EM (5% e 35%) após EVEC isotônica e hipertônica, respectivamente. Resultado similar foi observado em relação ao conteúdo de DA no SON (89% e 42%) e na EM (26% e 35%) após EVEC isotônica e hipertônica, respectivamente. No PVN apenas a EVEC-I produziu redução no conteúdo de DA (73%) enquanto a EVEC-H, aumento (37%). A EVEC-I ou EVEC-H não produziram alteração significativa no número de duplas marcações Fos-TH no PVN. No SON não foi observada expressão de Fos-TH em resposta a estes estímulos.

CONCLUSÃO Nossos dados sugerem que regiões hipotalâmicas possam estar envolvidas na modulação de vias simpáticas centrais recrutadas em resposta a expansão de volume sanguíneo e aumento de osmolalidade.

Palavras-Chave: noradrenalina, hipotálamo, osmolalidade, dopamina

Apoio Financeiro: CAPES, FAPESP, CNPq.

EFEITO DO HIPOTIROIDISMO INDUZIDO POR PROPILTIOURACIL NA OXIDAÇÃO DE NUTRIENTES ENERGÉTICOS NO SNC DE RATOS JOVENS

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Objetivo: Verificar o efeito da deficiência do hormônio da tireóide na oxidação de nutrientes energéticos (glicose, lactato e manose) em fatias de cerebelo de ratos com idades de 21 dias. Métodos: Foram utilizados 3 grupos (n=8filhotes+mãe): Controle, PTU e PTU+T3. Foi oferecido à rata(mãe) 0,05% de propiltiouracil(PTU) diluído na água de beber, do 1º dia pós-parto até o dia do experimento. No grupo PTU+T3 foi injetado a partir do 1º dia de vida até o dia do experimento 20µg/kg de 3,3',5-Triiodo-Tironina(Sigma), já no controle e no grupo PTU apenas NaOH 4uM. Foram incubadas fatias entre 45mg e 55mg em frascos, previamente aerados, por 1 hora com os respectivos nutrientes energéticos. Após parou-se a reação injetando 0,2ml de TCA 50% no frasco e 0,1ml de NaOH 2N no pocinho. A radioatividade foi medida em contador de cintilação líquida. Resultados: Os ratos hipotireoideos (PTU) mostraram maior incorporação de manose à proteína(7,17 cpm/mg) em relação ao grupo controle(5,19 cpm/mg) e ao grupo PTU+T3(5,64 com/mg), resultado igual ao da incorporação de manose à lipídio: controle(9,74 cpm/mg), PTU(12,34 cpm/mg) e PTU+T3(9,91 cpm/mg). Em relação ao lactato, o grupo PTU(1980 pmol) oxidou significativamente mais lactato do que o grupo controle(1455 pmol) e PTU+T3(1560 pmol), já na conversão de lactato à lipídio obtivemos o mesmo resultado: controle(92,31 pmol), PTU (218,89 pmol) e PTU+T3(123,25). Por último foi utilizada glicose, onde não encontramos diferença significativa na oxidação da mesma nos grupos utilizados: controle(625 pmol), PTU(609 pmol) e PTU+T3(610 pmol) e observamos na incorporação de glicose à lipídio que o grupo PTU(73,23 pmol) teve um resultado elevado em relação ao controle(52,03 pmol) e PTU+T3(58,41 pmol). Conclusões: A incorporação de manose à proteínas foi superior no grupo hipotireoideo em relação aos demais grupos, este resultado está de acordo com os resultados encontrados para a síntese protéica por outros grupos; sendo a justificativa para tal achado que o SNC dos ratos hipotireoideos é menos desenvolvido, portanto apresenta uma síntese maior de proteína. Tal explicação não cabe para a oxidação do lactato, pois ele aumenta com a idade.

Palavras-Chave: Hipotiroidismo, Propiltiouracil, Sistema Nervoso Central

Apoio Financeiro: CNPq, PROPESQ/UFRGS, FAPERGS, FINEP - Rede Brasileira de Neurociência (IBNnet)

E.01.006

Co-expression of leptin and estrogen receptors in the preoptic-hypothalamic area.

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Objectives: There is an interaction between the reproductive axis and the energetic homeostasis. A possible mediator of this interaction is the leptin, a hormone that acts in the control of metabolism, feed behavior and reproduction. Homozygous animals for the gene “ob” (ob/ob) do not produce leptin, and present obesity and infertility. These effects are reversed after systemic administration of leptin. On the other hand, the estrogen can modulate the leptin secretion from adipocytes through estrogen receptor (ER) -a and -b. The aims of this work were verify: 1- if there is co-expression of leptin and ER-a or -b in the medial preoptic area (MPOA) and in the hypothalamic nucleus arcuate (ARC), dorsomedial (DMH) and ventromedial (VMH); 2- if there is change of the leptin gene expression in these brain areas, pituitary and adipose tissue from estrogen-primed and -unprimed ovariectomized (ovx) animals. **Methods:** Wistar female rats with regular estrous cycles were utilized to verify the possible co-expression of leptin and ER-a or -b by immunofluorescence. To confirm the co-expression was used confocal microscopy. Estrogen-primed and -unprimed OVX rats were utilized to analyze the leptin gene expression by technique of Real Time-PCR. **Results:** It was verified co-expression of leptin and ER-a in the MPOA and DMH, but not in the ARC and VMH, as well as, of leptin and ER-b in the MPOA, DMH and ARC, but not in the VMH, in metestrous and proestrous. There was no statistical difference of leptin gene expression in the MPOA, DMH, ARC, pituitary and adipose tissue between estrogen-primed and -unprimed OVX animals. **Conclusion:** Our results indicate that estrogen may modulate directly the neurons that produce leptin in these brain areas related with reproductive function. Since the treatment with estrogen did not change the leptin gene expression, a putative modulation of estrogen on leptin neurons could be non genomic or a longer time of estrogen would be necessary to induce that expression.

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Palavras-Chave: Leptin, estrogen receptors, reproduction, MPOA, ARC, DMH.

Apoio Financeiro: FAPESP, CAPES, CNPq

E.01.007

A MUTAÇÃO RTbD337T EM CAMUNDONGOS ADULTOS ALTERA A EXPRESSÃO DAS PROTEÍNAS CNPase E MBP NO SISTEMA NERVOSO CENTRAL.

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Objetivo: Oligodendrócitos (OLs) pós-mitóticos expressam a isoforma beta1 de receptor de hormônio tireoideano (RTb-1) que é a isoforma envolvida nos efeitos de tri-iodotironina (T3) na maturação oligodendrocítica. Sendo assim, avaliamos a expressão da 2'3' nucleotídeo cíclico 3' fosfodiesterase (CNPase - marcador precoce de OLs, relacionado ao embainhamento) e da proteína básica de mielina (MBP – marcador tardio de OLs, expresso em mielina compacta), através da identificação por imunoblotting, no cerebelo e corpo caloso de camundongos adultos com uma mutação restrita no RTb (RTbD337T).

Métodos: Foram utilizados camundongos mutantes RTbD337T adultos heterozigotos (RTb^{mut/+}) e homozigotos (RTb^{mut/mut}), e selvagens. Os cerebelos e corpos calosos foram dissecados, homogeneizados e submetidos a Western blotting, utilizando-se anticorpos monoclonais anti-CNPase (Sigma 1:1000) e anti-MBP (Serotec 1:1000). A revelação foi feita através do método da avidina-biotina-peroxidase.

Resultados: A MBP não apresentou diferença significativa no cerebelo dos grupos mutantes, em comparação com os animais selvagens. Já no corpo caloso, um feixe compacto composto majoritariamente de fibras mielinizadas, os níveis das isoformas 14.0 e 21.5kDa da MBP se apresentaram aumentados no mutante RTb^{mut/+}. A expressão da CNPase, no corpo caloso e cerebelo dos mutantes RTb^{mut/mut} não apresentou diferença significativa. Já, os mutantes RTb^{mut/+} apresentaram a expressão da CNPase aumentada, em comparação com os selvagens, particularmente no cerebelo, uma região com fibras densamente compactadas na substância branca, mas também com fibras mielinizadas em padrão disperso nas folhas cerebelares.

Conclusão: A expressão da MBP e da CNPase é alterada nos animais RTb^{mut/+} adultos, sugerindo uma possível interferência da mutação restrita do RTb nas funções reguladoras do gene nos receptores normais (selvagens) *in vivo*. O fato dos marcadores apresentarem diferenças regionais, sugere uma função relevante do RTb na expressão normal daquelas proteínas, durante a maturação do oligodendrócito, influenciando na interação oligodendrócito-axônio e na mielinogênese.

Palavras-Chave: Oligodendrócito, Receptor beta do hormônio tireoideano, CNPase, MBP

Apoio Financeiro: CNPq, FAPERJ, SR2-UERJ

E.01.008

EFEITO DA ADMINISTRAÇÃO CENTRAL DE LOSARTAN NO APETITE AO SÓDIO EM RATAS OVARIECTOMIZADAS E SUBMETIDAS À DIETA HIPOSSÓDICA.

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Diversos estudos demonstram que a sede e o apetite ao sódio constituem comportamentos homeostáticos dependentes da ativação de receptores de angiotensina II (ANGII) cerebrais, AT1. Portanto, o objetivo deste trabalho foi avaliar o efeito da administração intracerebroventricular (*icv*) de losartan (LOS), um antagonista AT1, no apetite ao sódio e resposta dipsogênica em ratas ovariectomizadas (OVX) e OVX tratadas com estrógeno submetidas a uma dieta crônica deficiente em sódio.

Ratas Wistar (60 dias) foram distribuídas em 4 grupos experimentais: **I.** Ratas OVX tratadas com óleo, *sc* + salina 0,9% (2µl, *icv*) – grupo OVX-CONT; **II.** Ratas OVX tratadas com óleo, *sc* + LOS (50µg/2µl, *icv*) - grupo OVX-LOS; **III.** Ratas OVX repostas com benzoato de estradiol (E2) 20µg/animal/dia, *sc* + salina, (2µl, *icv*)- grupo OVX-E2; **IV.** Ratas OVX repostas com E2, *sc* + LOS (50µg/2µl, *icv*) - grupo OVX-E2-LOS. O tratamento com E2 foi iniciado 24h após a ovariectomia e, decorridos 8 dias de reposição estrogênica, iniciou-se a dieta carente em sódio (fubá de milho). Os animais foram colocados em gaiolas metabólicas providas de bebedouros graduados, contendo água como única fonte líquida. Após 4 dias, salina 1,8% foi oferecida em bebedouro simultaneamente à água, logo após a administração *icv* de LOS ou salina, sendo a avaliação da ingestão feita no período noturno, por 60-300min e após 24h. Em resultados preliminares, o grupo OVX-LOS apresentou uma diminuição na ingestão noturna de salina hipertônica em relação ao grupo controle (0,93±0,55ml vs 4,78±0,74ml, aos 240min, $p<0,05$ e 1,01±0,6ml vs 5,62±0,89ml, aos 300min, $p<0,01$) que se repetiu após 24h (2,0±0,82ml vs 9,62±2,17ml, $p<0,001$) não ocorrendo no grupo OVX-E2-LOS. Além disso, não houve alteração na ingestão hídrica.

Os resultados obtidos mostram a participação da ANGI na modulação do apetite ao sódio, através de receptores AT1 cerebrais em ratas OVX tratadas com dieta hipossódica. Enquanto que, o fato do grupo OVX-E2-LOS não ter apresentado alterações no apetite ao sódio como foi observado no grupo OVX-LOS, em relação aos respectivos controles, pode indicar uma possível modulação estrogênica da sinalização neuronal dos receptores AT1.

Palavras-Chave: Losartan, dieta hipossódica, angiotensina II

Apoio Financeiro: CNPq

E.01.009

Cholinergic stimulation in the Paraventricular Nucleus of the Hypothalamus (PVN) induces metabolic and cardiovascular responses

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Objective: The PVN is considered to be involved in cardiovascular and metabolic integration. The aim of this study was to assess if metabolic and cardiovascular adjustments that are controlled by the PVN are associated with cholinergic activity. We verified the effects of central cholinergic stimulation on plasma glucose and free fatty acid levels, and whether or not these effects are related to cardiovascular adjustments.

Methods: Male Wistar rats received unilateral Carbachol (1, 2 and 6 nmol) or NaCl (100nL) microinjections through guide cannulae chronically implanted into the PVN. A jugular catheter was used for blood sampling, and a cannula was inserted into the left femoral artery for cardiovascular monitoring, both also chronically implanted. Each animal was the control of itself in different days, and the injection of carbachol or NaCl was randomly chosen.

Results: PVN cholinergic stimulation increased, in a dose-dependent manner, mean blood pressure and plasma glucose levels, not affecting the mobilization of adipose tissue free fatty acids. Heart rate decreased with time, except for 1 nmol dose, which in minute 7 had a slight increase. Plasma glucose also increased its values with time, with a narrower correlation with blood pressure than with heart rate, its variation being probably due to baroreflex.

Conclusion: PVN cholinergic stimulation leads to an increase in specific sympathetic activity, as shown by the cardiovascular adjustments and the rise in hepatic production of glucose, not followed by an increase in the mobilization of adipose tissue free fatty acids.

Apoio Financeiro: CNPq, CAPES, Fapemig

Ovarian steroids regulate the activity of locus coeruleus and A2 noradrenergic neurons projecting to the medial preoptic area

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The preovulatory surge of luteinizing hormone (LH) is essential for ovulation and fertility in mammals. Brainstem noradrenergic neurons have long been implicated in LH regulation and are recognized as potential sites for ovarian steroids action in the brain. Because the rostral portion of the medial preoptic area (MPOA) is known to control LH release, we evaluated the effect of estradiol and progesterone on c-Fos expression in noradrenergic neurons projecting to the MPOA.

Ovariectomized (OVX) rats were injected with Fluoro-Gold (FG; 80 μ L) into the right MPOA. Ten days after FG injection, rats were treated with corn oil or 17 β -estradiol (OVE; 10 μ g/0.2mL/rat, s.c.) for 3 days. On the 4th day, OVX and OVE rats received a corn oil (OVX+O, n = 4; OVE+O, n = 4) or progesterone (2.5 mg; OVX+P4, n = 4; OVE+P4, n = 5) injection at 12 h and were perfused at 16 h. Coronal sections from the locus coeruleus (LC), A1 and A2 were triple-labeled to c-Fos, FG and tyrosine hydroxylase (TH). FG injections into the MPOA were placed within +0.12 and -0.24 mm from bregma. The percentage of TH-immunoreactive (ir) neurons retrograde-labeled with FG in the LC, A2 and A1 was 5.1 ± 0.7 , 8.7 ± 1.4 and 17.4 ± 3.5 %, respectively (mean $\pm$ SEM; n = 17). Progesterone significantly increased the percentage of FG/TH-ir neurons expressing c-Fos in the LC (P < 0.05), and estradiol did not alter this response (OVX+P4 vs. OVE+P4). On the other hand, the percentage of FG/TH-ir neurons expressing c-Fos was significantly augmented by estradiol in the A2 (P < 0.01), which in turn was not altered by progesterone treatment (OVE+O vs. OVE+P4). In A1, there was no significant expression of c-Fos in FG/TH-ir neurons in any of the hormonal conditions evaluated.

The present data show that ovarian steroids regulate the activity of brainstem noradrenergic neurons projecting to the MPOA in a selective manner. The A2 neurons are activated in the afternoon in estradiol-treated rats, whereas progesterone induces activation of the LC neurons. We therefore suggest that ovarian steroids effects upon LH secretion are, at least in part, mediated by A2 and LC projections to the MPOA.

Palavras-Chave: LH; Norepinephrine; Preoptic Area; Brainstem; Fluoro-Gold; c-Fos

Apoio Financeiro: FAPESP; NIH DK43200; DA19356; Bridge Grant from the Endocrine Society

THE ROLE OF NUMBING AND THE DEHYDROEPIANDROSTERONE HORMONE IN POST-TRAUMATIC STRESS DISORDER.

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Introduction: Posttraumatic stress disorder (PTSD) is characterized by re-experiencing, hyperarousal, avoidance and numbing symptoms. Several studies have associated PTSD with dysfunctions of the hypothalamic-pituitary-adrenal axis (HPA). However, the exact nature and direction of HPA hormone alterations remains unclear. Here we investigated the DHEA, a putative anti-glucocorticoid hormone secreted by the adrenal cortex in response to adrenocorticotrophin.

Methods: Morning secretory activity of salivary DHEA was measured in 10 PTSD patients and 10 trauma-exposed controls. A total of four saliva samples were collected: +0, +30, +45 and +60 minutes after awakening. We used enzyme-immunoassays to measure salivary DHEA. Psychometric scales assessed personality traits and PTSD symptoms.

Results: Morning levels of salivary DHEA were higher in the PTSD patients ($t=2.61$; $df=18$; $p=0.01$). More importantly, hormone levels in patients showed an inverse correlation with numbing symptoms (Spearman $r=-0.81$; $p=0.004$). Multiple regression analysis of the whole sample showed that numbing was a predictor factor of the changes in hormonal levels ($\beta=-1.69$; $p<0.001$) after controlling for group (patients and controls), depression and anxiety.

Conclusions: Our results indicate that numbing symptoms, an indicator of poor PTSD prognosis, have a significant influence on the DHEA morning secretory activity. The results of this study should therefore encourage the investigation of the neuroendocrine profile that develops in response to extreme stress and its relation to the pattern of neuropsychiatric and behavioral sequelae. **Palavras-**

Chave: Dehydroepiandrosterone; Post-Traumatic Stress Disorder; Numbing; HPA axis

Apoio Financeiro: Instituto do Milênio MCT/CNPq, PRONEX, FAPERJ, IBN-Net/FINEP, CAPES

Efeito da Pinealectomia e do Exercício Físico na Atividade Metabólica do Tecido Adiposo e Muscular de Ratos Tratados com Melatonina

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Objetivo: O tecido adiposo permite ao organismo adaptar-se a mudanças metabólicas, como o exercício físico. A pinealectomia (Pinx) prejudica a capacidade de adaptação do tecido adiposo, indicando que a presença da glândula pineal é extremamente importante para o animal enfrentar condições de estresse como o exercício (Ex). Assim o objetivo do estudo é investigar o efeito da Pinx e do Ex. no metabolismo do tecido adiposo e muscular de ratos tratados com melatonina.

Materiais e Métodos: Ratos Wistar machos, com luz e temperatura controladas, pinealectomizados, treinados por 8 semanas e divididos em 6 grupos: sedentário (Csd), treinado (Cex), pinealectomizado sedentário (Psd), pinealectomizado treinado (Pex), pinealectomizado sedentário com reposição de melatonina (Psdm), e pinealectomizado treinado com reposição de melatonina (Pexm). Após esse período foram sacrificados e extraídos o tecido adiposo peri epididimal e o músculo sóleo para análise de síntese de glicogênio, captação e oxidação de glicose.

Resultados: A análise de variância mostrou que a captação de glicose aumentou com o Ex. e diminuiu com a Pinx tanto na atividade metabólica do tecido adiposo quanto muscular. Foi revertida a diminuição com o Ex. + reposição de melatonina avaliada no tecido adiposo, e no muscular não houve diferença significativa. A síntese de glicogênio no tecido adiposo e muscular aumentou com Ex., diminuiu com a Pinx, e foi revertida a diminuição com Ex. + reposição de melatonina. A oxidação de glicose aumentou em ambos os tecidos com o Ex., reduziu com a Pinx e revertida a redução no tratamento, e na combinação Ex. + melatonina.

Conclusões : A pinealectomia modifica o metabolismo do tecido adiposo e muscular, reduzindo a capacidade adaptativa ao treinamento, visto que o exercício provoca adaptações benéficas e o tratamento parece reverter o quadro instaurado pela pinealectomia, é certo que a pineal através da secreção de melatonina está implicada com a função do tecido adiposo e muscular em resposta ao exercício.

Palavras-Chave: glândula pineal, tecido adiposo, músculo, exercício, atividade metabólica

Apoio Financeiro: FAPESP 04/06767-2

E.01.013

IMMUNOHISTOCHEMICAL DISTRIBUTION OF CD13 AND CD26 IN RAT HYPOTHALAMUS AND HIPPOCAMPUS.

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Objective: To explore the distribution of reactivity to polyclonal antibodies against the proteins CD13 (EC 3.4.11.2) and CD26 (EC 3.4.14.5) in the rat hypothalamus and hippocampus.

Methods and Results: Immunohistochemical analysis by ABC technique using five adult, healthy Wistar animals. Immunoreactivity (ir) to CD13 was localized in cell bodies of supraoptic, paraventricular, arcuate, periventricular and retrochiasmatic hypothalamic nuclei. Supraoptic, medial parvicellular part of paraventricular, and medial and lateral part of arcuate hypothalamic nuclei presented denser CD13-ir. The hippocampus presented CD13-ir only in the perikarya of dentate gyrus. Immunoreactive CD26 perikarya was present in the supraoptic, arcuate and periventricular hypothalamic nuclei, and in the hippocampal dentate gyrus. However, abundant hypothalamic CD26-ir was observed in the periventricular nucleus, just ventrally to the paraventricular nucleus. Immunoreactive CD26 fibers, but no perikarya, were found in the hippocampal fimbria.

Conclusions: This is the first mapping of CD26 protein in brain areas related to the hydrosaline balance, energetic metabolism, learning and memory. These results indicate that CD13 and CD26 may act as receptors and/or enzymes (neutral aminopeptidase and dipeptidyl peptidase IV, respectively) in these brain areas. The presence of CD26-ir in nerve fibers suggests its involvement in synaptic function in the hippocampus. **Palavras-Chave:** CD13, CD26, hypothalamus, hippocampus

Apoio Financeiro: FAPESP 05/04699-2, CNPq 131809/2006- 7

E.01.014

Efeitos do LPS sobre a ingestão seletiva de sódio em ratos desidratados.

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O lipopolissacarídeo (LPS), um componente da parede celular de bactérias Gram-Negativas, induz uma série de mudanças fisiológicas e patológicas que alteram a homeostase do organismo. Esse trabalho mostra os efeitos inibidores da administração de doses subsépticas dessa endotoxina bacteriana (25, 50, 100 e 300 µg/Kg de p.c.) sobre a ingestão preferencial de sódio em teste de privação de água e alimento por 36 horas e aumento na concentração plasmática de ocitocina (OT) e nitrato (NO₃⁻), um dos metabólitos exclusivos de óxido nítrico (NO). Além de teste associado de ingestão de água foram também avaliadas a osmolalidade plasmática e os níveis circulantes de sódio (Na⁺) e potássio (K⁺) sem, no entanto, apresentarem alterações significativas. Ratos machos Wistar (270–300g) foram submetidos ao protocolo de privação de água e alimento por 36 h, sendo que 30 min antes do final deste período receberam (via i.p.) doses subsépticas de LPS (25, 50, 100 ou 300 µg/Kg; *Escherichia coli* EH100). Após um período (2 h) de re-hidratação parcial foi oferecido por mais 1 hora água e NaCl 1,8%. Os animais foram sacrificados e amostras de sangue foram coletadas para posterior análise de NO, OT, Na⁺ e K⁺ e osmolalidade plasmática através das técnicas de Quimiluminescência NO/Ozônio, RIE, fotometria de chama e osmômetro respectivamente. Os resultados são expressos como média ± EPM e as diferenças estatisticamente significantes (*p<0,05, ***p<0,001) foram avaliadas por Teste *t* de Estudantes. Da dose mínima (25 µg/Kg) à máxima (300 µg/Kg) ocorreu inibição de 71,6% a 92,6% do apetite ao sódio, respectivamente. Em contraste, observamos que a endotoxina estimulou de maneira dose-dependente a produção periférica de nitrato (mínimo de 52,28% e máximo de 244%) e OT (mínimo 196,04% a máximo 1063,40%). O aumento de nitrato mostrou a eficácia do LPS em estimular a produção sistêmica de NO, independente das concentrações utilizadas serem consideradas subsépticas, e, portanto, não devem ter induzido alterações hemodinâmicas substanciais. Estes efeitos não foram seguidos de variações na ingestão de água, nos níveis de Na⁺, K⁺ e osmolalidade plasmática, sugerindo que a elevação da concentração plasmática de OT não estaria relacionada a um estímulo osmótico. Em contrapartida, a inibição na ingestão preferencial de sódio poderia estar relacionada à estimulação da secreção de OT, um neurohormônio reconhecidamente inibidor da ingestão de sal. Finalmente, este estudo mostrou que doses subsépticas de LPS podem induzir déficit na reposição de sódio (após re-hidratação parcial) relacionado ao aumento na secreção de OT de forma independente de variações nos níveis plasmáticos de Na⁺, K⁺ e osmolalidade.

Palavras-Chave: OT, ingestão de sódio, ingestão de água, NO, LPS

Apoio Financeiro: CAPES, UFPA, CNPq, FAPESP

Oxide nitric and carbon monoxide modulate hypertonic sodium induced-atrial natriuretic peptide release from *in vitro* olfactory bulb explants

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This study was performed to investigate the role of nitric oxide (NO) and carbon monoxide (CO) in the regulation of basal or hypertonic sodium-induced atrial natriuretic peptide (ANP) secretion from *in vitro* olfactory bulb (OB) explants. Male adult rats weighting 230-250 g were maintained under environmental controlled conditions of temperature (23-25 °C) and illumination (12 h light/12 h dark). The rats were killed by decapitation and OB extirpated and dissected under ice cold phosphate buffer solution (2-5 °C). The BOs were distributed in individual tubes and pre-incubated (60 min) into Dubnoff bath immersed in Krebs salts solution under atmosphere of 95% O₂ and 5% CO₂, at 37 °C, pH 7,4 and continuous shake (50 cycles/min). Samples of medium were collected and frozen at -70 °C for posterior measures of ANP by double antibody radioimmunoassay, nitrate (metabolite of NO) by quimiluminescence and C¹⁴-citruline by beta counter (indirect measure of NO sintase activity). The data were presented as median±SEM and difference between groups compared by one way ANOVA and Newman Keuls as post test. The results showed direct relation between ANP release (+25% or -42%) and sodium-induced osmolality changes (280-340 mOsm/Kg H₂O or 280-240 mOsm/Kg H₂O). In contrast, a inverse relation was observed for nitrate levels (-42% or +42) after similar changes on sodium concentration in the medium (0,15 to 0,19 or 0,15 to 0,13 M), suggesting that OB may to control increases in extracellular sodium levels by stimulating ANP release or inhibiting NO production. In fact, the use of donors as 0,3 mM sodium nitroprussiate (SNP) and 0,3 mM 3-Morpholinylnsydnoneimine Chloride (SIN-1) or the inhibitor of NOS 0,5 mM l-nytroarginine methyl ester (L-NAME) induced decrease (53% or 31%) or increase (+130%), respectively, in basal ANP release to the medium. All treatments induced similar effects in hypertonic sodium condition. These effects may be occurred via cyclic guanosine monophosphate (cGMP) synthesis, since that addition of 1H-[1,2,4]-oxadiazolo-[4,3-a]-quinoxalin-1-one (ODQ), a inhibitor of soluble guanilate cyclase (sGC), induced a significant increase (62%) in ANP release inhibited by SIN-1. ODQ individually, stimulated (73%) the basal ANP release only, suggesting that in hypertonic sodium condition the endogenous cGMP production followed the lower NO synthesis activity. These low NO production may depend of high CO levels since that blockage of Hemeoxygenase (enzyme that synthesize CO) by ZNDPBG induced decrease (59%) in ANP release and increase (30%) in C¹⁴-citruline production. This study showed evidences that suggest a ANP neurosecretor osmoresponsivity induced by sodium stimulation and that ANP secretion may be modulated by NO inhibitory mechanism via cGMP. Finally, NO and cGMP synthesis may depend of a negative feedback activated by CO production. **Palavras-Chave:** ANP, Olfactory Bulb, NO, CO, sodium

Apoio Financeiro: CAPES, FAPESP, UFPA

EFEITO PROTETOR DA VITAMINA C SOBRE O DÉFICIT NA INGESTÃO DE SÓDIO EM RATOS EXPOSTOS AO METILMERCÚRIO.

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Objetivo: Determinar se o metilmercúrio promove distúrbios na regulação da ingestão de sódio e água em animais desidratados e o papel da vitamina C como um possível fator citoprotetor. **Materiais e resultados:** Foram utilizados ratos Wistar 280-300 g submetidos a dois protocolos experimentais: um com exposição crônica (de 54 dias) e outro aguda (3 dias), em diferentes doses de metilmercúrio (1, 5 e 7,5 mg/Kg) na presença ou ausência da vitamina C. A administração de MeHg 1 mg/kg durante 54 dias provocou déficit na ingestão de água, grupo controle 27.7 ± 1.03 ml contra 20.1 ± 0.6 ml do grupo tratado ($P < 0.001$), e sódio, grupo controle 7.3 ± 0.6 ml contra 2.4 ± 0.6 ml do grupo tratado ($P < 0.001$), enquanto que administração de MeHg nas doses de 1, 5 e 7,5 mg/kg (3 dias) provocou déficit na ingestão de sal dose dependente, 2.8 ± 0.7 ml, 1.6 ± 0.4 ml e 0.6 ± 0.3 ml respectivamente dos grupos tratados contra 4.2 ± 0.4 ml do grupo controle ($P < 0.05$), sem alterar a ingestão de água, grupo controle 20.6 ± 0.5 ml contra 19.7 ± 0.5 ml grupo tratado ($P > 0.05$). A vitamina C (0,33 mg/kg) foi capaz de prevenir o déficit na ingestão de sal causada pelo MeHg nas doses de 1 e 5 mg/kg por 3 dias ($P < 0.05$), grupo com vitamina C 4.1 ± 0.4 ml contra 2.0 ± 0.6 ml do grupo sem vitamina C (dose de mercúrio 1mg/kg) e 4.4 ± 0.9 ml contra 1.6 ± 0.4 ml do grupo sem vitamina C (dose de mercúrio 5mg/kg). **Conclusão:** O MeHg causou déficit no comportamento de reposição seletiva para sódio um distúrbio atenuado por vitamina C, confirmando a participação deste agente antioxidante e mediador do metabolismo neuronal como um fator de proteção contra a intoxicação mercurial.

Palavras-Chave: hipotálamo, Homeostase Hidroeletrólítica, metilmercúrio, vitamina C.

Apoio Financeiro: EGPA

Investigação do efeito da administração de CNTF e da LEPTINA sobre a gordura marrom de ratos neonatos: análise morfológica dos adipócitos e da expressão da UCP1.

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O Fator Neurotrófico Ciliar (CNTF) tem reconhecida ação neuroprotetora sobre motoneurônios. Porém, testes clínicos em portadores de esclerose lateral amiotrófica verificaram efeitos colaterais importantes, como a redução do peso corporal. Sendo este efeito similar ao produzido pela Leptina (LEP), cresceu o interesse por sua ação no tecido adiposo. Sabe-se que CNTF e LEP estimulam a capacidade termogênica do tecido adiposo marrom pelo aumento da expressão da proteína UCP1 em ratos adultos. Contudo, há poucos estudos sobre a ação destas moléculas na expressão de UCP1 em ratos neonatos. Este estudo investigou o efeito do tratamento com CNTF e LEP sobre a expressão da UCP1 na gordura marrom desses animais. Ratos Wistar neonatos (P2) foram tratados por 5 dias com dose diária (s.c.) de CNTF (0,25 µg/g; n=13), LEP (0,30 µg/g; n=11) ou PBS (n=11). O peso corporal (pC) foi verificado diariamente e 24hs após a última dose (P7) a gordura marrom interescapular (GMI) foi coletada e pesada (pGM) para obtenção do índice de gordura marrom ($GMI_i = pGM/pC$). Foi realizada a análise da histologia e da expressão da UCP1, por Western blot, neste tecido. Os ratos tratados com CNTF ou LEP tiveram ganho de peso corporal (P2 a P7) inferior ao dos controles. Em P7, ratos tratados com CNTF, LEP ou PBS tiveram taxas de crescimento de $1,89 \pm 0,03$; $1,63 \pm 0,04$ e $2,07 \pm 0,02$ (média±epm; $p < 0,001$), respectivamente. O GMI_i também foi inferior nos grupos CNTF ($5,6 \pm 0,3$) e LEP ($5,3 \pm 0,2$) quando comparados ao grupo PBS ($6,7 \pm 0,2$; $p < 0,01$). A histologia da GMI corroborou estes resultados ao evidenciar redução das vesículas lipídicas nos grupos CNTF e LEP em comparação ao controle. Por sua vez, ambos os tratamentos reduziram a expressão da UCP1 (CNTF $0,34 \pm 0,06$; LEP $0,57 \pm 0,12$; Densidade Óptica Relativa ao PBS; $p < 0,05$). Sabe-se que em ratos neonatos há redução da expressão de mRNA UCP1 a partir do nascimento. Embora não tenha sido demonstrado, o mesmo deve ocorrer com a expressão da proteína. Nossos resultados sugerem que a administração de CNTF e LEP acelerou este processo. Além disso, a mobilização de lipídios promovida por essas moléculas parece não estar necessariamente relacionada com a termogênese na GMI.

Palavras-Chave: CNTF, LEPTINA, gordura marrom interescapular e UCP1

Apoio Financeiro: PIBIC/CNPq - PRP

INFLUÊNCIA DOS HORMÔNIOS GONADAIS SOBRE O VOLUME SOMÁTICO DOS NEURÔNIOS DO SUBNÚCLEO PÓSTERO-DORSAL DA AMÍGALA MEDIAL DE RATAS ADULTAS

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Objetivo: O volume somático neuronal (VSN) do subnúcleo pósterodorsal da amígdala medial (MePD) de ratos apresenta dimorfismo sexual e é influenciado pela fase do ciclo estral em fêmeas. O objetivo deste estudo foi avaliar a influência dos hormônios gonadais benzoato de estradiol e progesterona sobre o VSN do MePD de ratas adultas ovariectomizadas e submetidas à terapia hormonal substitutiva.

Métodos: Ratas Wistar adultas (N=3/grupo) foram ovariectomizadas e submetidas a injeções subcutâneas de veículo oleoso (OVX, 0,1 mL); benzoato de estradiol (BE, 10 µg/0,1 mL), progesterona (P, 500 µg/0,1 mL) ou BE + P (10 µg/0,1 mL e 500 µg/0,1 mL). Os animais foram anestesiados, perfundidos e seus encéfalos foram removidos, seccionados (100 µm) e processados para inclusão em Durcupan. Imagens do MePD de secções semifinas seriadas (1 µm) coradas com azul de toluidina 1% foram capturadas usando um microscópio acoplado a uma câmera CCD Pro-Series High Performance e Image Pro Plus Software 4.1. Foram analisadas ao menos 10 secções e contados ao menos 56 áreas/ponto por neurônio (15 neurônios/animal). A média dos 15 neurônios representou o valor estimado do VSN de cada animal. O VSN estimado do MePD foi adquirido com o método de Cavalieri associado à técnica de contagem de pontos, sendo obtido com a equação: $V = SP \cdot a/p \cdot T$, onde V=volume, SP=somatório de pontos contados, a/p =área/ponto=9,07 µm² e T=espessura da secção (1 µm), e comparado entre os grupos (média±erro-padrão) com ANOVA de uma via e o teste *pos-hoc* da diferença mínima significativa (LSD), com nível de significância $p < 0,05$ (SPSS11.5).

Resultados: No MePD foi observada uma diferença estatística significativa na média do VSN estimado entre os grupos ($p < 0,02$), sendo esta menor no grupo BE (710,07±33,23 µm³) em comparação aos grupos OVX, P e BE + P (1008,43±69,25; 1030,74±112,92; e 1123,89±27,23 µm³, respectivamente).

Conclusão: Os animais tratados com BE apresentaram uma redução no VSN do MePD, efeito este revertido quando associado à progesterona. Portanto, os hormônios gonadais parecem ser um dos componentes responsáveis pelo estabelecimento das diferenças observadas no VSN do MePD durante o ciclo estral de ratas. **Palavras-Chave:** volume somático neuronal; MePD; terapia hormonal substitutiva; hormônios gonadais; ratas

Apoio Financeiro: CNPq

ANGIOTENSIN IV MODULATION OF MELATONIN SYNTHESIS.

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Objectives: The pineal gland has a circadian rhythm of melatonin (MEL) synthesis and secretion that is imposed by nocturnal noradrenaline release and modulated by many peptidergic systems. In the rat pineal gland a local renin-angiotensin system (RAS) was described and the presence of aminopeptidases A and N, that converts angiotensin II into angiotensin III, and the last in angiotensin IV (Ang IV). In order to better understand RAS in the pineal gland, the objective of this work was to investigate the role of Ang IV on MEL synthesis. We studied the effects of angiotensin IV, evaluating the interactions between astrocytes and pinealocytes.

Methods: Dissociated cells from rat pineal glands were obtained by papain digestion. After the dissociation, the cells (pinealocytes and astrocytes) remained together for 16h, and after that pinealocytes were isolated. When both cellular types were cultivated together during 60h, this preparation was characterized as co-culture. The cells were maintained in DMEM + BSF (10%). MEL was evaluated by HPLC with electrochemical detection. Intracellular calcium was analyzed by confocal microscopy. IRAP receptor expression was evaluated by RT-PCR.

Results: Melatonin synthesis concentration-response curves for noradrenaline (NOR) were obtained for pinealocytes and co-culture, with the maximal responses between 0.1 μ M and 1 μ M. AngIV (10nM) increased melatonin synthesis induced by noradrenaline in isolated pinealocytes (NOR=82.35 $\pm$ 2.79ng/ml; NOR+AngIV=110.26 $\pm$ 6.07ng/ml), but when in co-culture this effect was not observed. Ang IV (10nM) induced a calcium increase in pinealocytes. The Ang IV receptor, IRAP, was identified in pinealocytes.

Conclusions: Ang IV is an important modulator of noradrenergic-induced melatonin synthesis. It is possible that an increase in intracellular calcium be responsible for this effect because AA-NAT, the rate-limiting enzyme to the MEL synthesis, is calcium dependent. In co-culture, Ang IV had no effect, what would indicate that, in this situation, AngIV effect already was occurring by the metabolism of AngII that is released under noradrenergic stimulation, as demonstrated before (Baltatu et al, 2002).

Palavras-Chave: Pineal gland, melatonin, pinealocytes, astrocytes, angiotensin IV

Apoio Financeiro: Fapesp and CNPq

E.01.020

Alterações do desenvolvimento neurocomportamental induzidas pelo hipotireoidismo pós-natal e reversão com tiroxina

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Objetivos: O objetivo deste trabalho foi avaliar se a reposição de T4 reverte as alterações neurocomportamentais tardias induzida pelo hipotireoidismo pós-natal em ratos. **Metodologia:** Ao nascimento, 25 ninhadas foram ajustadas de modo a se obter 4 casais que foram divididos entre os seguintes grupos: controle, PTU (4mg/kg), PTU (4mg/kg) + T4 (25ug/kg) e PTU (4mg/kg) + T4 (35ug/kg). Os animais foram tratados diariamente do dia 5 ao 22 pós-natal (PN). Após o sacrifício (PN 23 e 150), foram avaliados o peso dos órgãos, os níveis de T4 total no soro e o padrão histológico das células granulares do cerebelo. Alterações de emocionalidade e da atividade motora foram avaliados no teste do campo aberto (dia 80 PN). **Resultados e discussão:** No dia 23 PN, o tratamento com PTU reduziu significativamente a concentração de T4 total, acompanhado pelo aumento significativo do peso da tireóide, redução do timo e baço e atraso na migração das células granulares do cerebelo. Essas alterações foram transientes e no dia 150 PN os grupos PTU e controle apresentaram valores semelhantes. Entretanto, neste mesmo dia (150 PN) os animais hipotireoideos apresentaram hiperatividade e alterações da emocionalidade constatados pelo teste do campo aberto. Nos grupos tratados com PTU que receberam T4 (ambas as doses), o padrão de atividade motora e parâmetros ligados a emocionalidade se mostraram semelhantes ao controle sugerindo um efeito protetor do T4. Este efeito aparentemente óbvio do T4 não se mostrou consistente com relação aos outros parâmetros responsivos à TH. Nesses grupos, os valores de T4 totais no soro estavam significativamente aumentados comparados ao controle no dia 23 PN; já o peso do baço, timo e migração das células granulares do cerebelo apresentaram valores consistentes com o controle. **Conclusão:** Os resultados sugerem que a administração diária de T4 concomitante ao tratamento com PTU é capaz de reverter as alterações neurocomportamentais tardias produzidas pelo hipotireoidismo pós-natal.

Palavras-Chave: Neurocomportamento, Hormônios Tireoideanos, Campo Aberto, Ratos, Desenvolvimento

Apoio Financeiro: FAPERJ

E.01.021

NOREPINEPHRINE ACTIVATES NF- κ B TRANSCRIPTION FACTOR IN THE RAT PINEAL GLAND

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Objectives: During the darkness pineal gland synthesizes and releases the hormone melatonin in consequence of a sympathetic noradrenergic stimulation. In this process AANAT enzyme transcription and translation are the rate-limiting steps. The transcription factor NF- κ B plays an important role in many biological processes, including inflammation, neuroprotection and apoptosis and it can be activated by many factors, such as glutamate, cytokines, and others. The aim of this work was to investigate if NF- κ B could be activated by noradrenaline and if it plays a role on melatonin synthesis by cultured rat pineal gland.

Methods: Pineal glands from adult rats were maintained in culture for 48h and then stimulated by noradrenaline in association or not with PDTC (300 μ M) or sodium salicylate (SS) (1 mM) (inhibitors of NF- κ B translocation). Melatonin was quantified by HPLC with electrochemical detection and AANAT activity by radiometric assay. NF- κ B was analyzed by electrophoretic mobility shift assay in pineal glands or in isolated pinealocytes or astrocytes. AANAT expression was evaluated by real time PCR.

Results: Noradrenaline activated NF- κ B in the pineal gland and pinealocytes, but does not in astrocytes. Moreover, p50 seems to be the subunit involved. PDTC and sodium salicylate inhibited melatonin synthesis (nor=10,36 $\pm$ 3.74ng/ml, nor+PDTC=6,81 $\pm$ 4.52ng/ml, nor+SS=5,84 $\pm$ 3.88ng/ml) and AANAT activity and expression.

Discussion: The NF- κ B transcription factor is activated by noradrenaline in the rat pineal gland and this process has an important role in AANAT synthesis and activation that leads to melatonin secretion.

Palavras-Chave: Pineal gland, melatonin, pinealocytes, NF- κ B

Apoio Financeiro: Fapesp and CNPq

PARTICIPAÇÃO DO BULBO OLFATÓRIO ACESSÓRIO NA REGULAÇÃO DA INGESTÃO DE ÁGUA E SAL EM RATOS.

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Objetivos: Este estudo pretende avaliar a participação do bulbo olfatório acessório (AOB) na regulação da homeostase hidroeletrólítica em ratos normohidratados ou depletados de sódio.

Material e Métodos: Ratos machos Wistar (260-300g) normohidratados ou privados de água e alimento (48h) foram anestesiados e perfundidos com solução de paraformaldeído (4%) em PBS 0,1M + sacarose (30%). Em seguida os bulbos olfatórios foram cortados (30µm) e reagidos para imunomarcagem de c-fos. Implantes de cânulas de demora (8 x 0,7 mm) no AOB (6,2 mm antero-posterior ao bregma; 1,5 mm lateral; 1,5 mm vertical ao seio venoso) (1), de ratos machos Wistar (260–300g) normohidratados ou privados de água e sal (24 e 36h), foram realizados 5 dias antes dos experimentos. A ingestão de água e/ou NaCl 1,8% foi observada em intervalos de 20 min, por um período de 60 minutos, após a administração de veículo, Cloreto de Potássio (KCL 56mM), Angiotensina II (ANGII 100 µmoles), Nitroprussiato de Sódio (SNP 1nmol/µl, doador de óxido nítrico - NO) e N^G-nitro-L-arginina (L-NOARG 1nmol/ µl, um inibidor da síntese de NO) no AOB. Os resultados foram expressos como média±EPM e as diferenças estatisticamente significantes (p<0,05) foram avaliadas por Teste *t*.

Resultados: Maior número de neurônios imunorreativos para Fos (36%) foram identificados ao longo da camada mitral e granular do AOB em animais depletados de sódio por 48 h, sugerindo que esta região seja responsiva a déficits da osmolaridade plasmática dependente deste cátion. A injeção intrabulbar de KCl (56 mM) em ratos privados por 24 h, mostrou diminuição de 74% da ingestão de NaCl 1,8%. Por sua vez, a administração de ANG II (100 µmoles) ou SNP (1 nmol) aumentou (1000% ou 115%, respectivamente) e L-NOARG diminuiu (43%) a ingestão de NaCl 1,8% quando administrados no AOB de animais normohidratados. Todos os efeitos ficaram restritos ao tempo de 20 min de observação. Nenhuma alteração na ingestão de água foi observada para quaisquer dos tratamentos realizados.

Conclusão: O AOB pode ter um papel modulador inibitório sobre o balanço de sódio no organismo seguindo a tendência classicamente conhecida do Bulbo Olfatório onde está inserido.

Referencia: 1 - Paxinos & Watson 06:40, 1998.

Palavras-Chave: Bulbo Olfatório Acessório, Ingestão de Sal, Imunomarcagem para c-fos

Apoio Financeiro: CNPQ

E.01.023

Controle da secreção de tireotrofina (TSH) durante a realimentação: Há envolvimento de hormônios do trato gastrointestinal ou do tecido adiposo?

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Objetivo: A atividade tireoidiana é influenciada pelo estado metabólico do organismo e, durante o jejum, a redução na secreção de TSH representa uma adaptação ao estado catabólico. O PYY₃₋₃₆ é um hormônio intestinal secretado logo após a ingestão de alimento, capaz de estimular a secreção de TSH de ratos em jejum. Portanto, nossos objetivos foram: Avaliar se esse efeito do PYY₃₋₃₆ é por ação direta no sistema nervoso central; investigar a secreção de TSH e leptina poucas horas após a realimentação. **Metodologia:** Foram utilizados ratos machos, em jejum por 72 horas. Para investigar o efeito central do PYY₃₋₃₆, foram implantadas cânulas no 3º. ventrículo (-0,5/-8,5mm; AP/DV em relação ao bregma) 1 semana antes dos experimentos. Após 15 minutos da administração central de PYY₃₋₃₆ ou salina, os ratos foram guilhotinados e seu sangue coletado. Outro grupo de animais, foi utilizado para investigar o efeito da realimentação sobre a secreção de TSH e leptina. Para isso, ratos foram sacrificados em jejum, 1 ou 3 horas após o início da realimentação. A quantificação da concentração sérica de TSH e leptina, foi realizada por radioimunoensaios específicos. **Resultados:** Após 15 minutos da administração central (3º ventrículo) de PYY₃₋₃₆ (0,1 nmol por rato), houve redução na concentração sérica de TSH (2,02±0,29 vs 1,03±0,1 ng/mL; PYY vs controle, P<0,01), enquanto a dose menor (0,01nmol) não promoveu modificação (1,95±0,16 vs 1,67±0,20 ng/mL; P>0,05). Embora 1h de realimentação não tenha alterado a concentração sérica de TSH (0,98±0,18 ng/mL), após 3h houve incremento (2,99±0,40 vs 1,15±0,23 ng/mL; 3hs vs jejum, P<0,001). Por outro lado, a concentração sérica de leptina não modificou durante o período experimental (1,71±0,27, 1,75±0,23 e 2,12±0,19 ng/mL; jejum, 1 e 3 hs de realimentação, P>0,05). **Conclusões:** A concentração sérica de TSH aumenta 3 horas após a realimentação. Portanto, é possível que o PYY₃₋₃₆ esteja envolvido nesse efeito. Entretanto, sua ação estimulatória sobre a secreção de TSH não é por ação central direta. Embora a leptina seja capaz de estimular a secreção de TSH, não parece estar envolvida neste fenômeno durante a realimentação, pois sua concentração não se elevou.

Palavras-Chave: PYY, TSH, leptina

Apoio Financeiro: CNPq

EFEITOS PROTETORES DA PROLACTINA NOS CULTIVOS DE ASTRÓCITOS INOCULADOS COM VÍRUS OROPOUCHE

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Objetivo: Avaliar efeitos protetores da prolactina (PRL) após infecção dos cultivos primários de astrócitos (CPA) com *vírus Oropouche* (OROV).

Métodos: A pureza do CPA foi confirmada pela marcação anti-GFAP (glial fibrillary astrocytic protein) e a quantidade de células viáveis (98,7%) mensurada por citometria de fluxo e por 3-(4,5-dimetiltiazol-2-yl)-2,5-difenil brometo tetrazólio (MTT). CPA foram inoculados com diferentes diluições do OROV (0,045; 0,09; 0,18 e 0,36 UFP/ml) e tratados, separadamente, com diferentes concentrações de PRL (0,1; 0,5; 1 e 10 nM), a diferentes intervalos de tempo (24, 48 e 72 horas). A quantificação dos níveis de citocinas foi realizada por técnica de ELISA.

Resultados: Os resultados demonstraram que o efeito citopático (ECP) e a diminuição da viabilidade celular dos CPA evoluíram dependentes da diluição viral e do tempo. A adição das diluições de 0,18 e 0,36 UFP/ml resultou em significativa diminuição (36,8% $\pm$ 7,3 e 43,3% $\pm$ 2,1 [p<0,05], respectivamente) da viabilidade celular em 24 h de infecção, chegando ao seu efeito máximo (74% $\pm$ 0,7-77% $\pm$ 0,4 [p<0,001]) em 72 h. A infecção com o OROV (24 h) resultou em efeito bifásico na liberação das citocinas TNF- α (curva em “U”), IFN- γ (curva em mista) e IL-12 (curva em “sino”). Os níveis de proteínas das citocinas estudadas aumentaram significativamente em relação ao controle, 24 h pós-tratamento com as diferentes diluições de PRL. Quando os CPA foram tratados com PRL (1 nM) e inoculados com vírus (0,18 UFP/ml) conjuntamente, observou-se uma atenuação do ECP na viabilidade celular em relação ao CPA inoculado apenas com OROV, em 24 h. Nestes cultivos, a liberação das citocinas (TNF- α , IFN- γ e IL-12) pelos astrócitos aumentou significativamente em relação aos cultivos apenas inoculados com OROV (0,18 UFP/ml).

Conclusões: O vírus estudado causou ECP nos CPA, nas diferentes concentrações utilizadas, e a PRL na concentração de 1 nM atenuou a ação viral na diluição de 0,18 UFP/ml do OROV (24 h) tanto pelo aumento da viabilidade quanto pela liberação das citocinas pró-inflamatórias mostrando um papel imunoregulador e citoprotetor da PRL em astrócitos cultivados *in vitro* e infectados pelo OROV.

Palavras-Chave: vírus Oropouche, Prolactina, Astrócitos

Apoio Financeiro: CAPES/UFPA/IEC

E.02.002

Does sleep deprivation alters neurochemical response to lipopolysaccharide in mice?

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Objectives: To investigate the effects of sleep deprivation (SD) upon neurochemical alterations induced by lipopolysaccharide (LPS) administration in mice.

Methods: Male C57BL/6J mice were submitted to 72h of SD whereas the control (CTRL) group was maintained in home-cage. Both groups received injection of LPS (5, 10 or 20µg/animal-ip) or saline (Sal) and were returned to their cages for 2h prior to euthanasia. The brains of the animals were dissected for collection of striatum for monoamines concentration analysis (High Performance Liquid Chromatography) and for cyclooxygenase 2 (COX-2) expression in the cortex and hypothalamus (Western Blott).

Results: Our results shown that norepinephrine (NE) is increased at the group CTRL-LPS 20ug in comparison to CTRL-Sal (5.2 ± 0.6 vs. 2.8 ± 0.1 ng/mg protein), however, at the dose of 10ug, SD increased the NE release in comparison with your respective CTRL (5.4 ± 0.7 vs. 3.6 ± 0.1 ng/mg protein). In the CTRL group, dopamine (DA) was significantly increased only at the dose of 20 ug (69 ± 11.2 vs. 14.6 ± 2.2 ng/mg protein), however, in the SD animals, DA increased in both 10 (63.9 ± 9.1 vs. 27.3 ± 3.0 ng/mg protein) and 20 (97.3 ± 4.3 vs. 27.3 ± 3.0 ng/mg protein) ug/animal. SD also increased DA at the doses of 10 (63.9 ± 9.1 vs. 36 ± 3.3 ng/mg protein) and 20 (97.3 ± 4.3 vs. 69 ± 11.2 ng/mg protein) ug in comparison with their respective controls. DA turnover represented by the HVA/DA ratio were strongly decreased by SD (0.5 ± 0.07 vs. 1.3 ± 0.2) and all doses of LPS: 5ug (0.3 ± 0.03 vs. 1.3 ± 0.2), 10ug (0.2 ± 0.02 vs. 1.3 ± 0.2) and 20ug (0.1 ± 0.009 vs. 1.3 ± 0.2). When compared with the SD-Sal, only the SD-LPS 20ug group decreased DA turnover (0.1 ± 0.003 vs. 0.5 ± 0.07). Regarding the COX-2 expression in the hypothalamus, neither SD nor LPS administration changed this pro-inflammatory protein expression. In the cortex, an increase in the COX-2 expression were found at all groups that received the LPS injection, both SD and control.

Conclusions: Our results indicates that the alterations caused by SD in the sickness behavior induced by LPS are directly related with alterations in the neurotransmission pathways of NE and DA, and are not affected by the expression of COX-2.

Palavras-Chave: Sickness behavior, sleep deprivation, lipopolysaccharide, dopamine, cyclooxygenase 2

Apoio Financeiro: AFIP, CNPq and FAPESP (CEPID #98/14303-3 to S.T. and 06/58275-1 to A.Z.).

THE ROLE OF SALBUTAMOL (A β_2 ADRENERGIC RECEPTOR) IN THE DEVELOPMENT OF ELECTROCARDIOGRAPHIC ABNORMALITIES AND CARDIAC AUTONOMIC MODULATION IN EXPERIMENTAL MODEL OF ACUTE CHAGAS MYOCARDITIS IN MICE.

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INTRODUCTION: Recent evidences showed that norepinephrine and epinephrine, through stimulation of the β_2 -adrenoreceptor–cAMP-protein kinase A pathway, inhibit the production of type-1 cytokines, whereas stimulate type-2 cytokines. Chagas disease, caused by hemoflagellate *Trypanosoma cruzi*, is one of the most common forms of cardiac disease in the Latin America. In the acute phase of the infection, there is a myocarditis that causes electrocardiographic (ECG) changes and cardiac-autonomic dysfunction. The aim of the present study was to characterize the effects of the β_2 -adrenoreceptor stimulation with salbutamol on acute chagasic myocarditis.

Materials and Methods: Forty male FVB mice were inoculated with 1000 blood forms of the y strain of *T. cruzi*. During 20 days twenty animals were treated with Salbutamol (SALB group) 200 microgram ip, the other twenty chagasic mice (CHG group) received only vehicle. Fifteen normal FVB mice, matched by age, were used as control (CON group). Parasitemia was evaluated at different time points after infection by counting the number of trypomastigotes in peripheral blood. Two conventional ECG recordings (before and twenty days after the infection) were performed on anesthesia with ketamine/xylazine. RR, QaT, QaTc and PR intervals, P and QRS wave durations and heart rate were measured. Electrocardiographic (ECG) electrodes (lead I) and venous canula were implanted on anesthesia with sodium pentobarbital. After 24 hours, ECGs were taken in conscious animals during 30 minutes. Following, autonomic blockade with propranolol and atropine was performed. Time series of RR interval, extracted from baseline ECG recordings, were analyzed using autoregressive spectral analysis. Total variance and spectral densities in very low (VLF, <0.1Hz), low (LF, 0.1-1.0Hz) and high frequency (HF, 1.0 - 5.0Hz) were calculated. Changes in heart rate after propranolol or atropine were used to estimate cardiac autonomic effects.

Results: Parasitemia levels in SALB group were higher than CHG group on the 8th and 12th day post infection ($p=0.015$ and 0.05 , respectively). After 20 days of infection, all electrocardiographic parameters in CHG group were higher than CON group ($p<0.001$). However, a significant reduction in P wave duration ($p<0.001$), dQRS ($p<0.001$) and QaT interval ($p<0.001$) was observed on SALB group when compared CHG, suggesting that salbutamol treatment was able to prevent some electrocardiographic changes observed in chagasic mice. When the spectral densities of heart rate variability were compared among CON, CHG and SALB groups, no significant differences were found, despite of a trend ($p=0.08$) of improvement in HF density (a marker of vagus nerve modulation) in SALB group. After cardiac autonomic blockade with propranolol and atropine, a marked increase in the vagal and sympathetic effect was observed SALB group when compared to CHG group ($p=0.007$ and $p<0.05$, respectively).

CONCLUSION: The present results revealed a pivotal role of β_2 -adrenoreceptor stimulation in controlling the course of infection (represented by parasitemia levels) and inflammation in the heart, since salbutamol treatment was able to prevent changes in ECG abnormalities and to improve the cardiac autonomic dysfunction in acute chagasic mice. Thus, the pharmacological manipulation of the sympathetic-immune interface, using β_2 -adrenoreceptor agonists, could be used as a new strategy in the treatment of Chagas disease.

Palavras-Chave: neuroimmunomodulation, spectral analysis, Chagas disease, electrocardiogram

Apoio Financeiro: FAPEMIG, CAPES, CNPQ, FUNEPU

E.02.004

The expression of DARPP-32 is reduced in leukocytes of patients with schizophrenia or bipolar disorder

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Bipolar disorder (BPD) and schizophrenia (SCZ) are severe disorders representing an enormous social, familiar and individual burden, being SCZ the most disabling psychiatric disorder characterized by psychosis and cognitive impairment. It is well known that SCZ and BPD are associated with abnormalities in dopamine signaling pathway. Recent data in the literature have demonstrated altered expression levels of some proteins involved in the modulation of this pathway in both brain and peripheral tissues. It was shown that protein and mRNA levels of dopamine and cAMP regulated phosphoprotein (DARPP-32) was downregulated in dorsolateral prefrontal cortex (DLPFC) of patients with SCZ or BPD when compared to controls. Due to the difficulty to access brain tissue and the absence of objective laboratory tests for bio-markers, we measured DARPP-32 expression in blood cell sub-populations (CD4+ T lymphocytes, CD56+ NK cells, CD19+ B lymphocytes and CD14+ monocytes) taking advantage of the close relation of nervous and immune systems. Using flow cytometry as the analytical method, our results have shown that the DARPP-32 expression was diminished in CD4+T lymphocytes, CD19+ B lymphocytes and CD14+ monocytes of BPD patients and was also decreased in CD4+ T lymphocytes and CD56+ NK cells of SCZ patients. These results showed that DARPP-32 expression in immune cells agrees with reports of reduced DARPP-32 protein in the DLPFC of BPD or SCZ patients. Our data suggest that DARPP-32 expression in lymphocytes and monocytes could be used as a source of bio-markers to help in the diagnosis and/or treatment response of neuropsychiatry disorders.

Palavras-Chave: leukocytes, bipolar disorder, schizophrenia, flow cytometry, DARPP-32, dopamine

Apoio Financeiro: CNPq and FAPEMIG

POSSIBLE ACTIVATION OF NON-NEURONAL CHOLINERGIC SYSTEM MITIGATES MUSCLE INFLAMMATION IN *MDX* MICE

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Introduction: *Mdx* mice, the animal model of Duchenne Muscular Dystrophy, develop an X-linked recessive inflammatory myopathy characterized by progressive degeneration of skeletal muscle fibers and connective tissue replacement. Acetylcholine receptor (AChR) activation has been shown to regulate the production of pro-inflammatory cytokines via the “cholinergic anti-inflammatory pathway”. The present work aimed to assess the involvement of non-neuronal $\alpha 7$ AChR subunit (nAChR $\alpha 7$) in the production of TNF α (TNF α) and metalloproteinases -9 and -2 (MMP9, MMP2) in the muscular lesion of *mdx* mice.

Methods: Gastrocnemius skeletal muscles of male control C57 and *mdx* mice were used at the following ages: 2 weeks (w), predominance of myonecrosis (4w), regeneration (8 and 12w) and fibrosis (24 and 48w). Western blot and RT-PCR were used for analysis of protein and mRNA content of nAChR $\alpha 7$ and TNF α . Local activity of MMPs was determined by zymography and immunohistochemistry for demonstrate nAChR $\alpha 7$ co-localization on F4/80⁺ macrophages.

Results: Soluble TNF α production in *mdx* was higher at 4w than 2w (48 $\pm$ 19%, p<0.05), with reduction at 12w (61 $\pm$ 5%, p<0.001) and levels remained steady towards 24w. MMP9 activity involved with leukocyte migration and proteolytic cleavage of TNF α , was decreased at 12w when compared to 4w (87 $\pm$ 27%, p<0.05). Comparing nAChR $\alpha 7$ expression to C57, *mdx* showed significant increase from 4 until 12w (74 $\pm$ 15%, p<0.01), and diminished expression at 24w. Expression of the nAChR $\alpha 7$ protein was inversely related with the amount of soluble TNF α and MMP9 at all ages but 2w. Analysis of active-MMP-2, a marker of tissue remodeling, was markedly increased at 8 and 12w (830 $\pm$ 13%, p<0.01). *Mdx* mice at 12w showed 2.9 fold increases of F4/80⁺ macrophages in the muscular lesion co-expressing nAChR $\alpha 7$.

Conclusion: Altogether the results suggest that nAChR $\alpha 7$ may be relevant in the pathophysiology of *mdx* muscular lesion by influencing migration of inflammatory cells, local production of TNF α and tissue remodeling through a non-neuronal and peripheral cholinergic system.

Palavras-Chave: Muscular dystrophy, *mdx*, acetylcholine receptor, macrophage

Apoio Financeiro: CAPES; UFF

E.02.006

COULD SLEEP DEPRIVATION CHANGE THE SURVIVAL TIME OF SKIN ALLOGRAFT IN MICE?

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OBJECTIVE: This study aimed to assess whether paradoxical sleep deprivation (PSD) and sleep restriction (SR) would affect the survival time of skin allograft (STSA) in mice. **METHODOLOGY:** Adult male BALB/c mice were used as donors and C57BL/6J mice as recipients. The C57BL/6J groups were subjected, before and after the transplantation, to PSD for 72 hours or to SR for 15 days by the modified multiple-platform method (placing five mice inside cages containing 14 circular platforms with water up to 1 cm of their upper surface) or maintained in their home-cage (control group). The SR protocol consisted of 21 hours of PSD and 3 hours of sleep. The skin graft was inspected daily to determine survival time. **RESULTS:** SR significantly increased the STSA in relation to the control group (n=10) prior to (14 ± 1.9 vs. 9 ± 1.8 , $p < 0.001$; n=8) or after (16 ± 3.3 vs. 9 ± 1.8 , $p < 0.001$; n=9) transplantation. In turn, PSD increased the STSA in relation to control only after transplantation (17 ± 4.2 vs. 9 ± 1.8 , $p < 0.001$; n=9). There were no significant differences between SR performed before and after transplantation (14 ± 1.9 vs. 16 ± 3.3 , $p > 0.05$). **CONCLUSIONS:** Our findings suggest that sleep deprivation altered the immunologic response. In this model, chronic SR for 15 days as well as PSD for 72h increased the STSA. Such alteration in time of survival probably occurred because PSD impaired the mechanisms involved in the rejection process.

Palavras-Chave: Sleep Deprivation; Sleep Restriction; Skin Allograft; Mice;

Apoio Financeiro: FAPESP (#07/55445-6 to F.S.R. and CEPID #98/14303-3 to S.T.), CNPq, and Associação Fundo de Incentivo à Psicofarmacologia (AFIP).

AVALIAÇÃO COMPORTAMENTAL, NEUROQUÍMICA E MORFOLÓGICA DE CAMUNDONGOS NUDE SUBMETIDOS AO MODELO EXPERIMENTAL DA PILOCARPINA

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Objetivos: A epilepsia do lobo temporal tem como principal característica a esclerose hipocampal. O camundongo Nude é um animal transgênico que apresenta atrofia de timo, o que confere baixa eficiência do sistema imunológico e baixa rejeição a induções tumorais. Nos últimos anos, são crescentes os trabalhos que mostram a participação do sistema imunológico na regulação de mecanismos envolvidos com a morte neuronal causada por crises epiléticas. No presente trabalho, foram avaliadas as alterações comportamentais, neuroquímicas e morfológicas causadas pelo *status epilepticus* (SE) induzido pela pilocarpina no camundongo Nude comparado ao Balb/C.

Métodos e Resultados: A pilocarpina foi injetada (i.p.) a camundongos machos adultos, Nude e Balb/C, nas doses de 320mg/Kg e 270mg/Kg, respectivamente. Após 2 horas de SE, os animais (n=5/grupo) foram perfundidos por via transcardíaca com formaldeído tamponado 4% e os cérebros processados para imuno-histoquímica. A análise dos aminoácidos neurotransmissores no córtex e hipocampo de ambos os grupos foi feita por HPLC. Camundongos Nude apresentaram menor sensibilidade à pilocarpina, maior latência para a ocorrência do SE, menor duração das crises e maior mortalidade (~70%) comparados ao Balb/C. Observou-se um aumento significativo no número de células imuno-reativas para c-fos no grupo Nude em relação ao Balb/C, nas seguintes áreas: estriado (p<0,05), CA3 (p<0,05), giro denteado (p<0,001) e amígdala basomedial (p<0,01). A análise neuroquímica revelou um aumento significativo (p<0,01) na concentração de aspartato, glutamato, serina e histidina no córtex do Nude comparado ao Balb/C.

Conclusões: O Nude apresenta um padrão de alterações comportamentais distintas do Balb/C quanto à expressão do SE: foi mais resistente à pilocarpina, apresentou maior latência para início da crise e maior mortalidade. Houve uma maior marcação de c-fos nas diversas regiões cerebrais do Nude comparado ao Balb/C, porém sem alterações significantes na concentração dos aminoácidos excitatórios ou inibitórios. O Nude parece ser um interessante modelo animal para investigar o envolvimento do sistema imunológico na epileptogênese.

Palavras-Chave: Camundongo Nude, epilepsia, pilocarpina

Apoio Financeiro: Fapesp, CNPq e Capes

Stress-induced changes in T-lymphocytes function is attenuated in trained rats

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Objectives. Modulations of the immune function by stress are a well-known phenomenon. Acute restraint stress may induce impaired T-lymphocyte responses. Moderate physical training is associated with beneficial effects on immunological functions. We investigated the effects of a moderate physical training on function of T-lymphocytes of rats submitted to acute restraint stress.

Methods. Thirty male Wistar rats weighing 210–226 g were randomly divided into four groups: Non-trained (NT, n=7), and non-trained submitted to stress (NT+S, n=8); Trained (T, n=7), and trained submitted to stress (T+S, n=8). Trained rats were submitted to a program of moderate-running training over a period of 8 weeks (5 days/week, 60min/day, at 60% of VO_{2max}). Twenty-four hours after the last session of exercise, rats submitted to restraint stress were kept immobilized in glass cylinders (8 cm diameter × 24 cm long) during 60 minutes. Thymic lymphocyte were collected and dispersed in a steel wire mesh. Lymphocytes were stimulated by ConA. Proliferation data were determined as the incorporation of ³H-thymidine through the stimulation rate. Plasma concentrations of corticosterone were measured by radioimmunoassay. Values are express in means ± SE, the ANOVA test was used with p<0.05.

Results: Plasma concentration of corticosterone in NT+S animals was higher in comparison with both NT and T+S rats (NT = 55.6 ± 0.9 ; NT+S = 66.9 ± 1.8 ; T+S = 51.9 ± 3.1 ; p<0.05). There was no difference between the NT and T groups (T = 53.7 ± 2.7). There was a lower lymphoproliferative response of the NT+S (13.2 ± 0.9) when compared to NT (16.1 ± 0.7) and T animals (16.3 ± 1.3). T+S animals (15.3 ± 0.3) did not show any change when compared to NT.

Conclusions. Our data confirm that acute restraint stress is associated with changes in T-lymphocyte function. Moreover, moderate physical training attenuates the effects of acute stress by a mechanism that involves hypothalamic-pituitary-adrenal axis and increase in tolerance of leukocytes.

Palavras-Chave: Neuroimmunomodulation, physical training, cross-tolerance, stress

Apoio Financeiro: FACEPE, FAPESP, CNPq

E.02.009

Stress-induced downregulation of macrophage phagocytic function is attenuated by exercise training in rats

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Objectives. Acute restraint stress may induce impaired macrophage phagocytic function. Moderate physical training is associated with beneficial effects on immunological functions. We investigated the effects of a moderate physical training on phagocytic function of alveolar macrophage in rats submitted to acute restraint stress.

Methods: Thirty male Wistar rats weighing 210–226 g were randomly divided into four groups: Non-trained (NT, n=7), and non-trained submitted to stress (NT+S, n=8); Trained (T, n=7), and trained submitted to stress (T+S, n=8). Trained rats were submitted to a program of moderate-running training over a period of 8 weeks (5 days/week, 60min/day, at 60% of VO_{2max}). Twenty-four hours after the last session of exercise, rats submitted to restraint stress were kept immobilized in glass cylinders (8 cm diameter $\times$ 24 cm long) during 60 minutes. A sample containing 1×10^6 alveolar macrophages was incubated for 1 h at 37°C in 1 mL of medium with 2×10^7 *Escherichia coli* cells labeled with FITC and evaluated in a flow cytometry. Macrophages (1×10^6) were also incubated for 40 min at 37°C in 1 mL RPMI 1640 medium with opsonized particles of *zymosan*. The phagocytic capacity was defined as the number of macrophages that internalized three or more *zymosan* particles. Values are express in means $\pm$ SE, the ANOVA test was used with $p < 0,05$.

Results: Macrophages from NT+S animals showed a reduced rate of phagocytosis stimulated by *Escherichia coli* in comparison with both NT and T + S rats (NT = 72.9 ± 1.5 ; NT + S = 61.5 ± 1.2 , T + S = 75.5 ± 1.2 , $p < 0.05$). In the animals submitted only to the protocol of training, there was no changes in the rate of phagocytosis by macrophages (T = 74.7 ± 0.8 ; $p < 0.05$). The same results were observed when macrophages were stimulated to phagocyte *zymosan* particles (NT = 76.3 ± 1.1 ; NT + S = 56.0 ± 0.8 ; T = 73.7 ± 1.8 ; T + S = 69.7 ± 2.8).

Conclusion: Our data confirm that acute restraint stress is associated with impaired function of macrophages. Moreover, moderate physical training attenuates the effects of acute stress by a mechanism that involves an increase in tolerance of macrophages.

Palavras-Chave: Neuroimmunomodulation, physical training, macrophages, stress

Apoio Financeiro: FACEPE, FAPESP, CNPq

E.02.010

Evidence for a role of 9-O-acetyl GD3 ganglioside as a co-receptor for *Mycobacterium leprae* in Schwann Cells

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Leprosy is a chronic and infectious disease caused by the intracellular pathogen *Mycobacterium leprae* (ML), which internalization into Schwann cell (SC) leads to peripheral nerves disfunction. Laminin-2, α -dystroglican and Erb-B2 receptor have been described as important molecules for adhesion of ML on SC. Gangliosides are surface glycolipids which have several biological roles in developing, mature and injured neural cells. The expression of 9-O-acetyl GD3 ganglioside is up regulated after sciatic nerve lesion in adult rats and our preliminary data showed high colocalization between 9-O-acetyl GD3 and ML. The main goal of this work was to investigate the potential role of 9-O-acetyl GD3 as a ML co-receptor in SC. For that, we analyzed the colocalization of this ganglioside with the molecules described above *in vitro* and *in vivo*, as well as the effect of 9-O-acetyl GD3 immunoblockage on ML invasion.

Human primary SC were expanded in DMEM F-12+ 10% fetal bovine serum for 48h on cover slips pre-treated with poly-L-lysine and laminin. ML pre-conjugated with FITC or PI was added to the culture medium and incubated for 2, 24, 48 and 72h. After these time periods, cultures were washed with PBS 10mM and fixed with 4% PF. For *in vivo* experiments, NUDE mice received one injection of 500 μ L of RPMI medium containing 2×10^8 of ML/mL. Three months after infection both sciatic nerves were removed, fixed and longitudinal sections were obtained by cryostat. Fixed cultures were blocked with 5% NGS (30') and double labeled for 9-O-acetyl GD3 and S-100, Laminin-2, α -dystroglican, Erb-B2 or Erb-B3. Longitudinal sections were blocked with 10% NGS+1% BSA (1h) and triple labeled under following combinations: 9-O-acetyl GD3/Laminin-2/ α -dystroglican and 9-O-acetyl GD3/Erb-B2/Erb-B3.

Quantitative analyses showed 16% of SC expressing 9-O-acetyl GD3 24h after incubation with ML compared with 8% in non-infected SC *in vitro*. All 9-O-acetyl GD3 positive cells were infected by ML. Immunoblockage of 9-O-acetyl GD3 *in vitro* reduced in about 16% the number of SC infected by ML. Confocal analyses *in vivo* and *in vitro* showed high colocalization of 9-O-acetyl GD3 with Laminin-2, α -Dystroglican and Erb-2. Taken together these data showed evidences for 9-O-acetyl GD3 as co-receptor in SC for ML adhesion and infection.

Palavras-Chave: Leprosy, Schwann cell, *Mycobacterium Leprae*, Ganglioside, Cell adhesion.

Apoio Financeiro: CNPq, FAPERJ, FIOCRUZ

E.02.011

Participation of nitrergic system in the pathogenesis of experimental avian model of cerebral malaria

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Objectives: The aim of present study was to develop an experimental model of cerebral malaria in domestic chickens, utilizing *Plasmodium gallinaceum* as etiologic agent, as well as, to verify the involvement of nitrergic system in this model.

Methods: Chickens with 10 days old were inoculated intravenously with 10^6 erythrocytes containing *P. gallinaceum* and clinically examined daily from the 3rd day post-infection (pi). Brain histology was made by Hematoxylin-Eosin stain. NOS activity in chick brain was evaluated as described by Bredt and Snyder (1990) and NO production in macrophage was determined by nitrite content.

Results: Clinical manifestations (temperature and hematocrit values) were correlated with blood parasitemia. In infected animals maximum parasitemia observed was $57.8\% \pm 15.81$ with decreased hematocrit values ($20.3 \pm 2.62\%$) relation with the control 30%. Was also observed elevation of temperature in infected animals ($42.6^\circ\text{C} \pm 0.39$) when compared with normal value (41°C). Brain histology showed neural alterations in the infected animals and the presence of erythrocytes sequestration in the microvasculature. Hystopatological analysis demonstrated presence of malaric pigment in infected cerebral tissue. In addition, NOS activity in infected brain was related with parasitemia (P%) values (P16% $1,6 \pm 0,3$, P30% $3,1 \pm 0,6$, P45% $4,3 \pm 0,4$ and P65% $10,3 \pm 1,3$ fmoles of citruline/mg protein/min), as well as, NO production in macrophage (P25% 10,7, P40% 14,6 e P68% 19,7 μM) in comparison with noninfected animals (3,4 μM).

Conclusions: *Plasmodium gallinaceum* causing neural alterations in chick brain, which provoke cerebral malaria, ratifying the avian model as tool to study the mechanisms related with pathogenesis of disease. The nitric oxide results suggest that oxidative stress can represent an important way of toxicity induced by the cerebral malaria. **Palavras-Chave:** cerebral malaria, nitric oxide, Plasmodium gallinaceum

Apoio Financeiro: PADCT/CNPq, FINEP, CAPES, FAPESPA

**CONTRIBUTION OF THE CAROTID BODY TO CARDIOVENTILATORY PLASTICITY
INDUCED BY INTERMITTENT HYPOXIA**

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Intermittent hypoxia (IH), characterized by short cyclic hypoxic episodes followed by normoxia, induces long-term facilitation of ventilatory motor activity, hypertension and reduced the heart rate variability (HRV). Since reflex responses to acute hypoxia rely on carotid body (CB) chemoreception, it is likely that the CB contributes to the IH-induced cardioventilatory acclimatization. Thus, we studied the effects of short and long-term IH on cat and rat CB chemosensory and cardioventilatory reflexes induced by acute hypoxia. Cats exposed to IH (PO₂ ~75 Torr, 9 times/hour for 8 hours) for 4 days showed enhanced CB chemosensory and ventilatory responses to acute hypoxia (PO₂ < 100 Torr). These animals had normal arterial pressure and heart rate, but presented reduced baroreflex sensitivity, increased hypoxia-induced tachycardia and a shift of HRV spectral indexes to lower frequencies. Rats exposed to IH (PO₂ ~37.5 Torr, 10 times/hour for 8 hours) for 12 days presented enhanced hypoxic ventilatory chemoreflexes, without changes in arterial pressure. However, after 21 days of CIH baseline arterial pressure was increased. Our results suggest that the enhanced CB reactivity induced by short-term IH contributes to potentiate the ventilatory reflexes to acute hypoxia, as well as to change HRV. These early alterations occurred without and increase in arterial blood pressure. Thus, our results suggest that cyclic hypoxic stimulation of the CB by short-term IH produces selective alterations of HRV and baroreflex sensitivity in normotensive animals.

Palavras-Chave: Carotid body, intermittent hypoxia, cardioventilatory response, heart rate variability

Apoio Financeiro: FONDECYT 1070584

E.03.002

Activation of α_2 -adrenergic receptors into the lateral parabrachial nucleus decreases renal responses to acute salt load in rats.

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Objectives: The activation of α_2 -adrenergic receptors in the lateral parabrachial nucleus (LPBN) by bilateral injections of moxonidine (α_2 -adrenergic and imidazoline receptor agonist) increases 1.8% NaCl and water intake induced by the treatment with sc furosemide + captopril or in rats previously treated with intragastric load of 2 M NaCl. Moxonidine injected into the LPBN also decreases natriuresis and diuresis induced by intragastric load of 2 M NaCl, but the receptors involved in these renal effects were not studied yet. Therefore, in the present study we investigated the effects of bilateral injections of moxonidine alone or combined with RX 821002 (α_2 -adrenergic receptor antagonist) into the LPBN on urinary volume and sodium excretion during acute sodium load.

Methods: Male Holtzman rats (n=7) with stainless steel cannulas implanted into the LPBN were submitted to an intragastric load of 2 M NaCl (2 ml/rat). One h later, moxonidine or vehicle was injected bilaterally into the LPBN. RX 821002 or saline was injected into the LPBN 15 min before moxonidine. Urine samples were collected at every 20 min during 2 h beginning 15 min after moxonidine injection.

Results: Moxonidine (0.5 nmol/0.2 ml) injected into the LPBN decreased natriuresis (1.647 ± 290 mEq/120 min, vs. vehicle: 2530 ± 167 mEq/120 min) and urinary volume (4.6 ± 0.7 ml/120 min vs. vehicle: 7.4 ± 0.6 ml/120 min). Previous injection of RX 821002 (10 nmol/0.2 ml) abolished moxonidine effects on natriuresis (2223 ± 197 mEq/120 min) and diuresis (6.5 ± 0.7 ml/120 min).

Conclusion: The results suggest that moxonidine activates α_2 -adrenergic receptors in the LPBN to decrease renal responses to acute salt load in rats. **Palavras-Chave:** sodium excretion, moxonidine, imidazoline, dehydration

Apoio Financeiro: CNPq, FAPESP

E.03.003

Prenatal nicotine exposure decreases the activity of raphé neurons in neonatal mice

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Nicotine is the main neuroteratogen in tobacco and it has been associated with Sudden Infant Death Syndrome (SIDS). Infants who died from SIDS, showed reduced arousal responses induced by hypoxia and hypercarbia. Neonatal mice exposed to prenatal nicotine hypoventilate and have reduced ventilatory responses to hypercarbia and reduced central chemoreception. Medullary raphé nuclei are involved in arousal responses and, its subdivision the raphé obscurus (ROb), in the respiratory chemosensory drive. Based on that, we studied the effects of prenatal nicotine on passive and active electrophysiological properties of ROb neurons and their responses to hypercarbic challenge. Using whole-cell recordings in mouse brainstem slice preparations

Osmotic minipumps were implanted subcutaneously into CF1 mice at 1 to 7 days of pregnancy to deliver nicotine ditartrate 60 mg Kg⁻¹ day⁻¹ (nicotine exposed) or saline (controls) until the end of gestation. Mice at the postnatal day 3 (P3) were anesthetized with ether, decapitated, and their brains removed. Transverse brainstem slices of 300 µm containing the ROb were cut with a vibratome, incubated for 30 min at 37°C and then transferred to a recording chamber at room temperature (24°C) while superfused with artificial cerebrospinal fluid and gassed with 5% CO₂ and 95% O₂ pH 7.4. Glutamatergic and gabaergic synapses were blocked with kynurenic acid (1.5 mM) and bicuculline (20 µM). Hypercarbic test was done by increasing CO₂ in the superfusion medium from 5% to 10%.

Raphé neurons of nicotine-exposed mice had similar neuronal properties (RI, Cm and Vm) than those of control neurons. However, in nicotine-exposed preparations in the absence or presence of glutamate/gaba blockers, firing frequency was reduced compared to the controls ($p < 0.05$ y $p < 0.01$, respectively. Mann-Whitney U Test). Our results indicate that the hipoactivity (low firing frequency) of ROb neurons observed in brainstem slices of neonates exposed to prenatal nicotine may contribute to the reduction in respiratory chemosensory responses induced by nicotine, and in turn, through this mechanism contribute to the pathogeny of SIDS.

Palavras-Chave: Prenatal Nicotine, Central Chemoreception, Raphé Neurons

Apoio Financeiro: CONICYT AT-4040216 and FONDECYT 1060110

INFLUÊNCIA DA PRIVAÇÃO DE SONO PARADOXAL NO REFLEXO BARORRECEPTOR EM RATOS MACHOS E FÊMEAS

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A privação de sono tem recebido particular atenção em virtude das conseqüências deletérias sobre a saúde dos indivíduos, principalmente por meio das alterações do sistema cardiovascular. As diferenças hormonais entre homens e mulheres sugerem influências distintas do sexo tanto no sono quanto nas características cardiovasculares. Desse modo, o objetivo do presente estudo foi avaliar se a privação de sono paradoxal (PSP) por 24 e 96 horas altera parâmetros como pressão arterial média (PAM), frequência cardíaca (FC) e índice de sensibilidade barorreflexa. Para tanto, foram utilizados ratos adultos Wistar machos e fêmeas (n=10/grupo) distribuídos em controle (CTRL), PSP24h e PSP96h comparados entre si. Foram canuladas artéria e veia femorais, objetivando o registro direto da PAM e FC e a infusão de drogas, respectivamente. Para analisar o controle barorreflexo da FC foram realizadas infusões em bôlus de fenilefrina (3, 5 e 10 µg/kg, i.v. para avaliar o ganho vagal da FC) e nitroprussiato de sódio (5, 15 e 20 µg/kg, i.v. para avaliar o ganho simpático da FC). De acordo com os resultados, a PSP24h e a PSP96h não alteraram a PAM. No entanto, no grupo PSP96h houve aumento significativo de 12% na FC em machos e de 15% nas fêmeas em relação ao respectivo grupo CTRL. A PSP96h ainda culminou em redução no índice de sensibilidade barorreflexa quanto ao ganho simpático nos machos em todas as doses administradas da droga (60%, 37% e 50%, respectivamente), bem como, redução em 47% na maior dose da droga, em fêmeas. Nossos resultados indicam que períodos mais prolongados de PSP (96h) causam taquicardia e atenuação do ganho barorreflexo simpático nas fêmeas e principalmente nos machos. Esses dados sugerem que a deficiência no controle autonômico pode estar envolvida no aumento do risco cardiovascular encontrado em indivíduos em condição de privação de sono, com maior influência sobre os machos.

Palavras-Chave: privação de sono, barorreflexo cardíaco, gênero

Apoio Financeiro: AFIP

E.03.005

EFEITO DA OBESIDADE NA SENSIBILIDADE BARORREFLEXA EM RATAS ZUCKER

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A obesidade está intimamente relacionada à ocorrência de doenças cardiovasculares. No entanto, os mecanismos envolvidos permanecem pouco explorados. Diversos estudos consideram a sensibilidade barorreflexa cardíaca como marcador de alterações cardiovasculares que podem ocasionar doenças, entre elas, a hipertensão arterial. Desse modo, o objetivo do presente estudo foi avaliar a sensibilidade barorreflexa associada à obesidade e envelhecimento. Para tanto, foram utilizadas ratas Zucker com 15 meses de idade obesas (O, n=6) e magras (M, n=6). Todas as fêmeas foram utilizadas nos experimentos quando estavam na fase diestro do ciclo estral. Os animais foram anestesiados para a implantação de cânulas na artéria e veia femoral, para o registro direto da pressão arterial média (PAM) e frequência cardíaca (FC) e para a infusão de drogas, respectivamente. Para avaliar o barorreflexo cardíaco nos animais acordados, foram realizadas infusões em bôlus de fenilefrina (3, 5 e 10 µg/kg, i.v. para avaliar o ganho barorreflexo para FC quanto ao componente vagal) e nitroprussiato de sódio (5, 15 e 20 µg/kg, i.v. para avaliar o ganho barorreflexo quanto ao componente simpático). De acordo com os resultados, não houve alteração na PAM entre os grupos. No entanto, foi observado aumento de 10% na FC nas magras em comparação às obesas. O índice de sensibilidade barorreflexa foi significativamente menor nas obesas em todas as doses de fenilefrina (redução de 67%, 55% e 63%, respectivamente) e nitroprussiato de sódio (redução de 71%, 47% e 67%, respectivamente). Assim, nossos resultados sugerem que, embora a obesidade não tenha culminado em hipertensão, o índice de sensibilidade barorreflexa foi significativamente menor nas obesas, evidenciando prejuízo tanto no ganho barorreflexo vagal quanto no simpático. Em conjunto, esses dados sugerem que a disfunção barorreflexa não é um fator preponderante para causar hipertensão arterial na obesidade.

Palavras-Chave: Obesidade, envelhecimento, fêmeas, barorreflexo cardíaco

Apoio Financeiro: AFIP

E.03.006

Morfologia e neuroquímica do sistema nervoso entérico (SNE) no estômago e intestino de *Megalobulimus abbreviatus* (Gastropoda, Pulmonata)

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Objetivos: O SNE atua na regulação do comportamento alimentar de moluscos. Identificar os elementos nervosos e a neuroquímica envolvida é fundamental para a compreensão deste processo. Assim, investigou-se a presença de mediadores comuns do sistema nervoso central e periférico de gastrópodes nos plexos entéricos de *M. abbreviatus*. **Métodos:** Identificação dos neurônios periféricos monoaminérgicos que controlam a atividade do estômago e intestino (método do ácido glioxílico-AG;05), atividade da acetilcolinesterase (AChE, método Karnovski e Roots;03), atividade nicotinamida adenina dinucleotídeo fosfato diaforase (NADPH-d;04) e imunoistoquímica para o neuropeptídeo FMRF-amida (04) em caracóis adultos *M. abbreviatus*. **Resultados:** O SNE do estômago é uma rede mais densa na região de transição com o esôfago (cárdia) do que na região pilórica, com fibras nervosas entre e em torno dos feixes musculares. Fibras AChE são mais abundantes na submucosa que na muscular. Não foi encontrada atividade AChE em corpos celulares. A imunoreatividade ao FMRF-amida (Fa-ir) foi maior na mucosa e submucosa, onde foram observados corpos celulares e muitas fibras que se dirigem até a túnica muscular. A ampla atividade da NADPH-d em todos os tecidos analisados permitiu evidenciar os tipos de neurônios e sua organização na parede do estômago e intestino. Na região cárdica existe uma grande concentração de neurônios e fibras nervosas, principalmente entre as fibras musculares, tornando-se mais esparsa na moela e piloro. Predominam neurônios bipolares, mas existem multipolares e poucos unipolares. A malha nervosa no intestino parece ser uma continuidade do arranjo pilórico, mantendo-se contínua e uniforme por todo órgão. Um maior número de neurônios encontra-se junto às duas tênias longitudinais dorsais, na porção ascendente do órgão. Os demais aspectos não diferem do observado no estômago. Não foram detectadas monaminas no SNE com o método do AG. **Conclusões:** A ausência de corpos celulares AChE-positivos sugere origem extrínseca desta inervação. A atividade NADPH-d e o peptídeo Fa-ir são intrínsecos e estão amplamente difundidos entre os tipos celulares da parede dos órgãos. **Palavras-Chave:** sistema nervoso entérico, NADPH-d, AChE, FMRF-amida, molusco pulmonado

Apoio Financeiro: CAPES; CNPq

LOCALIZAÇÃO DOS NEURÔNIOS GABA-IMUNORREATIVOS NO SISTEMA NERVOSO CENTRAL DO CARACOL MEGALOBULIMUS ABBREVIATUS E VARIAÇÃO DA IMUNORREATIVIDADE SOB ANOXIA

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Introdução: O caracol terrestre *Megalobulimus abbreviatus* é uma espécie anaeróbia facultativa, que permanece enterrada sob períodos de baixa umidade e temperatura, sujeita a déficits prolongados de oxigênio. Em trabalhos anteriores verificou-se uma tendência à depressão metabólica do sistema nervoso central (SNC) como um todo – uma estratégia para resistir aos efeitos da anoxia experimental nesta espécie. **Objetivo:** Avaliar uma possível alteração na concentração de ácido γ -aminobutírico imunorreativo (GABAir) em diferentes regiões do SNC de *M. abbreviatus*. Também foi realizado um mapeamento dos neurônios GABAir em condições basais, ainda não descrito para esta espécie. **Métodos:** Animais adultos, mantidos sob condições controladas de temperatura (22 – 26°C) e fotoperíodo (12h claro / 12h escuro), foram expostos a 0% de O₂ por 3 ou 12h de anoxia. Para a reoxigenação, os animais foram submetidos à anoxia de 3h, seguido de 15h de recuperação aeróbica. Os controles foram mantidos aeróbicos, mas nas mesmas condições (alimentar, horários de dissecação) dos anóxicos. Por meio de imunoistoquímica e análise da densitometria óptica (DO) foi avaliada a distribuição de GABAir nos gânglios subesofageais e cerebrais do caracol *M. abbreviatus* em condições basais e sob anoxia ou recuperação. **Resultados:** Neurônios GABAir estão presentes em todos os gânglios analisados, mas em maior número nos gânglios pedais (PE), seguido dos gânglios cerebrais (GC). Fibras GABAir estão presentes nos neuropilos dos GC e subesofageais. A DO foi obtida de corpos celulares e de áreas neuropilares dos GC e PE, e do neuropilo dos gânglios pleurais (PL), parietais (PA) e visceral (V). A anoxia provocou diminuição da imunoreatividade (ir) nos neuropilos do PE (às 12h anoxia), PL (3h), PAesquerdo (12h) e V (3h), e diminuição da ir nos somas do PE (3h). Não houve alteração da ir nos neuropilos PAdireito e GC. Os somas do GC aumentam a ir sob 3h anoxia. **Conclusão:** Esta análise *in situ* revelou que o nível de GABAir diminui nos gânglios subesofageais sob anoxia e não sofre alteração ou aumenta nos neurônios cerebrais, sugerindo uma resposta diferenciada dos gânglios centrais à anoxia. **Palavras-Chave:** GABA, imunoistoquímica, molusco pulmonado, sistema nervoso central, anoxia

Apoio Financeiro: FAPERGS, CNPq, PIBIC-UFRGS

Regulação da atividade na região pericardial pelos gânglios nervosos subesofageais no caracol *Megalobulimus abbreviatus*

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Objetivos. Estudos têm sido realizados sobre a farmacologia do coração em moluscos pulmonados, mas pouco se conhece sobre os neurônios ganglionares que participam do controle cardiovascular. Como o caracol *Megalobulimus abbreviatus*, vem se consolidando como modelo em neurobiologia, este trabalho teve como objetivos identificar a organização e localização dos neurônios envolvidos na regulação da atividade cardíaca. **Métodos.** Animais adultos foram coletados em Minas do Leão (RS) e mantidos sob fotoperíodo, temperatura e umidade controlados. Após anestesiados (mentol por 30 min), 6 animais foram dissecados para a descrição anatômica da inervação da região pericardial e 12 para as técnicas de traçamento retrógrado de vias com cloreto de cobalto e biocitina pelo ramo renopericardial. **Resultados.** A região pericardial é innervada pelo gânglio visceral (V), via nervo intestinal, que se bifurca no nervo renopericardial (nRP), o qual emite o ramo renal e o pericárdico. Este penetra no pericárdio e se bifurca emitindo o ramo que adentra o átrio. Foram identificados um grande número de neurônios médios (40-65 µm de diâmetro) e grandes (70-150µm) marcados na região dorsal do gânglio parietal direito (PAd) e medial do V e um número menor de neurônios médios marcados no gânglio parietal esquerdo (PAe). Um grande número de neurônios pequenos (até 30µm) dos gânglios pleurais (PL) também participam desta inervação primária do sistema coração-rim. Apenas um pequeno número de somas pequenos dos gânglios pedais envia axônios ao nRP. Não foram encontrados axônios de neurônios cerebrais no nRP. Três neurônios gigantes (180-365µm), dos V, PAd e PAe, também enviam fibras para a região renopericardial. **Conclusão.** A região pericardial de *Megalobulimus abbreviatus* é innervada com a participação de todos os gânglios subesofageais, especialmente por neurônios grandes do PAd e V e neurônios pequenos do PL direito. Projeções de neurônios gigantes dos PAd, PAe e V também se innervam diretamente esta região visceral. Estão sendo realizados outros traçamentos anterógrados e retrógrados para esclarecer a participação diferenciada destes neurônios subesofageais na inervação do rim e do coração. **Palavras-Chave:** Molusco pulmonado, marcação de vias, cardiovascular, sistema nervoso central

Apoio Financeiro: CNPq, CAPES, PIBIC-UFRGS, BIC-PROPESQ-UFRGS

FMRFamida-imunorreativa e serotonina no coração do caracol terrestre *Megalobulimus abbreviatus*

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Introdução: Os estudos sobre a distribuição das monoaminas e do peptídeo FMRFamida no sistema nervoso central e periférico e suas ações nos moluscos evidenciam a importância destes mediadores nestes animais. As suas ações no controle cardíaco são bem documentadas. Menos amplamente reconhecida é a presença destes em circuitos intrínsecos em corações miogênicos. Por esta razão, este trabalho investiga a presença e padrão de distribuição das monoaminas e do neuropeptídeo FMRFamida no coração miogênico e bicavitário de *Megalobulimus abbreviatus*. **Métodos:** Animais adultos provenientes de Minas do Leão (RS) foram mantidos sob condições controladas de temperatura, umidade e fotoperíodo. Após a anestesia dos animais (mentol), realizou-se imunoistoquímica para FMRFamida (4 animais) e o método histoquímico do ácido glioxílico (De La Torre e Surgeon, 1976 modificado, Zancan et al.1997; 4 animais) para a identificação de monoaminas no coração. **Resultados:** O método do ácido glioxílico revelou a presença de terminações serotoninérgicas e não foi detectada a presença de catecolaminas. Foram identificadas muitas fibras finas serotoninérgicas (5HT) no átrio (A), em maior número, e no ventrículo (V). Observou-se uma densa inervação 5HT na junção átrio-ventricular (AV) e fibras 5HT partindo desta região em direção à aorta. Não foram encontrados corpos celulares 5HT. Foi detectada uma extensa rede de feixes imunorreativos à FMRFamida (Fa-ir) de diversos calibres. Feixes calibrosos são mais encontrados no A, desde a região próxima às veias pulmonares até a junção AV, onde inervam a região da valva (Va) com grande densidade de fibras, varicosidades e somas neuronais Fa-ir. Ramificações Fa-ir percorrem o V em direção à aorta. Das fibras Fa-ir menores originam-se numerosos axônios com varicosidades, distribuídas por todo o miocárdio. **Conclusão:** A inervação 5HT provém do nervo pericardial (nP) e predomina sobre a região do A e na região AV. A inervação FMRFamida é tanto extrínseca, do nP (penetra pelo A e distribui-se para todo o coração), quanto intrínseca, com somas neuronais no V. Os dados indicam, ainda, um controle especial do neuropeptídeo FMRFamida na região da valva AV **Palavras-Chave:** Molusco pulmonado, FMRFamida, monoaminas, 5HT, cardiovascular

Apoio Financeiro: CNPq, PIBIC-UFRGS, BIC-PROPESQ-UFRGS

E.03.010

ENTOURAGE EFFECT OF PALMITOYLETHANOLAMIDE IN THE SPINAL CORD OF ANESTHETIZED RATS

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The effects of arachidonylethanolamide (anandamide; AEA) can be enhanced by structurally related, endogenous fatty acid derivatives termed entourage compounds. We have reported that intrathecal (it) injection of AEA to urethane-anesthetized rats induced a hypotensive effect due to the activation of both cannabinoid CB₁ and vanilloid TRPV1 spinal receptors (García et al., Naunyn-Schmiedeberg's Arch. Pharmacol. 368: 270, 2003). The aim of this study was to examine whether palmitoylethanolamide (PEA) behaves as an entourage compound for the hypotensive effects of endocannabinoids administered to the spinal cord. AEA (25, 50 and 100 nmol; it) induced a dose-dependent decrease in the mean blood pressure (Δ MBP: -1.2 ± 2.4 , -10.5 ± 1.3 and -17.3 ± 1.3 mmHg, respectively; $n=4-6$). PEA (100 nmol; it) did not modify *per se* the baseline MBP but increased the hypotensive response to AEA (25 and 50 nmol; it; $p < 0.05$). Moreover, PEA enhanced the hypotensive effects of the endocannabinoid N-arachidonoyldopamine (25 nmol; it) and to the metabolically stable analog of AEA methanandamide (25 and 50 nmol). The hypotensive response induced by co-injection of PEA with 25 nmol AEA was prevented by pre-treatment with either the TRPV1 receptor antagonist capsazepine (20 nmol; it) or the CB₁ receptor antagonist rimonabant (20 nmol; it) and the TRPV1 receptor agonist capsaicin (0.3 nmol; it). By contrast, a significant enhancement of PEA effect was observed after pretreatment with the URB602 (3.5 nmol; it), a putative inhibitor of PEA hydrolysis in microglia cells. It is suggested that PEA positively modulates the hypotensive responses to endocannabinoids in the spinal cord. This facilitatory effect involves the activation of both CB₁ and TRPV1 spinal receptors and seems modulated by enzymatic hydrolysis.

Palavras-Chave: blood pressure, endocannabinoids, entourage effect, anandamide, palmitoylethanolamide

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HISTAMINE-INDUCED RESPONSES IN THE RABBIT PETROSAL GANGLION.

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The carotid body (CB) is sensory innervated, through the carotid sinus nerve (CSN), by petrosal ganglion (PG) neurons. It is accepted that these neurons are driven by transmitters released by CB chemoreceptor cells, but the role of several putative transmitter is still debated. Histamine (HA) has been suggested to play an excitatory role in the CB, but little information is available on the effects of HA on PG neurons. We studied HA-induced responses and the effects of the HA receptor blocker pyrilamine (Pyr) on the discharge frequency of PG neurons projecting through the CSN.

The PGs were removed from adult male New-Zealand White rabbits anaesthetized with a ketamine/xylazine mixture (75/7.5 mg/kg), placed in a recording chamber, and superfused with Hanks' solution, with 5 mM HEPES, pH 7.43 at 38 ± 0.5 °C. The CSN was placed on paired electrodes and the discharge frequency (f_{CSN} , in Hz) was recorded. HA (0.1-200 µg) and ACh (500 µg) were applied in 10 µl boluses over the PG, while Pyr (0.2-10 µM) was applied in the superfusion medium.

Histamine produced a dose-related increase in f_{CSN} , with a threshold dose of 0.1-0.2 µg. About half of the preparations reached a plateau of maximal activity for doses of 20-50 µg, with a mean half effective dose ~2 µg. The remaining ones reached maximal discharge at doses from 2-5 µg; larger doses produced a dose-dependent reduction in the response. Maximal responses induced by HA (82 ± 13 Hz) were significantly lower ($p < 0.01$; paired t-test; $n=10$) than those evoked by ACh (216 ± 23 Hz). Pyr (200 nM) produced a right-shift of the HA dose-response curve, with larger concentrations (≥ 1 µM) blocking HA-induced but not ACh-induced responses.

Our results show that HA applied to the PG increases f_{CSN} , suggesting that HA could be involved in the generation and/or maintenance of chemosensory activity in the rabbit CB.

Palavras-Chave: Sensory neurons; Peripheral nerve; Respiration

Apoio Financeiro: Grants FONDECYT 1040638 and DID ENL 08/11

OXIDATIVE STRESS IN THE PARAVENTRICULAR NUCLEUS IS ASSOCIATED WITH HIGH BLOOD PRESSURE IN SUCROSE FED RATS

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High sucrose fed rats, used as a metabolic syndrome model, present increased arterial blood pressure (BP), hyperinsulinemia and elevated angiotensin II (Ang II) plasma levels. It is well known that Ang II can stimulate the reactive oxygen species production contributing to the maintenance of high BP. Considering that the paraventricular nucleus (PVN) is a site of integration for autonomic and endocrine-mediated cardiovascular responses, the aim of this study was to investigate the relationship between the central and peripheral oxidative stress and the BP, renal sympathetic nerve activity (RSNA) and insulinemia of rats submitted to a metabolic syndrome model.

Male Wistar rats were treated for 30 days with 20% sucrose solution (SCvC, SC) or tap water (C). SCvC received vitamin C (150 mg/kg/d) by gavage during the last 7 days of the experimental protocol, while SC and C received vehicle instead. Oxidative stress was evaluated by thiobarbituric acid reactive substance (TBARS) serum levels and in the PVN by real-time polymerase chain reaction quantification of AT1r, p47phox, gp91phox and CuZnSOD mRNA expression. Mean arterial pressure (MAP), insulinemia, glycemia and RSNA were analyzed.

Compared to C, SC presented higher ($P<0.05$) MAP ($C=111 \pm 3$; $SC=136 \pm 3$ mmHg), RSNA ($C=115 \pm 5$; $SC=152 \pm 4$ spikes/s), insulinemia ($C=1.8 \pm 0.25$; $SC=4.2 \pm 0.76$ ng/mL) and TBARS serum levels ($C=3.0 \pm 0.1$; $SC=4.1 \pm 0.1$ nmol/mL), although no difference was found in glycemia ($C=6 \pm 0.1$; $SC=6 \pm 0.2$ mmol/L). In the PVN of SC there was an increase ($P<0.05$) in AT1r ($SC=48 \pm 9$; $C=1 \pm 0.1$ AU), p47phox ($SC=6 \pm 0.9$; $C=1.4 \pm 0.3$ AU) and gp91phox ($SC=2.1 \pm 0.4$; $C=1.0 \pm 0.06$ AU) mRNA expression levels and a decrease in gene expression of CuZnSOD ($SC=0.2 \pm 0.1$; $C=0.8 \pm 0.1$ AU) when compared with C. The TBARS levels ($SCvC=3.7 \pm 0.1$ nmol/mL) as well as MAP ($SCvC=105 \pm 1$ mmHg) and RSNA ($SCvC=95 \pm 5$ spikes/s) were lower ($P<0.05$) in SCvC than in SC. In addition, the gene expression of AT1r ($SCvC=2.6 \pm 0.3$ AU), p47phox ($SCvC=0.4 \pm 0.04$ AU) and gp91phox ($SCvC=0.2 \pm 0.05$ AU) decreased and CuZnSOD ($SCvC=0.6 \pm 0.06$ AU) increased in the PVN of SCvC compared to SC.

The data suggest that vitamin C administration ameliorates the peripheral oxidative stress and its markers abundance into the PVN that are probably related to hypertension and increased RSNA in SC.

Palavras-Chave: AT1 receptors, sucrose feeding, arterial pressure, paraventricular nucleus, oxidative stress

Apoio Financeiro: FAPESP

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Papel de Receptores Gabaérgicos em regiões distintas bulbares sobre o controle tônico e reflexo Simpático Renal

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Objetivos: O objetivo deste trabalho foi avaliar qual o efeito do bloqueio de receptores GABAérgicos (subtipo A) na região rostroventrolateral (RVLM) ou área pressora caudal (APC) sobre o controle tônico e reflexo cardiovascular. Ratos Wistar (250 – 350 g, n=13), foram anestesiados com uretana (1,4mg/Kg, IV) e instrumentados para análise da atividade nervosa simpática renal (RSNA) e registro direto da pressão arterial.

Métodos: Microinjeções bilaterais de bicuculina (400 pmol, 100 nL) foram realizadas na APC ou RVLM em grupos distintos de animais. Microinjeções de glutamato foram realizadas para a localização da RVLM ou APC. Os parâmetros analisados foram: pressão arterial média (PAM) e RSNA. O barorreflexo renal foi estimado pela administração endovenosa de fenilefrina (10 ug, IV, *in bolus*). Os resultados foram expressos em porcentagem do basal $\pm$ erro padrão, admitindo-se $P < 0,05$ como nível de significância (teste *t*-student).

Resultados: A administração de fenilefrina produziu aumentos significantes de PAM $53 \pm 5,8\%$ e queda da RSNA RVLM ($62 \pm 6,0\%$). A microinjeção de bicuculina na RVLM e APC produziu um aumento significativo da PAM (RVLM $45 \pm 8,1\%$; APC $18 \pm 6,0\%$) e RSNA (RVLM $50 \pm 19,4\%$; APC $14 \pm 3,0\%$). As quedas de RSNA em resposta à ativação barorreflexa pela fenilefrina apresentou um decréscimo de $16 \pm 5,1\%$ e $51 \pm 9\%$ para RVLM e APC, respectivamente.

Conclusões: Os resultados obtidos sugerem: 1) a RVLM e a APC são tonicamente inibidas por sinapses GABAérgicas, a ação dessas sinapses aparentemente é mais intensa na RVLM; 2) A integridade de sinapses GABAérgicas na RVLM porém não na APC é importante para o controle barorreflexo da RSNA. A origem das eferências GABAérgicas à APC é ainda desconhecida. **Palavras-Chave:** RVLM, APC, Atividade Simpática Renal e Barorreflexo

Apoio Financeiro: FAPESP

POTENTIAL BIOCHEMICAL SERUM MARKERS OF VASCULAR INJURY IN CHRONIC CYCLOSPORINE TREATED RATS

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Objective: Cyclosporine (CsA), a potent immunosuppressant agent that has been extensively used in transplanted patients, is related to a variety of side effects. Vascular disease is a major cause of morbidity and mortality among renal and cardiac transplant recipients, but the underlying mechanisms of vascular injury caused by CsA are poorly understood. In the present study, we investigated blood parameters that could be useful as markers of adverse effects on the vasculature induced by chronic CsA administration to non-transplanted rats.

Material and Methods: Male Wistar rats were divided in three groups. The control group received vehicle (corn oil), and treated groups received CsA 5 mg/kg or 15 mg/kg, by daily gastric gavage during 8 weeks. Concentrations of CsA, fibrinogen, platelets, total homocysteine and uric acid levels were assessed, as well as ATP, ADP and AMP hydrolysis in blood serum. CsA 15mg/kg treatment induced cell injury and inflammatory response involving the endothelium and the intimal layer of the aorta artery, as well as statistically significant increase in total homocysteine (Control: 4.7 ± 0.7 ; CsA 5mg/kg: 5.4 ± 0.5 ; CsA 15mg/kg: $8.2 \pm 1.2^* \mu\text{mol/L}$, $P < 0.01$), fibrinogen levels (Control: 362 ± 94 ; CsA 5mg/kg: 377 ± 132 ; CsA 15mg/kg: $596 \pm 259^* \text{mg/dL}$, $P < 0.01$), and platelets number (Control: 58.4 ± 3.8 ; CsA 5mg/kg: 64.4 ± 5.6 ; CsA 15mg/kg: $68.4 \pm 9.3^* \times 10^4/\text{mm}^3$, $P < 0.05$). Additionally, CsA induced a decrease in uric acid levels and ATP, ADP and AMP hydrolysis and there was a statistically significant correlation between uric acid levels and the amount of hydrolysis of ATP ($r = 0.678$, $p < 0.001$), ADP ($r = 0.642$, $p < 0.002$) and AMP ($r = 0.764$, $p < 0.001$).

Conclusion: CsA long-term treatment induced inflammatory vascular disturbances that were corroborated by changes in serum biochemical markers – total homocysteine, fibrinogen, uric acid and platelets - as well as morphological alterations in the aorta artery. Furthermore, we encourage bringing to mind that ectonucleotidase activity may be considered as an additional marker of vascular disturbance caused by CsA.

Palavras-Chave: Cyclosporine, homocysteine, vascular injury

Apoio Financeiro: CNPq and IBN.net

EFEITO DA ISQUEMIA/REPERFUSÃO INTESTINAL SOBRE O RECEPTOR PURINA P2X₇ E O CÓDIGO QUÍMICO DOS NEURÔNIOS DO PLEXO MIOENTÉRICO DO ÍLEO DE RATOS

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Objetivos: O ATP (5-adenosina trifosfato) é conhecido como um co-transmissor e seus receptores são P2X₁₋₇, foi verificada a presença destes receptores no sistema nervoso entérico. A isquemia/reperfusão intestinal (I/R-i) causa alterações morfológicas nos neurônios entéricos. Este trabalho tem como objetivo estudar possíveis alterações na distribuição do receptor P2X₇ e nas classes dos neurônios mioentéricos do íleo de ratos submetidos a I/R-i.

Métodos: A isquemia intestinal foi obtida pela oclusão da artéria ileal por 30 minutos, com períodos de reperfusão de 24 e 72 horas. Foram utilizados fragmentos do íleo para métodos de imunohistoquímica de simples e duplas marcações do receptor P2X₇ com o óxido nítrico sintase (NOS – marcador de neurônios inibitórios), a calretinina (Calr – marcador de neurônios excitatórios) e a calbindina (Calb – marcador de neurônios sensoriais). As análises foram feitas em microscópio de fluorescência e de confocal de varredura a laser.

Resultados: As análises qualitativas demonstraram nos neurônios do grupo I/R-i de 24h e 72h diminuição da expressão do receptor P2X₇ e degeneração dos neurônios NOS-ir (imunorreativos). As análises quantitativas demonstraram: as colocalizações do grupo controle (GC) dos neurônios NOS-ir, Calr-ir e Calb-ir com o receptor P2X₇-ir, foram 98±0,4%, 99±1% e 99±0,8%, respectivamente; no grupo I/R-i 24h foram: 98±2%, 99±1% e 98±0,4%, respectivamente e no grupo I/R-i 72h foram: 96±0%, 99±0,6% e 95±6%, respectivamente. Houve diminuição na densidade neuronal (neurônios/mm²) do grupo I/R-i 24h e 72h (p<0,05) quando comparado ao GC. A densidade do GC foi: neurônios receptor P2X₇-ir 168,4±0,7, neurônios NOS-ir 57,9±0,1, neurônios Calr-ir 65,8±0,3 e neurônios Calb-ir 21,0±0,1; grupo I/R-i 24h: neurônios receptor P2X₇-ir 131,6±0,1, neurônios NOS-ir 39,5±0,2, neurônios Calr-ir 47,4±0,1 e neurônios Calb-ir 13,2±0,1; no grupo I/R-i 72h: neurônios receptor P2X₇-ir 123,7±0,9, neurônios NOS-ir 34,2±0,1, neurônios Calr-ir 39,5±0,1 e neurônios Calb-ir 13,2±0,1.

Conclusão: A I/R-i afetou a expressão do receptor P2X₇ e classes de neurônios NOS-ir, Calr-ir e Calb-ir do íleo, isto sugere que a I/R-i possa alterar a motilidade intestinal.

Palavras-Chave: Receptor P2X₇, neurônio mioentérico, isquemia/reperfusão intestinal

Apoio Financeiro: FAPESP/CAPES

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ANALYSIS OF GLIAL FIBRILLARY ACIDIC PROTEIN IN BRAIN CARDIOVASCULAR AREAS OF WISTAR KYOTO AND SPONTANEOUSLY HYPERTENSIVE RATS

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Objective: Hypertension may be regulated by neurotransmission modulation in the central cardiovascular areas, which might be influenced by astrocyte function. The present study analyzed the distribution of the glial fibrillary acidic protein (GFAP), the major constitutive of the intermediate filament of the astroglial cytoskeleton, in areas of the brainstem, and the protein levels in areas of the central nervous system involved with cardiovascular regulation such as nucleus tractus solitarius (NTS), locus coeruleus (LC) and hypothalamic paraventricular nucleus (PVN) of the Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats.

Methods: Male WKY and SHR rats (3 months old) were used. The mean blood pressure (BP) was achieved through the tail-cuff method. One group was processed for GFAP immunoreactivity (IR), that was quantified by means of morphometric (area, μm^2) and microdensitometric (spMGV) image analysis. The other group, caudal and rostral NTS, was submitted to Western Blot in order to evaluate GFAP levels. Student's t test was applied and data presented as percentage of WKY.

Results: Highest labeling of GFAP was found in the NTS, LC and PVN compared to other areas of the same level. BP was increased in the SHR (85%) compared to WKY. The area of GFAP IR decreased ($p < 0.05$) in the caudal NTS (53%), rostral NTS (24%), LC (58%), PVN (32%) of the SHR. The spMGV also diminished ($p < 0.05$) in the caudal NTS (18%), rostral NTS (17%), LC (15%), PVN (23%) of the SHR. The levels of GFAP were decreased in the caudal NTS (25%) and rostral NTS (18%) of SHR.

Conclusions: The massive presence of the GFAP in central cardiovascular areas and the regulation of the protein in SHR animals open up the possibility of an astroglial function in the mechanism of the BP control.

Palavras-Chave: glial fibrillary acidic protein, nucleus tractus solitarius, hypertension

Apoio Financeiro: CNPq and FAPESP

EFFECT OF L-NAME MICROINJECTED INTO THE NUCLEUS TRACTUS SOLITARIUS ON THE JEJUNAL GLUCOSE ABSORPTION

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Objectives: The nucleus tractus solitarius (NTS) plays a role in the autonomic regulation, mainly, in the integration of the cardiovascular, respiratory and gastrointestinal system. In this study were evaluated cardiovascular parameters and investigated the effect of the NG-nitro-L-arginine-methyl ester (L-NAME), microinjected into NTS, on the jejunal glucose absorption.

Methods: Male Wistar rats (210g 250g) were anesthetized (urethan 1,4g/ml/Kg) and a cannula was placed into the femoral artery. Rats were submitted to midline laparotomy to expose and isolated 20 cm of jejunal loop. Saline (n=12) or L-NAME (10 nmol 100 nl⁻¹, n=7) and a subgroup vagotomized rats, saline (n=6) or L-NAME (10 nmol 100 nl⁻¹, n=7) were injected into the rostral NTS using a stereotaxic instrument. Tyrode solution pH=8, containing twice glucose, sodium and potassium concentrations was infused (0.5 mL min⁻¹) into the jejunal loop. Samples were taken at 10-min intervals during the 40-min experiment. The cardiovascular parameters were registered during all the experimental time. The glucose was measured by enzymatic method.

Results: There was no significant difference in blood pressure between the L-NAME and control groups. In addition, the microinjection of L-NAME (10nmol) into the NTS promoted significant increase of the jejunal glucose absorption when compared with the control group: 10'(1,9 ± 0,1 vs. 3,1 ± 0,3mg) 20'(2,0 ± 0,2 vs. 3,6 ± 0,3mg), 30'(2,0 ± 0,2 vs. 3,1 ± 0,1mg), 40'(1,2 ± 0,2 vs. 3,1 ± 0,3mg), p<0.05. The vagotomy associated with L-NAME did not modify the effect observed when L-NAME was administrated alone. Glucose absorption of the group that received L-NAME associated with vagotomy increased at 10'(2,3 ± 0,1 vs. 3,3 ± 0,4mg) and 40'(1,9 ± 0,2 vs 2,9 ± 0,2mg), p<0,05.

Conclusion: L-NAME microinjected into rostral NTS increased glucose absorption, which was not impaired by the vagal outflow tract. The sympathectomy effect will be evaluated.

Palavras-Chave: NUCLEUS TRACTUS SOLITARIUS (NTS), JEJUNAL ABSORPTION, GLUCOSE, L-NAME

Apoio Financeiro: Supported by FAPEMIG

E.03.018

Perfil do marcador bioquímico de lesão miocárdica creatinoquinase-MB (CK-MB) em primatas não humanos da espécie *Cebus apella* expostos ao metilmercúrio (metilHg)

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Objetivos: investigar, através da análise quantitativa, o perfil da CK-MB em macacos *Cebus apella* expostos ao metilHg. Secundariamente associaram-se as alterações músculo-esqueléticas, renais e hepáticas ao comprometimento cardíaco, através da análise quantitativa da CK, uréia, creatinina e transaminases séricas.

Métodos: Foram utilizados quatro espécimes machos adultos. As amostras de sangue foram coletadas por punção venosa, na região do antebraço do animal e na região proximal do membro inferior. E conduzidas ao Laboratório de Análises Clínicas do Hospital Universitário João de Barros Barreto. Os animais foram expostos por via oral com metilHg (4) utilizando-se uma quantidade de 0,5mg/kg dissolvida no leite condensado cinco vezes por semana durante 120 dias.

Resultados: Os resultados demonstraram que houve elevação gradativa da CK-MB durante o tempo de exposição (69 - 634 UI/mL no animal de exposição completa e de 76 – 106 UI/mL no de exposição em andamento). As demais substâncias mantiveram-se com valores de referência normais.

Conclusão: Visto que os valores de referência para CK-MB variam de 0 – 24 UI/mL (1,2 e 3), o grau de elevação desta enzima nos animais tratados com metilHg, demonstra que a mesma pode ser indicadora de lesão miocárdica na referida exposição.

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Palavras-Chave: metilmercúrio. Creatinoquinase-MBEnvolvimento cardíaco.

Apoio Financeiro: Financiado pela Secretaria de Ciência e Tecnologia do Estado do Pará/PRONEX.

ÁCIDOS GRAXOS LIVRES (AGL) E GLICOSE COMO MARCADORES METABÓLICOS DE ESTRESSE AGUDO EM RATOS

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OBJETIVOS: Os vários tipos de estresse são situações que envolvem a integração de sistemas neuroendócrinos, tendo como respostas alterações comportamentais, hormonais e metabólicas. Estudos de indicadores de estresse tem sido feitos, mas não existe a definição de marcadores ideais até o momento. A dosagem da corticosterona foi usada como marcador convencional do estresse. O objetivo do presente trabalho foi verificar se a dosagem plasmática de ácidos graxos livres (AGL) e glicose podem ser usadas como marcadores metabólicos alternativos de estresse em animais submetidos a três diferentes estressores: imobilização (IMO), nado forçado (NADO) e labirinto em cruz elevado (LCE). **MÉTODOS:** Foram utilizados ratos Wistar machos adultos, pesando em média 250g. Os animais foram submetidos a canulação da veia jugular para repetidas amostragens. Os animais foram divididos em 3 grupos: G1= IMO, sessão de 1h, coleta de sangue 0, 30 e 60 min após a retirada do animal do modelo; G2=NADO, sessão de 15 min, coleta de sangue 0 e 30 e 60 min após a retirada do animal do modelo; G3 = LCE, sessão de 5 min, coleta de sangue 0 e 30 e 60 min após a retirada do animal do modelo. Para os três grupos foram feitos grupos controles, animais não submetidos à sessão de estresse. Também foram coletadas amostras de sangue 90 min antes de iniciar o experimento (basal). Todos os animais foram submetidos ao jejum de 8 h, sendo recolocado o alimento 4 h antes do experimento. As amostras sanguíneas foram usadas para dosagem de AGL, glicose e corticosterona. **RESULTADOS:** Os resultados indicam que houve aumento estatisticamente diferente dos níveis de glicose e AGL nos três modelos de estresse utilizados. A resposta lipolítica máxima nos animais submetidos à IMO e ao NADO foi observada no tempo 0, sendo esse valor 139% e 96% acima do valor basal, respectivamente. Animais submetidos ao LCE apresentaram aumento dos níveis plasmáticos de AGL no intervalo de tempo 0-60 min, o valor máximo foi no tempo 30 com 300% acima do valor basal. Nos animais submetidos à IMO e ao NADO, o valor máximo da glicemia foi observado no tempo 0, 20% e 32% acima do valor basal, respectivamente. A exposição ao LCE promoveu aumento da glicemia, o valor máximo foi no tempo 30, com 47% acima do valor basal. O nível sérico de corticosterona manteve-se significativamente aumentado dos 0-60 min, nos três grupos experimentais, chegando esse aumento a 54% (IMO), 83% (NADO) e 75% (LCE) acima do valor basal, todos no tempo 30 do período experimental. **CONCLUSÃO:** Nossos resultados corroboram os dados existentes na literatura, onde tem sido demonstrado que o aumento da concentração de substratos energéticos no sangue podem ser indicativos do estado de estresse. Assim, a concentração de AGL e glicose plasmática pode ser um valioso marcador para a caracterização da resposta individual para vários tipos de estressores.

Palavras-Chave: marcadores de estresse, ácidos graxos livres, glicose

Apoio Financeiro: Universidade do Planalto Catarinense - UNIPLAC

E.04.002

Short-term environmental enrichment increases adult neurogenesis in the dentate gyrus of type 1 diabetic mice.

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Objectives Diabetes affects several body systems including the CNS. The hippocampus is involved in cognitive processing, and neurogenesis in the hippocampal dentate gyrus (DG) is essential for some types of learning and memory. We previously found diminished neurogenesis in diabetic mice which is reversed by antidepressant treatment (Eur J Neurosci, 23:1539, 2006). In aging or depression, the exposure to a rich environment is able to increase neurogenesis in the DG. Our aim was to test if a short environmental enrichment (EE) was capable of reversing the low neurogenesis in the DG of type 1 diabetic mice.

Methods Adult male C57BL/6 mice were rendered diabetic by streptozotocin. Diabetic and control mice were exposed for 10 days to either standard caging (SC) or EE. The EE involved more mice per cage, larger cages with toys, tubes, houses and nesting material. No running wheel was present to avoid the influence of physical exercise on neurogenesis. Bromodeoxyuridine (BrdU) was injected 24 h before starting the EE. We performed immunohistochemistry to study cell survival (BrdU), neuronal differentiation (BrdU-Tuj1 double immunofluorescence) and dendritic length of new neurons (DCX).

Results Cell survival was reduced in diabetic animals in SC, and exposure to EE significantly increased it (CTL-SC 1076±71.44; CTL-EE 1254±130.0; DIAB-SC 579.2±100.5, P<0.05 vs CTL-SC; DIAB-EE 1194±186.9, P<0.05 vs DIAB-SC; mean±SEM number of BrdU+ cells/DG). Neuronal differentiation was lower in diabetic animals with SC and exposure to EE augmented it (CTL-SC 0.89±0.01; CTL-EE 0.93±0.009; DIAB-SC 0.79±0.02, P<0.05 vs CTL-SC; DIAB-EE 0.89±0.02, P<0.01 vs DIAB-SC; mean±SEM ratio of BrdU+Tuj1+/BrdU+ cells). We found decreased dendritic length in DCX+ cells of diabetic animals and EE increased length to control levels.

Conclusions Our results showed that a short-term exposure to a rich environment increased adult hippocampal neurogenesis and positively modulated the dendritic length of newborn neurons in diabetic mice. Further investigation is needed to identify potential mediators of these events and to correlate neurochemical results with cognitive impairments associated with the disease.

Palavras-Chave: Diabetes, environmental enrichment, dentate gyrus, neurogenesis, dendritic morphology

Apoio Financeiro: Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (BID 1728/OC-AR PICT 2006 N° 1845), Facultad de Medicina, Universidad de Buenos Aires.

PHARMACOLOGICAL INTERVENTIONS OF SEROTONERGIC MODULATION IN SWIM STRESSED RATS

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Saint Johns Wort (SJW), a herbal medicinal compound, is well known for its broad pharmacological spectrum. Present study has investigated acute effects of SJW (500mg/kg) administration on behavioral, neuroendocrine responses and serotonergic (serotonin, 5HT) activity following forced swim test (FST) exposure in rats. 5-hydroxytryptamine (5HT) and its metabolite 5-hydroxyindoleacetic acid (5HIAA) in brain regions were quantified with an HPLC coupled to an electrochemical detector. SJW increased swimming and climbing behavior of rats during FST exposure. Swim stress produced significant reduction in serum total tryptophan ($P<0.01$), increase in corticosterone ($P<0.01$) and increase in 5HT turnover in hypothalamus by 100 % ($P<0.01$), amygdala by 148.07 % ($P<0.01$), and hippocampus by 41.3 % ($P<0.05$) when compared with unstressed saline injected group. SJW in swim stressed rats when compared with saline injected stressed rats altered neither lowered serum tryptophan nor enhanced HPA axis response, however 5HT was found to be increased by 109.86% ($P<0.01$), 162.89% ($P<0.01$) and 171.69% ($P<0.01$), in hypothalamus, amygdala and in hippocampus respectively. 5HIAA was also found increased in hypothalamus by 73.54 % ($P<0.01$), amygdala by 44.6 % ($P<0.01$) and hippocampus by 143.5 % ($P<0.01$). Further SJW administration in unstressed rats showed decrease in tryptophan ($P<0.01$), increase in corticosterone ($P<0.01$), 5HT was found to be decreased in hypothalamus (46.59%, $P<0.01$) and in amygdala (13.15%, $P<0.05$) with no change in hippocampus, while 5HIAA was found increased in hypothalamus by 57.64% ($P<0.01$), amygdala by 203.06% ($P<0.01$) and hippocampus by 170.9% ($P<0.01$). In conclusion, SJW increases intraneuronal 5HT metabolism but inhibits its release under adverse conditions proving its anxiolytic property.

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Palavras-Chave: Forced Swim Stress, Behaviour, St. Johns Wort, 5HT

Apoio Financeiro: Department of Biochemistry, University of Karachi

E.04.004

Effects of CRH-R₁ receptor antagonist on gonadotrophins secretion in female rats under restraint stress.

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Objectives: Acute stress modulates hypothalamus-pituitary-gonadal (HPG) axis activity at different levels. Corticotropin-releasing hormone (CRH), the principal regulator of the hypothalamus-pituitary-adrenal (HPA) axis, exerts its actions through CRH-R₁ and CRH-R₂ receptors. CRH-R₁ receptor has greater expression principally in central nervous system and participates in disorders that involved hyperactivity of HPA axis. Antalarmin is a selective non-peptide CRH-R₁ receptor antagonist. In the present study we evaluated if the effect of acute restraint stress associated with estrogen action on luteinizing hormone (LH) and follicle-stimulating hormone (FSH) is mediated by CRH through CRH-R₁ receptors. **Methods:** estrogen-primed ovariectomized rats bearer of intravenous (i.v.) catheter received either antalarmin (0,1 or 0,5mg/kg i.v.) or vehicle before restraint stress by 40 minutes. Blood samples were collected before (1000h) and after stress for evaluating early (until 1400h) and late stress effects (until 1800h). **Results:** Restraint stress induced a large increase in early LH ($P<0,001$) but not FSH secretion, that was blocked by antalarmin ($P<0,001$). Afternoon LH surge induced by estrogen was delayed by stress while the FSH surge was inhibited by stress ($P<0,05$). Antalarmin did not affect these responses. **Conclusion:** these data suggest that CRH-R₁ receptors are involved in early effects induced by restraint stress on LH secretion, but not in LH and FSH surges induced by estrogen at afternoon. **Palavras-Chave:** Restraint stress, antalarmin, luteinizing hormone, follicle-stimulating hormone.

Apoio Financeiro: FAPESP and CNPq from Brazil.

INFLUÊNCIA DA EXPOSIÇÃO A DOIS DIFERENTES ESTRESSORES NOS NÍVEIS PLASMÁTICOS DE ÁCIDOS GRAXOS LIVRES (AGL) E GLICOSE EM RATOS

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OBJETIVOS: Estudos anteriores em nosso laboratório demonstraram que o aumento da concentração de substratos energético no sangue pode ser indicativo do estado de estresse. Assim, a concentração de AGL e glicose plasmática podem ser valiosos marcadores para a caracterização da resposta individual para vários tipos de estressores. O objetivo do presente trabalho foi verificar a influência da exposição a dois diferentes estressores, nado forçado (NADO) e labirinto em cruz elevado (LCE), nos níveis plasmáticos de AGL e glicose em ratos. **MÉTODOS:** Foram utilizados ratos Wistar machos adultos, pesando em média 250g. Os animais foram submetidos à canulação da veia jugular para repetidas amostragens. Os animais foram divididos em 2 grupos: G1= NADO (15 min) seguido do LCE (5 min), coleta de sangue 0, 30 e 60 min após a retirada do animal do LCE; G2= LCE (5 min) seguido de NADO (15 min), coleta de sangue 0 e 30 e 60 min após a retirada do animal do modelo. Para os 2 grupos foram feitos grupos controles, animais não submetidos às sessões de estresse. Também foram coletadas amostras de sangue 90 min antes de iniciar o experimento (basal). Todos os animais foram submetidos ao jejum de 8 h, sendo recolocado o alimento 4 h antes do experimento. As amostras sanguíneas foram usadas para dosagem de AGL, glicose plasmática e corticosterona. **RESULTADOS:** Os resultados indicaram que houve aumento estatisticamente significativo dos níveis de AGL e glicose nos grupos submetidos a dois diferentes tipos de estressores. A resposta lipolítica nos animais submetidos ao NADO/LCE e ao LCE/NADO foi máxima no tempo 0, atingindo 95% e 132% acima do valor basal, respectivamente. Nos animais submetidos ao NADO/LCE, o aumento da glicemia foi observado no intervalo de tempo 0-30 min, quando comparado com o controle no mesmo intervalo de tempo. O valor máximo foi obtido no tempo 0 (32% acima do valor basal). Nos animais submetidos ao LCE/NADO o aumento da glicose plasmática foi significativamente diferente somente no tempo 0 (54% acima do valor basal), quando comparado com o grupo controle no mesmo intervalo de tempo. O nível sérico de corticosterona manteve-se significativamente aumentado dos 0-60 min, nos dois grupos experimentais, chegando esse aumento a 80% (NADO/LCE) e 83% (LCE/NADO) acima do valor basal, no tempo 0 do período experimental. **CONCLUSÃO:** Nossos resultados mostraram que houve influência da exposição a dois diferentes estressores, nos níveis plasmáticos de AGL e glicose em ratos.

Palavras-Chave: marcadores de estresse, ácidos graxos livres, glicose

Apoio Financeiro: Universidade do Planalto Catarinense - UNIPLAC

E.04.006

EFEITO DO ESTRESSE PROVOCADO POR CONTENSÃO NA PRODUÇÃO DE LEITE, NA SECREÇÃO DE PROLACTINA E CORTICOSTERONA EM RATAS LACTANTES

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Objetivo: analisar o efeito do estresse provocado por contensão (EC) na produção de leite, no ganho de peso da ninhada, na secreção de prolactina (Prl) e corticosterona (Cort) em ratas lactantes.

Métodos: fêmeas lactantes Wistar foram divididas em 2 grupos: grupo 1 (controle; N=12) não sofreu EC e grupo 2 (N=12) foi submetido diariamente ao EC por 30 min. No 1º, 3º, 5º, 7º e 9º dia de idade, os filhotes foram pesados e, no 10º dia pós-parto (DPP), a fêmea foi decaptada para coleta de sangue. Esta foi realizada em tubo heparinizado, o sangue foi centrifugado a 3500 rpm por 10 min, o plasma foi separado e estocado em freezer -20°C para posterior análise das concentrações plasmáticas de Prl e Cort. As médias ($\pm$ E.P.M.) do ganho de peso (gramas) dos filhotes e das concentrações hormonais foram comparadas pelo teste *t* de Student ($p < 0,05$).

Resultados: o EC realizado diariamente no grupo 2 alterou significativamente o ganho de peso da ninhada apenas no 1º DPP ($53,4 \pm 3,2$; $t = 0,387$, $p = 0,02$) quando comparado ao grupo 1 ($52,0 \pm 1,7$). Entretanto, não houve diferença significativa no 3º ($68,7 \pm 2,8$ e $69,4 \pm 2,3$), 5º ($90,8 \pm 3,5$ e $91,1 \pm 3,0$), 7º ($112,0 \pm 4,7$ e $115,3 \pm 3,5$) e 9º DPP ($140,0 \pm 5,6$ e $138,2 \pm 4,5$; grupo 2 e 1, respectivamente). A análise das concentrações plasmáticas de Prl e Cort está sendo realizada e, neste momento, temos apenas resultados preliminares.

Conclusão: O ganho de peso dos filhotes reflete diretamente a produção de leite das fêmeas e amamentação. Nossos resultados indicam que o EC da fêmea, após o parto, não ocasionou redução na produção de leite. No entanto, somente após as dosagens de Prl e Cort poderemos analisar se o EC modifica a concentração destes marcadores fisiológicos ao estresse. Além disso, poderemos correlacionar a concentração destes marcadores com a produção de leite e a amamentação de ratas.

Apoio Financeiro: UFCSPA

E.04.007

INTERVENÇÕES NO PERÍODO NEONATAL: POSSÍVEL MODIFICAÇÃO DA SUSCEPTIBILIDADE AO ESTRESSE CRÔNICO EM RATAS ADULTAS

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Objetivos

O estudo visou verificar se ratos que passaram pela separação materna (SM) por períodos prolongados poderiam ter afetada a susceptibilidade a parâmetros relacionados a comportamentos do tipo deprimido ou ansioso após serem submetidos ao estresse crônico variado (ECV) na idade adulta.

Métodos

Durante os dias 1-10 pós-natal, ratos Wistar foram submetidos à SM, permanecendo 3h/dia afastados da mãe. Quando adultas, ratas fêmeas passaram pelo ECV, que consiste em diversos agentes estressores moderados, por 40 dias. Após esse período, foram analisados parâmetros comportamentais, relacionados a depressão (tempo de imobilidade no nado forçado), ansiedade (tempo de permanência nos quadrados centrais (TQC) do campo aberto) e atividade motora (atividade locomotora e exploratória na mesma tarefa), e bioquímicos, como níveis de corticosterona plasmático (CORT) e da proteína S100B no líquido (por kits comerciais de ELISA). Diferenças consideradas significativas quando $P < 0,05$.

Resultados

No nado forçado, os animais separados no período neonatal apresentam menor tempo de imobilidade; dados expressos em média±EPM: [intacto (I)=3,55±0,19; ECV=3,94±0,25; SM=2,94±0,27; SM+ECV=2,86±0,34]. Na tarefa do campo aberto, foi observada uma diferença no grupo SM+ECV (dia do teste), permanecendo menos tempo nos TQC, indicando maior ansiedade (I=3,76±1,09; ECV=4,84±1,33; SM=8,17±1,74; SM+ECV=3,28±0,89). Para a atividade exploratória (respostas de orientação) e atividade locomotora (cruzamentos), foi observado efeito do ECV, diminuindo tais comportamentos (respostas de orientação: I=18,5±2,65; ECV=8,9±2,41; SM=26,5±2,56; SM+ECV=12±3,40; cruzamentos: I=100,7±5,33; ECV=82,8±7,38; SM=108,6±6,05; SM+ECV=89,7±5,83). Há efeito do ECV, aumentando os níveis de CORT plasmática (I=197,2±259; ECV=214,1±163,1; SM=186,4±185,1; SM+ECV=238,5±315,8) e de S100B no líquido (I=0,82±0,10; ECV=2,39±0,49; SM=1,22±0,53; SM+ECV=1,98±0,55).

Conclusões

A SM levou a um menor comportamento do tipo deprimido nas ratas fêmeas, sugerindo um papel protetor dessa intervenção no período neonatal. Por outro lado, nos demais parâmetros avaliados, a separação não foi capaz de alterar os efeitos induzidos pelo ECV.

Palavras-Chave: separação materna, estresse crônico variado, corticosterona, S100B, depressão, ansiedade, atividade exploratória, atividade locomotora

Apoio Financeiro: CNPq, FINEP/IBNnet.

Localization in plasma membrane lipid microdomain and transmembrane domain self-association of neuronal membrane glycoprotein, M6a

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We have previously identified M6a as stress/antidepressant-responsive gene in the hippocampus in different animal models. This gene is down regulated in both physically and socially stressed animals. Antidepressant treatment can block these stress effects. M6a is a protein with four transmembrane domains (TMs), two extracellular loops and N- and C- terminal extensions toward the cell cytoplasm. This glycoprotein is involved in neuronal plasticity through an unknown mechanism. The self-association of membrane proteins and/or with specific microdomains such as lipids rafts in the plasma membrane is important in the regulation of extra and intracellular signaling pathways and can give us a clue of how the protein exerts its function. **Objectives:** To study protein-protein and protein-lipid interactions of M6a in rat hippocampus and neuroblastoma cell line (N2A). **Methods:** To determine if M6a could be part of a lipid raft, a discontinuous sucrose gradient in the presence/absence of different non ionic detergents followed by ultracentrifugation and SDS-PAGE-WB technique in a rat hippocampus and N2A was developed. In addition, to establish if M6a is organized as a monomer or an oligomer with a homo/hetero-protein-protein interaction in the plasma membrane, a chemical crosslinking assay and an *in vitro* homodimerization of TMs using a TOXCAT system were performed. **Results:** We identified the presence of M6a in detergent resistant membranes (DRMs) from both hippocampal tissue and N2A cell line. Also, we tested for the formation of stable M6a oligomers in the presence/absence of chemical cross-linkers and under increasing urea concentrations. We show that under non reducing conditions M6a form different stable multimers. Similar results were obtained when we used a chemical crosslinker, although the participation of other proteins cannot be excluded. Using a TOXCAT oligomerization assay for the four M6a-TMs we find self-interaction particularly in TM1 and TM2. **Conclusion:** We show that lipid raft microdomain association is an intrinsic property of M6a. Furthermore, our data suggest a strong propensity to self-association of M6a-TMs that could be involved in the formation of oligomers. **Palavras-Chave:** stress, neuronal glycoprotein, protein-protein interaction, lipid raft

Apoio Financeiro: Agencia Nacional de Promoción Científica y Tecnológica de Argentina

Supplementation with fish oil and coconut fat prevents changes on postnatal development caused by prenatal stress.Borsonelo, E.C.¹; Suchecki, D¹; Galduroz, J. C.¹ - ¹UNIFESP - Psicobiologia

Prenatal stress (PNS) has been associated with impairment of offspring's development. In addition, nutritional factors during pregnancy and lactation could influence fetal and postnatal growing. This study assessed the postnatal development of rat offspring exposed to PNS whose mothers were fed with different diets during pregnancy and lactation. Female Wistar rats were assigned to one of three groups: regular diet (R), diets supplemented with coconut fat (C) or fish oil (F). When pregnancy was confirmed, they were distributed into control (CLT) or PNS groups. PNS was accomplished by restraint stress during the last week of pregnancy. Body weight, righting reflex and locomotion were assessed during different phases of development. At birth, PNS males and females were lighter than their respective CLT [males: 6.87±0.08g (N=86); 7.22±0.10 (N=60), p=0.007; females = 6.51±0.07g (N=87); 6.81±0.09 (N=67), p=0.006]. At 21 days of age, PNS+R males exhibited lower body weight than CTL+R [47.59±1.45g (N=33); 55.06±1.25 (N=15), p=0.000, respectively], whereas C and F diets prevented this effect [PNS+C= 60.50±1.71g (N=21), p=0.000] and PNS+F [55.92±0.87 (N=32), p=0.000, respectively]. Similar results were seen in PNS+R females compared CLT+R [44.90±1.30g (N=28); 52.82±0.90 (N=18), p=0.000], being also prevented by the diets [PNS+C= 59.84±1.68g (N=25); p=0.000] and [PNS+F=55.04±0.77 (N=34), p=0.000, respectively]. C diet decreased the latency of righting reflex in 1 day-old females compared to the other groups [C=11.54±1.61 sec (N=48); R=21.54±3.60 (N=46), p=0.02; F=20.04±2.54 (N=60), p=0.02]. In males, CLT+F diet impaired the reflex compared CLT+R [18.31±3.53 sec (N=21); 6.45±2.12 (N=15), p=0.05]. In regards to locomotion in 13 day-old, PNS+R males ambulated more than CTL+R males [14.48±1.48 (N=33); 5.93±0.74 (N=15), p=0.000]. In females, the effects were similar [PNS+R= 14.53±1.10 (N=28); CTL+R=6.33±0.57 (N=18), p=0.000] but prevented by F diet [8.94±1.05 (N=34), p=0.01]. In conclusion, the postnatal development of pups, from birth until weaning, was influenced by supplemented diets and by PNS. Some of the alterations induced by PNS were prevented by C and F diets.

Palavras-Chave: prenatal stress, development, fish oil, pregnancy, diet

Apoio Financeiro: Capes and AFIP

E.04.010

FENOTIPOS ASOCIADOS A LA DISFUNCION DE M6 EN *Drosophila*.

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En situaciones de estrés crónico el cerebro responde mediante procesos de remodelación neuronal y plasticidad sináptica. Identificamos un gen, *m6a*, cuya expresión en el hipocampo disminuye en los modelos de estrés crónico por inmovilización en ratones y de estrés psicosocial en musaraña. Este efecto se revierte mediante el tratamiento con antidepresivos. M6a es una glicoproteína, miembro de la familia 'Proteolipid proteins' (PLP) y tiene un rol importante en la extensión de neuritas y formación de filopodios/espinas (PNAS. 102:17196, 2005). Objetivo: Para desentrañar la función de M6a a nivel molecular y celular se decidió iniciar la caracterización del gen ortólogo, *m6*, en *Drosophila melanogaster*. Los estudios evolutivos indican que *m6* es el único representante de la familia PLP en artrópodos y el análisis bioinformático de las secuencias reveló que M6a y M6 presentan homología estructural. Métodos: Se dispone de una línea mutante en la que demostramos que los niveles de ARNm de *m6* están disminuidos (40%±8%) con respecto al control debido a la inserción del transposón. Para analizar si esta mutante presenta defectos asociados a su función en sistema nervioso se la evaluó en distintos paradigmas comportamentales -geotaxis, actividad locomotora, sobrevida y diversas condiciones de estrés (exposición a temperaturas extremas, agentes oxidantes y ayuno). Resultados: En ensayos de actividad locomotora observamos que la línea mutante para M6 posee menores niveles de actividad (44%±4%) al comparar con el control mientras que los demás paradigmas testeados no mostraron diferencias. Además se observó un fenotipo no esperado: las hembras mutantes para M6 son estériles. No observamos alteraciones severas en la ovogénesis temprana e intermedia al comparar los ovarios de la mutante para M6 con el control, sin embargo los huevos depositados tienen serias malformaciones morfológicas que impiden su desarrollo. Actualmente estamos evaluando si M6 estaría involucrada en la formación de la cubierta del huevo. Conclusión: La disminución de M6 determina no solamente alteraciones en el sistema nervioso si no también en el sistema reproductivo en *Drosophila melanogaster*.

Palavras-Chave: *Drosophila*, comportamiento, esterilidad, PLP

Apoio Financeiro: CONICET

E.04.011

Mutational analysis of the stress regulated glycoprotein M6a

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A neurobiological understanding of depression that could lead to the development of improved treatments requires the identification of the genes that make individuals vulnerable to the syndrome. In our previous work, we identified the gene encoding the glycoprotein M6a whose expression is diminished by chronic stress exposure in the hippocampal formation. This effect is counteracted by treatment with antidepressants. Furthermore, we demonstrated that M6a plays an important role in neurite/filopodium outgrowth. Based on our data we suggested that reduced M6a expression by chronic stress might be directly related to the morphological alterations found in the hippocampus of chronically stressed animals. The molecular mechanism that would explain how the stress-regulated neuronal glycoprotein M6a mediates its effects on neurite/filopodium outgrowth and its involvement in chronic stress response remains unclear.

Objective: To define the biologically critical part of the M6a molecule essential for the function of the protein in filopodial protrusion formation.

Methods: Using site-directed mutagenesis we constructed a panel of M6a mutants with appropriate point mutations at the key positions expected to interfere with the function of the protein. The cellular localization of these mutants as well as the effect on filopodial protrusions formation were assessed in neuroblastoma cell line and primary hippocampal neurons by immunomicroscopy.

Results: We show that four cysteines C162, C174, C192 and C202 within the large extracellular loop (EC2) are critical. The presence of two cysteines C162 and C202 is required for the efficient cell surface expression of M6a. On the other hand, mutations of C174 and C192 do not affect the cell surface expression of M6a protein. Interestingly, neurons overexpressing C174A and C192A mutants display decreased filopodia number. In addition, the function blocking monoclonal antibody against the M6a protein fails to recognize C174A and C192A mutants in non-permeabilized cells.

Conclusion: Taken together, we demonstrated that the EC2 cysteines are critical for proper folding of the EC2 domain and the role of M6a in filopodia formation.

Palavras-Chave: M6a glycoprotein, chronic stress, depression, mutational analysis, neurite/filopodia outgrowth

Apoio Financeiro: CONICET

Efeitos de dexametasona (DX) e adrenalectomia (ADX) na expressão de receptores para glicocorticóides (GRs) em neurônios gabaérgicos do núcleo paraventricular (PVH).

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Objetivos: Neurônios gabaérgicos controlam a atividade do eixo hipotálamo-hipófise-adrenal (HPA) via glicocorticóides (Gc). O objetivo deste trabalho foi investigar a expressão de GRs em neurônios GABA na região parvocelular do PVH em diferentes condições de atividade do eixo HPA. **Métodos:** Após 5 dias de ambientação no biotério, ratos machos Wistar foram divididos em 6 grupos (I a VI): I e II (normais); III e IV (*sham*, cirurgia fictícia) e V e VI (ADX bilateral). No 6º dia, os animais receberam injeção intraperitoneal de salina (NaCl 0,15M, grupos I, III e IV) ou DX (2,5 mg/100g de peso corporal, grupos II, V e VI) às 19.00h. Após 12h, foram anestesiados para coleta de sangue (para dosagem de corticosterona-CT por radioimunoensaio), seguida de perfusão para retirada do cérebro que foi congelado a -70°C até a secção em criostato para imuno-fluorescências para GABA e GRs. **Resultados:** Injeção de DX reduziu a secreção de CT em animais normais ($62,2 \pm 8,7$ no grupo I para $3,3 \pm 0,5$ ng/mL no grupo II) e *sham* ($68,9 \pm 5,9$ no grupo III para $4,8 \pm 2,5$ ng/mL no grupo V). Houve expressão de GABA em neurônios da área periventricular anterior (PVA) nos grupos II e V, tanto em secções da porção anterior do PVH (PVHap) ($0,2 \pm 0,1$ no grupo II e $1,0 \pm 0,3$ no grupo V) quanto na porção medial do PVH (PVHmp) ($0,4 \pm 0,1$ no grupo II e $2,8 \pm 0,9$ no grupo V). A DX aumentou o número de neurônios com expressão de GRs tanto em secções da PVHap ($8,7 \pm 1,0$ no grupo IV versus $37,7 \pm 14,0$ no grupo VI), quanto da PVHmp ($1,5 \pm 2,3$ no grupo IV versus $68,8 \pm 14,8$ no grupo VI). DX induziu aumento do número de neurônios com expressão de GRs na PVHmp ($6,0 \pm 7,0$ no grupo II versus $68,8 \pm 14,8$ no grupo VI), mas não na PVHap. A porcentagem de neurônios em que houve co-expressão GABA e GRs na PVHmp foi maior no grupo V ($19,7 \pm 7,4\%$) que no grupo II ($6,2 \pm 3,7\%$). **Conclusão:** Gc podem agir diretamente em neurônios GABA. A expressão maior de neurônios GABA na PVHmp e o aumento da co-expressão de GABA/GRs nesta região, induzido pelo tratamento com corticóides, indicam um possível sítio de retro-alimentação negativa para controle da atividade do eixo HPA.

Palavras-Chave: GABA, Glicocorticóides, HPA,

Apoio Financeiro: CAPES, FAPESP, CNPq

E.04.013

INTERAÇÕES ENTRE ESTRESSE CRÔNICO E CONSUMO DE CAFEÍNA EM RATOS: AVALIAÇÃO DO DANO AO DNA EM HIPOCAMPO E DO COMPORTAMENTO

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Objetivo: verificar interações entre o consumo crônico de cafeína e o estresse crônico em ratos machos e fêmeas, sobre o comportamento do tipo ansioso e sobre o dano ao DNA no hipocampo.

Métodos: foram utilizados ratos Wistar de 60 dias, 58 machos (250-300g) e 48 fêmeas (150-200g), divididos em 6 grupos (controle, estresse, cafeína 0,3g/L, estresse+cafeína 0,3g/L, cafeína 1g/L, estresse+cafeína 1g/L). No grupo estresse os animais foram imobilizados 1 h/dia, 5 dias/semana, por 40 dias. A dieta consistiu de ração padrão e água "ad libitum", ou cafeína na concentração de 0,3 ou 1,0 g/L na água de beber como única fonte de líquido disponível. A dose média do consumo de cafeína no grupo 0,3 g/L foi de 40mg/kg/dia e no grupo 1 g/L, 108mg/kg/dia, não havendo efeito do estresse e do sexo sobre este consumo. A ansiedade foi avaliada pelo teste do labirinto em Cruz Elevado (5 min), utilizando como parâmetros o tempo de permanência e número de entradas nos braços abertos e fechados. O dano ao DNA foi avaliado pela técnica do Cometa.

Resultados: foram analisados por ANOVA de duas vias, sendo expressos como média $\pm$ EPM. Nos ratos machos, a cafeína e o estresse diminuíram o % de tempo nos braços abertos (controle: $57 \pm 5,9$; estresse: $45 \pm 6,1$; cafeína 0,3g/L: $38 \pm 5,4$; estresse+cafeína 0,3g/L: $34 \pm 7,4$; cafeína 1g/L: $39 \pm 7,3$; estresse+cafeína 1g/L: $22 \pm 6,7$). Nas fêmeas, não houve diferença entre os grupos em nenhuma das medidas avaliadas. Nos ratos machos, observou-se que a cafeína, em ambas as doses, e o estresse aumentaram o dano ao DNA, havendo interação entre as variáveis (controle: $17 \pm 2,7$; estresse: $35 \pm 2,7$; cafeína 0,3g/L: $35 \pm 2,5$; estresse+cafeína 0,3g/L: $39 \pm 2,5$; cafeína 1g/L: $34 \pm 3,3$; estresse+cafeína 1g/L: $45 \pm 3,8$), enquanto que nas fêmeas não houve diferença.

Conclusão: O estresse e a cafeína crônica aumentam comportamentos relacionados à ansiedade e o dano ao DNA no hipocampo em ratos machos. Por outro lado, as fêmeas parecem ser mais resistentes aos parâmetros analisados, pois nenhuma diferença foi encontrada.

Referência: Pharmacol Rev. 51:83,1999

Palavras-Chave: cafeína, estresse, dano ao DNA, ansiedade

Apoio Financeiro: CNPq, FINEP/IBNnet.

E.04.014

A cafeína previne os efeitos comportamentais do estresse crônico variado nas tarefas do campo aberto e do nado forçado.

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Objetivo: Verificar efeitos do consumo de cafeína e do estresse crônico variado sobre os parâmetros comportamentais do tipo depressivo e ansioso em ratos machos.

Métodos e Resultados: ratos Wistar machos de 90 dias de idade, pesando em torno de 250 a 350g foram divididos nos seguintes grupos: controle, estresse crônico variável (ECV), ECV+água, cafeína 1,0 g/L, administrada na água de beber como única fonte de líquido (grupo CAF), ECV + CAF. Os ratos dos grupos ECV foram submetidos a diversos estressores (privação de comida, caixa inclinada, imobilização, imobilização+frio, ruído, luz piscante e isolamento) aplicados em horários diferentes, de modo que os ratos não possam prever quando serão estressados. A avaliação da ansiedade foi realizada utilizando o teste do labirinto em cruz elevado. Na tarefa do nado forçado foi medido o tempo de imobilidade e, na tarefa de campo aberto, observamos respostas de orientação e número de cruzamentos. Não observamos diferença entre os grupos na tarefa do labirinto em cruz elevado. Houve uma interação entre o ECV e CAF, onde os animais submetidos ao ECV apresentaram uma diminuição na atividade exploratória e aumentaram o tempo de imobilidade no nado forçado, efeitos esses que foram prevenidos pelo consumo crônico de CAF ($p < 0,05$). Os resultados foram analisados utilizando ANOVA de duas vias, sendo expressos como média + EPM na tabela abaixo.

	Controle	ECV	CAF1	ECV+CAF1
Rearings	13,6 ± 3,26	2,4 ± 0,40	8,4 ± 2,40	15,8 ± 3,66
Cruzamentos	28,8 ± 5,36	9,2 ± 2,00	19,2 ± 1,42	26,4 ± 5,43
Tempo imobilidade (s)	87,8 ± 18,6	183,6 ± 31,7	166,8 ± 27,7	125,8 ± 13,5

Conclusão: a cafeína 1 g/L foi capaz de prevenir os comportamentos do tipo depressivo provocados pelo ECV.

Palavras-Chave: Cafeína, estresse crônico variado, campo aberto, nado forçado.

Apoio Financeiro: Apoio: CNPq, FINEP/IBNnet

EFEITOS DA MANIPULAÇÃO NEONATAL SOBRE A ATIVIDADE SEROTONÉRGICA, NORADRENÉRGICA E FOSFORILAÇÃO DO CREB NO BULBO OLFATÓRIO DE FILHOTES DE RATOS

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A manipulação neonatal reduz a preferência pelo odor maternal em filhotes de ratos. Esta preferência é dependente da ativação da noradrenalina (NA), serotonina (5-HT) e do CREB (proteína ligante ao elemento responsivo do AMPc) no bulbo olfatório (Learning & Memory. 6:608, 1999).

Objetivo: avaliar os efeitos da manipulação neonatal sobre a atividade noradrenérgica, serotonérgica e fosforilação do CREB no BO em filhotes de ratos.

Métodos: Foram utilizados filhotes não-manipulados (NM) e manipulados. O grupo manipulado foi subdividido em 2 grupos: manipulação única (MU; realizada no 7º dia) e manipulação repetida (MR, realizada do dia 1º ao 7º pós-natal). Quinze minutos após a manipulação, o BO foi retirado para dosagem de NA, 5-HT (pg/µg proteína; n=7-9) e seus metabólitos MHPG, 5-HIAA (pg/µg proteína; n=7-9) por HPLC e CREB (n=5) e CREBp (n=6) por *Western blot*.

Resultados: Foram expressos como média±EPM e analisados pela ANOVA seguida de Newman-Keuls (p<0,05). Os grupos MU e MR apresentaram aumento do conteúdo de NA (1,28±0,07; 1,44±0,07) no BO comparado ao grupo NM (0,93±0,05). No grupo MR a taxa de MHPG/NA (atividade) foi menor (0,47±0,04) que nos grupos NM (0,71±0,06) e MU (0,62±0,04). Quanto ao metabólito da serotonina (5-HIAA) os grupos MR (1,91±0,15) e MU (2,07±0,10) apresentaram um aumento quando comparado ao grupo NM (1,14±0,08). A taxa 5-HIAA/5-HT (atividade) foi maior no grupo MR (2,73±0,04) que nos grupos MU (2,07±0,32) e NM (1,42±0,09). O CREBp diminuiu no grupo MU (72,1±3,91) em relação aos grupos MR (94,6±3,02) e NM (100±0). Enquanto para o CREB um aumento foi verificado apenas no grupo MR (132,7±10,2) comparado aos demais grupos.

Conclusão: A manipulação repetida reduziu a atividade noradrenérgica, mas aumentou atividade a serotonérgica no bulbo olfatório de filhotes aos 7 dias de idade. Essa combinação de efeitos seria a provável causa da manutenção dos níveis de fosforilação do CREB.

Palavras-Chave: Manipulação neonatal, Bulbo Olfatório, Noradrenalina, Serotonina, CREB

Apoio Financeiro: CNPq, FAPESP, PUC-RS

PSYCHOSOCIAL STRESS TASK IN PEACEKEEPERS: IMMOBILITY REACTIONS AND CORTISOL RESPONSE.

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Objectives: Glucocorticoids responses to stressful events are known to vary substantially among individuals. Higher levels of those hormones were found in animals that react with more fear paralysis (tonic immobility) under life threat. Here we investigated if cortisol response to an acute psychosocial stress task would be related to the degree of immobility felt during its performance. **Methods:** We tested 52 Brazilian peacekeeper militaries 4 months after returning from a mission in Haiti. Psychometric scales estimated PTSD symptoms, anxiety and negative affect traits. We used an adapted version of the Trier Social Stress Task (TSST) for militaries. Saliva samples were collected at -25, -10, +10, +25, +40 minutes relative to the beginning of the stress task. Three questions assessed the intensity of paralysis/immobility during the task. We used linear regression model to test the impact of immobilization during stress on the cortisol response. **Results:** Immobility reports significantly predicted cortisol increase in response to the stress task (coefficient=0.28, $p<0.05$). This association remains significant after controlling for anxiety trait, negative affect trait and PTSD symptoms (coefficient=0.49, $p<0.01$). **Conclusions:** The results showed that, in a sample of militaries previously exposed to chronic stressors, proneness to react with immobility predicts cortisol increase to acute stress. These findings highlight the importance of evaluating somato-motor reactions to stress and its connections with cortisol reactivity in humans.

Palavras-Chave: Stress, Cortisol, Immobility and Peacekeepers

Apoio Financeiro: CNPq; CAPES - Projeto Pro-Defesa and Projeto Institutos do Milênio (CNPq # 420122/2005-2)

SEMELHANÇA NA ATIVAÇÃO DO EIXO HIPOTÁLAMO-PITUITÁRIA-ADRENAL ENTRE RATOS SENSÍVEIS E RESISTENTES À CORRIDA SELVAGEM

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O objetivo do presente estudo foi investigar a atividade do eixo Hipotálamo-Pituitária-Adrenal (HPA), via secreção de corticosterona e hipertrofia das adrenais, no modelo animal de pânico baseado na manifestação de corrida selvagem (CS). Para tanto, ratos Wistar machos adultos foram testados quanto à susceptibilidade à CS pela estimulação acústica de 112 dB por 60 segundos. Os animais que apresentaram CS foram classificados como sensíveis, e os que não manifestaram CS, como resistentes. Grupos compostos por esses 2 tipos de ratos foram expostos às seguintes condições de estimulação do eixo HPA: manutenção em ambiente refrigerado à 21°C por 12 horas para indução de estresse térmico; e submetidos ao estresse de imobilização pela contenção em cilindro de PVC por 1 hora. Em seguida, os animais foram sacrificados para a realização da coleta de sangue do tronco e pesagem das glândulas adrenais. Outros ratos sensíveis e resistentes, sem manipulação experimental, foram sacrificados da mesma forma e serviram para determinação da condição basal. As médias dos níveis de corticosterona basal dos ratos sensíveis e resistentes foram de 4,653 µg/100ml ± 0,984 (EPM) e 7,287 µg/100ml ± 1,367, respectivamente; sob estresse térmico, de 22,743 µg/100ml ± 1,643 nos sensíveis, e de 28,963 µg/100ml ± 3,530 nos resistentes; e sob estresse de imobilização de 44,735 µg/100ml ± 1,640 nos sensíveis, e de 41,858 µg/100ml ± 2,187 nos resistentes. Os valores sob ambas as condições de estresse diferiram em relação ao basal (ANOVA de duas vias; p<0,05) e houve diferença do estresse térmico para o de imobilização (Teste de Tukey, p<0,05). Não houve qualquer diferença entre animais sensíveis e resistentes dentro de cada condição. As médias de peso das adrenais não diferiram nem entre sensíveis e resistentes, nem entre as condições. Conclui-se que a ativação do eixo HPA não difere em função da susceptibilidade à CS, o que aproxima esse modelo animal das condições dos portadores de transtorno de pânico.

Palavras-Chave: Pânico, Corrida Selvagem, Estresse, Corticosterona

Apoio Financeiro: FAPESP (2008/50616-0)

E.04.018

B vitamins supplementation attenuates the stroke of SHRSP rats

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Objectives: The stroke prone rat (SHRSP) a recognized animal model for study of severe hypertension and stroke presents an elevated tissue levels of free radicals and the consequences of an oxidative stress such as mitochondrial damage, endothelial injury among others factors (Arch Neuroci 10 (3)146, 2005). In addition, there is an increased interest from a novel molecules that might reduces oxidative damage and cerebral edema following acute stroke (Eur J Clin Nutr 61(10)1237,2007). Therefore, the objective of this study was to identify the effect of B vitamins, closely involved on oxidative phosphorylation and homocysteine metabolism as well, on SHRSP rats.

Methods: 18 male SHRSP rats (10 wk) receiving Nuvilab (Nuvital) and water ad libitum, after 10 days of baseline period, were subdivided into three groups (n=6) to receive by oral gavage vehicle (water) or riboflavin 10mg/kg of body weight(B2), pyridoxine plus folic acid (1200mg(B6)+200mg(B9)/Kg of body weight)during 03 months. Daily were determined: body weight, water and food intake, diuresis; physical aspects and behavior. Sensory-motor responses were monitored by tail-flick meter analgesia and systolic blood pressure determined by plethysmography. The data was evaluated by ANOVA two-way and p<0.05significant.

Results: Supraphysiologic doses did not cause toxic effects. There was a significant (p<0.05) decrease of systolic blood pressure on animals under B vitamins supplementation: 228,76±3,52mmHg (control), 210±5,0mmHg (B6 plus B9) and 205,81±0,55mmHg (B2).The treatment also prevented the stroke in 79,7% of the rats, since some signs such as ataxia, loss of balance, partial paralysis were not detectable. The supplementation clearly protect the animals against the stroke since this event naturally expected at 20 weeks of age, was not observed in those treated animals.

Conclusion: This study strongly suggests that B vitamins therapy was effective on the control of systolic blood pressure and should be thought of as one of the alternative therapies to prevent stroke.

Palavras-Chave: B vitamins, oxidative stress and stroke

Apoio Financeiro: CNPq and CAPES.

Effects of litter-overlapping on emotionality, stress response and reproductive functions in male and female rats

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In the rat, mating at postpartum estrus and late weaning of the young would result in the overlapping of two different-age litters and ultimately in changes in pups' early experience.

Objectives: We aimed to analyze how changes in the family composition of the male and female newborn pups [single (SR) vs. overlapped reared (OR) litters] affect their emotionality, stress response and reproductive functions at adulthood.

Methods: Adult SR and OR male and females' anxiety-like behaviors were assessed in the open field test (1m² arena/5 min) and their plasmatic levels of corticosterone were quantified following a 20-min restrain period. Spermatogenesis (sperm count), ovulation (oocytes in estrus morning) and sexual behavior of SR and OR males and females were determined.

Results: Adult OR animals increased the time (s) spent in central quadrants of the open field [♀: SR: 9.9 ± 2.4, OR: 20.3 ± 5.1; ♂: SR: 9.1 ± 1.9, OR: 20.3 ± 4.3, means ± SE, p<0.05, Student t test]. While the stress response did not differ between SR and OR males [F(1,50)= 4.45, p=ns], only SR females exhibited enhanced corticosterone levels following stress [F(1,50)= 4.97, p<0.05]. The frequency and latencies of mounts and intromissions as well as the sperm count, the daily sperm production and the transit time through epididymis, did not differ between the SR and OR males. OR females showed reduced sexual behavior compared with SR ones [lordosis quotient, OR: 0.6 ± 0.1, SR: 0.4 ± 0.1], without changes in the number of oocytes.

Conclusions: The early-life experience in overlapping litters reduced the anxiety-like behavior of males and females, and diminished the endocrine stress response and reproductive behavior of female offspring during adulthood. These long-term effects could be attributed to a more complex precocious rearing environment than the one provided in single litters, which probably results in an altered quality/quantity of stimulation from mothers and/or siblings.

Palavras-Chave: early experience, stress, anxiety, reproduction, rat pups, family composition

Apoio Financeiro: CNPq, FAPERGS

Effects of decylubiquinone supplementation on systolic blood pressure and renal histology in SHRSP rats

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Objective: Stroke is a leading cause of death, a major cause of disability in the world and it's also closely associated to hypertension (Free Radic Biol Med. 39:841, 2005; Stroke. 35:1908, 2004). At the same way, high blood pressure also accelerates loss of kidney functions (Kidney Int. 61:62, 2002). In this context, studies with alternative therapies have been done with the objective of investigate possible effects of such maneuvers on treatment and prevention of hypertension (Clin Chim Acta. 351:101, 2005). Therefore the main objective of this work was to identify the effects of decylubiquinone supplementation on systolic blood pressure and target organs of SHRSP.

Method: Twelve SHRSP male rats, 8wk age maintained on Bioterium with controlled conditions, receiving Nuvilab chow and water ad libitum; after a basal period of 10 days were divided into two groups: treated and control (n=6, each). The treated rats were supplemented daily with a dose of 10 mg/kg/body weight of decylubiquinone (Sigma) by oral gastric gavage during four weeks; and the control group received the vehicle: coconut oil. The systolic blood pressure was determined using the noninvasive method of tail-cuff plethysmography and Kidney morphology by Scherle and microscopy with HE (hematoxylin-eosin). Statistical analysis was determined by student- t test and p<0.05 was accepted as statistically significant.

Results: The decylubiquinone supplementation showed a significant modification (p<0.05) on systolic blood pressure values when compared with control group (214.21±0.83 mmHg versus 226.22±0.47mmHg). Curiously, the SHRSP control group did not present the expected morphological alterations on renal histology. In addition, the administration of decylubiquinone did not show histological modifications between control and treated groups.

Conclusion: The decylubiquinone treatment attenuates the severe hypertension of SHRSP however the absence of kidney injury here reported might be attributed to the age of those animals. Therefore, the findings did not discharge the hypothesis of a modification in kidney histology on older SHRSP.

Palavras-Chave: Decylbiquinone, stroke, hypertension, renal histology

Apoio Financeiro: CNPq e Capes

E.04.021

Noradrenalina administrada centralmente induce un efecto dosis dependiente en el comportamiento y en la densidad de receptores GABA_A en sinaptosomas.

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Los receptores GABA_A-sensibles a flunitrazepam, medidos “ex vivo” en sinaptosomas a 4°C incrementaron, sin cambios en la afinidad, por el estrés agudo en pollos, posiblemente mediante la fosforilación de dichos receptores y/o sus proteínas asociadas. Por otro lado, adrenalina inyectada sistémicamente aumentó la densidad del receptor de una manera dosis dependiente sólo en los pollos estresados; por lo que el efecto de la adrenalina es dependiente y aditivo al estrés quizás por evocación central de noradrenalina. Por lo tanto, es posible que la administración central de noradrenalina module el comportamiento en una prueba de campo abierto cuando es inyectada inmediatamente antes de la prueba y esta acción farmacológica esté mediada por receptores GABAérgicos. Dosis bajas (0,0025, 0,005 y 0,05 µg/µl) y altas (1 µg/µl) de noradrenalina administrada intracerebroventricularmente, en pollos de 4-6 días de edad, indujeron un aumento en la emocionalidad y un efecto inhibitor sobre la actividad locomotora. Este comportamiento jugaría un rol fundamental en la respuesta al estrés agudo donde la inmovilidad observada a estas dosis podría ser considerada como una respuesta al ansiedad/miedo inducido por la prueba. Además, este efecto dependiente de la dosis fue observado sobre la densidad del receptor GABA_A en sinaptosomas de cerebro anterior sin cambios en la afinidad. Estos resultados sugieren que la liberación central de noradrenalina seguida a un estresor agudo modifica el comportamiento en un paradigma de estrés en aves y modula la densidad de receptores GABA_A de manera similar al inducido por adrenalina sistémica.

Palavras-Chave: noradrenalina-receptor GABA A-estres agudo

Apoio Financeiro: SECyT- UNC, CONICET

MANIPULAÇÃO NEONATAL, OCITOCINA E COMPORTAMENTO DE AGREGAÇÃO SOCIAL EM FILHOTES DE RATOS.

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A habilidade de reconhecer indivíduos de mesma espécie é a base dos comportamentos sociais que iniciam logo após o nascimento, na interação mãe-filho e entre irmãos. A ocitocina (OT) é um peptídeo central que modula comportamentos de interação social, atuando inclusive no reconhecimento olfatório da mãe pelo filhote, além de responder a estímulos estressantes. A manipulação neonatal (MN) envolve separações breves e repetidas da mãe e pode promover um déficit no reconhecimento olfatório durante a fase inicial do desenvolvimento. O objetivo deste trabalho foi investigar o efeito da MN sobre o comportamento de agregação social entre filhotes do mesmo sexo e avaliar os níveis de OT plasmática após o procedimento de manipulação.

Utilizamos 48 ratos Wistar divididos em: não-manipulados (NM; n=22) e manipulados (M), em que toda ninhada era manipulada por 1 min do 1º ao 10º dia pós-natal (n=26). No 11º dia, os filhotes foram submetidos ao teste de agregação social (AS), que consistiu em colocar 2 irmãos do mesmo sexo em uma caixa de acrílico transparente (40X30X24cm) em cantos opostos diagonalmente durante 5 min. Avaliou-se o tempo (s) em que os filhotes permaneceram separados. Em outro experimento, coletou-se plasma de filhotes de 10 dias idade logo após a manipulação (n=28) para medida da concentração plasmática de OT por radioimunoensaio (NM, n=28).

A MN em machos e fêmeas (média $\pm$ EPM; 13,30 $\pm$ 2,75; 7,68 $\pm$ 1,11) induziu um aumento do nível plasmático de OT comparado (ANOVA, seguida do teste de Newman-Keuls, $p<0,05$) com o grupo NM (10,04 $\pm$ 1,71; 5,13 $\pm$ 0,92). A MN em machos e fêmeas induziu também um aumento do tempo (s) de permanecer separado do irmão (média $\pm$ EPM; 257,50 $\pm$ 10,56; 294,40 $\pm$ 2,91) comparado (ANOVA, seguida do teste de Newman-Keuls, $p<0,05$) com o grupo NM (160,70 $\pm$ 28,11; 189,00 $\pm$ 32,66).

Verificou-se que a MN reduziu o comportamento de AS em filhotes machos e fêmeas aos 11 dias. Além disso, a MN aumentou a concentração plasmática de OT em filhotes de 10 dias, indicando que a MN pode ser considerada um evento estressor, o que possivelmente esteja associado à redução da interação social. **Palavras-Chave:** Manipulação neonatal, estresse neonatal, ocitocina, reconhecimento olfatório.

Apoio Financeiro: CNPq, CAPES e FAPESP.

E.04.023

Prenatal stress effect on glutamate metabolism, evaluated with mass spectroscopy-liquid chromatography.

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Episodes of stress suffered by the mother during pregnancy generate changes in the fetal environment affecting central nervous system development of the offspring. Previous studies from our laboratory have shown that adult offspring of rats stressed during the last week of pregnancy exhibited higher levels of glutamate receptors than control rats concomitantly with a long-lasting astroglial reaction and a reduced dendritic arborization with synaptic loss in the brain of adult offsprings. Since metabolism of glutamate and GABA is intimately linked to a substrate cycle between neurons and surrounding astroglia, our results prompted us to suggest that both glutamate and GABA neurotransmitter pathways might be impaired in the brain of a prenatally stressed rat. To study the effect of prenatal stress (PS) on the metabolism of glutamate, pregnant rats were subjected to restraint stress during the last week of gestation. Brain extracts of PS rats were evaluated by liquid chromatography and mass spectroscopy to measure the content of glutamate, GABA and others metabolites. This procedure was performed in frontal cortex and caudate putamen brain extracts of animals of 28 and 60 postnatal day (PND). Our results show that glutamate content in adult male (PND 60) PS rats is significantly reduced than in control rats. This would indicate that prenatal stress produces long-term changes in the metabolism and/or synthesis of glutamate thereby modulating the expression of glutamate receptors and altering the normal synaptic transmission of the adult brain.

Palavras-Chave: Prenatal Stress, glutamate

Apoio Financeiro: CONICET

EFEITO DA MANIPULAÇÃO NO PERÍODO NEONATAL SOBRE O NÚMERO DE NEURÔNIOS VASOPRESSINÉRGICOS E OCITOCINÉRGICOS EM RATOS ADULTOS.

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A manipulação neonatal (MN) interfere na relação mãe-filhote e causa alterações comportamentais e neuroendócrinas estáveis em ratos. Ocitocina (OT) e vasopressina (VP) agem como moduladores comportamentais. Este trabalho avalia o número de neurônios ocitocinérgicos e vasopressinérgicos do núcleo paraventricular (PVN) do hipotálamo, em ratos machos e fêmeas submetidos à MN.

Ratos Wistar foram divididos em manipulados (M, n=6) e não-manipulados (NM, n=6). Nos M toda ninhada era manipulada por 1 min do 1º ao 10º dia pós-natal. Perfundiu-se no 90º dia (fêmeas em diestro) e os cérebros congelados e seccionados a 50 µm de espessura no PVN. Foi feita imunistoquímica com anticorpos monoclonais para OT e VP. Os neurônios foram divididos em magno e parvocelulares e contados separadamente por hemisférios direito (LD) e esquerdo (LE). Obteve-se uma média (±EPM) por secção e utilizou-se ANOVA de duas vias, considerando hemisfério como medida repetida ($p<0,05$).

Verificou-se que em machos não houve alteração no número de neurônios OT por secção de cérebro em magno (M, LD=6,8±0,9/LE=7,1±0,6; NM, LD=6,4±1,06/LE=6,1±1,1) e em parvo (M, LD=2,5±0,3/LE=2,6±0,3; NM, LD=2,8±0,3/LE=2,8±0,3). Entretanto, as fêmeas M apresentaram uma redução no número de neurônios OT parvo (M, LD=1,6±0,1/LE=1,7±0,05; NM, LD=2,±0,04/LE=1,9±0,07). Quanto aos neurônios VP magno houve um aumento na média por secção em machos M comparados aos NM (M, LD=5,4±0,2/LE=4,8±0,1; NM, LD=3,6±0,2/LE=3,3±0,2), e nas fêmeas observou-se uma redução (M, LD=3,89±0,2/LE=3,7±0,2; NM, LD=4,8±0,21/LE=4,8±0,2). Nos neurônios VP parvo não houve diferença entre os grupos, tanto em machos (M, LD=1,28±0,19/LE=1,22±0,1; NM, LD=1,24±0,09/LE=1,19±0,1) quanto em fêmeas (M, LD=1,2±0,1/LE=1,22±0,08; NM, LD=1,28±0,13/LE=1,17±0,07).

Em fêmeas, a MN induziu a uma diminuição do número neurônios OT na região parvocelular, e neurônios VP na região magnocelular do PVN. Nos machos, a MN aumenta o número de neurônios VP na região magnocelular. A MN induz alterações morfológicas de longa duração no sistema OT e VP do PVN de ratos. Esses efeitos tardios são dependentes do sexo, e indicam que os efeitos da MN podem ser modulados pelos hormônios sexuais.

Palavras-Chave: Manipulação neonatal, estresse neonatal, ocitocina, vasopressina.

Apoio Financeiro: CNPq, FAPERGS e FAPESP.

EXPRESSION OF DOPAMINERGIC TRANSCRIPTION FACTORS AND MESENCEPHALIC CONNECTIVITY IN PRENATALLY STRESSED ADULT RATS.

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Rats exposed to different types of stress during the last week of pregnancy produce offspring that show severe anomalies in neural development and brain morphology that persist into adulthood particularly in dopaminergic neurotransmission. It has recently been identified two transcription factors (TFs), Nurr1 and Pitx3 which are expressed at critical moments of DA neurons differentiation. Their genetic expression is activated immediately after these neurons determination and maintained through adult life. Employing an immunocytochemistry approach, we studied the expression levels of these factors in mesencephalon slices of prenatally stressed adult rats. In addition, we evaluated the prenatal stress (PS) effects over the growth of a triple organotypic cultures of substantia nigra (SN), caudate putamen (Cpu) and frontal cortex (FC). The cultures of PS rats showed less growth when it is compared with the control group, showing that the PS could be affecting the normal connectivity of the corticonigrostriatal system. Nurr1 expression was localized in compact and reticular Substantia Nigra, (SNc and SNr), ventral tegmental area (VTA) and cortex regions, whereas Pitx3 expression was restricted to SNc, SNr and to VTA. Quantifications of Nurr1 and Pitx3 expression showed that both TFs increased in prenatally stressed adult offspring in the VTA area, whereas no changes were observed in SN areas. Since both TFs modulates the expression of tyrosine hydroxylase, the key enzyme in dopamine synthesis, the increase of TFs in the adult prenatally stressed rat might be a compensatory mechanism to counteract the decrease of dopamine levels observed previously.

Palavras-Chave: prenatal stress, dopamine, nurr1, pitx3

Apoio Financeiro: CONICET

REPEATED IMMOBILIZATION STRESS INCREASES p-CREB EXPRESSION IN CRH NEURONS OF THE RAT LATERAL BED NUCLEUS OF THE STRIA TERMINALIS. DESIPRAMINE ADMINISTRATION PREVENTS THIS EFFECT

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Repeated immobilization stress (RIS) has been shown to induce long-lasting anxiety and a sustained increase in corticotropin-releasing hormone (CRH) expression in the dorsal and ventral lateral bed nucleus of the stria terminalis (IBNST) and that desipramine (DMI) administration prevents this effect. It has been also shown that cAMP-response element binding protein (CREB) and the inducible cAMP early repressor (ICER) are involved in the regulation of CRH expression, and ICER in the antidepressant effect of DMI in the paraventricular nucleus of the hypothalamus (PVN). The present work was aimed at studying whether these transcription factors are involved in the effects of RIS and DMI administration on CRH expression in the IBNST and PVN. For this purpose, a group of rats was subjected to RIS consisted of a daily session of immobilization (15 days, 2 h/day). A group of stressed rats and its respective *naïve* controls received DMI (10 mg/kg, once a day), and another group of stressed and *naïve* animals received vehicle (water, once a day) during the 15 days of stress. On day 16, animals were sacrificed to study the expression of p-CREB protein, ICER mRNA and the number of CRH cells co-expressing these transcription factors in the dorsal and ventral IBNST and the PVN by means of immunohistochemistry and *in situ* hybridization.

The results showed that RIS-induces a significant increase in the number of cells expressing p-CREB in both ventral and dorsal IBNST, and PVN. DMI administration fully prevents this effect. RIS also increases the number of CRH cells co-expressing p-CREB in both the ventral and dorsal IBNST, and PVN. However, DMI administration only prevents this effect in the ventral IBNST and PVN, but not in the dorsal IBNST. Interestingly, RIS-induces a significant decrease in the number of cells expressing ICER and in the number of CRH cells expressing ICER in all the structures studied. These results suggest that an increased activation of CREB and a decreased expression of ICER might be involved in the sustained increase of CRH expression induced by RIS in the IBNST and PVN. The results also suggest that the effect of DMI administration is mediated by CREB, but not by ICER.

Palavras-Chave: BNST, p-CREB; CORTICOTROPIN-RELEASING FACTOR; ICER

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AVALIAÇÃO DA INFLUÊNCIA DA PRIVAÇÃO DE SONO REM E DO NADO FORÇADO NA MORFOLOGIA DE HEMÁCIAS DE RATOS *Wistar*

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Introdução: Estudos em animais demonstraram que situações de estresse, como a privação de sono REM (pREM) e o nado forçado (NF), podem induzir alterações comportamentais e fisiológicas. Outros dados mostraram que células nervosas podem sofrer lesões através de mecanismos excitotóxicos ou oxidativos sob estas condições. O objetivo deste trabalho foi avaliar o efeito da privação de sono REM e do nado forçado na morfologia de hemácias de ratos *Wistar*.

Métodos: Ratos *Wistar* machos (n=16, 3-4 meses) foram submetidos à pREM (1, 2, 3 e 4 dias) pela técnica do pote de flor invertido. Outros animais (n=12) foram submetidos ao NF (2,5; 5 e 20 minutos). Os animais foram anestesiados (tiopental 65 mg/kg) e amostras de sangue (0,5 ml), com anticoagulante, foram retiradas, preparadas as distensões sangüíneas e coradas (May-Grünwald-Giemsa). As imagens foram capturadas (5 campos por lâmina, 5 lâminas para cada tratamento) e realizada a análise qualitativa e morfométrica (relação perímetro/área) com programa específico (Image ProPlus Software). Como controle (n=8), animais não pREM e não NF.

Resultados: Os dados obtidos para relação perímetro/área foram ($X \pm DP$): $0,65 \pm 0,012$ (controle); $0,66 \pm 0,021$ (1 dia pREM); $0,65 \pm 0,009$ (2 dias pREM); $0,64 \pm 0,013$; $0,64 \pm 0,006$ (4 dias pREM); $0,64 \pm 0,012$ (2,5 min NF); $0,84 \pm 0,056$ (5 min NF) e $0,70 \pm 0,059$ (20 min NF). Houve diferença significativa ($p < 0.05$) na relação perímetro/área de hemácias de ratos 1 dia pREM e 5 minutos NF em comparação ao grupo controle.

Conclusão: Os resultados apresentados neste trabalho sugerem que ratos *Wistar* submetidos à privação de sono REM e ao nado forçado poderiam apresentar alterações na forma das hemácias. **Palavras-Chave:** Hemácias, Morfologia, Nado Forçado, Sono REM, Estresse

Apoio Financeiro: CNPq, FAPERJ, UERJ e UFRN.

E.04.028

Cuidado Maternal , Estresse e Corticosterona em Ratos Neonatos.

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Objetivos:

O cuidado maternal (CM) é crucial nas primeiras semanas de vida do rato, pois “programará”, de forma duradoura, o comportamento e sua fisiologia e neurobiologia subjacente. Ratos que receberam diferentes níveis de CM podem apresentar diferenças na ansiedade e nas respostas ao estresse (E) na vida adulta. Neste trabalho investigamos se as variações de CM influenciam as respostas da corticosterona (CORT), um dos hormônios envolvidos no E, em ratos no período neonatal submetidos a um estressor.

Métodos:

Utilizamos 60 ratas Wistar prenhas e no final do dia 0, definido como dia do nascimento, cada ninhada foi padronizada em 8 filhotes. A partir do dia 1 foi realizado o registro do CM até o dia 10, no qual foi avaliada a frequência de lambidas (FL) da mãe nos filhotes. Foram realizados 4 registros/dia, 72 min cada, nos horários: 9:30, 12:30, 15:30 e 18:30 h. A média de FL da população foi $5,52 \pm 0,1863$. Foram selecionadas as ratas que apresentam maior e menor FL, definidas como Muito-Cuidadoras (MC, média > 6,991, n=10), Pouco-Cuidadoras (PC, média < 4,1814, n=12), respectivamente. No dia 13 pós-parto, foram utilizados 3 machos (M) e 3 fêmeas (F) por ninhada, 2 casais submetidos a um E por frio (0°C, 6 min), sendo que um casal foi sacrificado 15 min (M15 e F15) e outro 30 min pós-E (M30 e F30). O casal não submetido ao E foi usado como controle (M0 e F0) e sacrificados no início do experimento. O sangue do tronco foi coletado para análise da CORT plasmática (ng/ml) por radioimunoensaio.

Resultados:

A ANOVA de três vias com Post Hoc de Duncan mostrou um efeito do E aumentando os níveis de CORT tanto em filhotes de ratas MC quanto em filhotes de ratas PC ($P < 0,001$).

Mãe	MC						PC					
Filhote	M0	M15	M30	F0	F15	F30	M0	M15	M30	F0	F15	F30
Média	8,4	22,2	19,8	7,0	12,3	19,3	13,8	18,9	20,6	9,3	22,9	22,5
Erro-padrão	3,9	4,9	4,7	0,9	1,9	5,6	4,4	2,8	4,0	0,9	3,8	2,1

Conclusões:

Ao contrário da literatura com ratos adultos, não houve efeito do CM na resposta da CORT ao estresse, o qual pode ser devido ao fato de que os animais estarem no período hiporresponsivo ao estresse. Isso indica que os efeitos das variações de CM nas respostas diferenciadas ao E surgem posteriormente no desenvolvimento. **Palavras-Chave:** cuidado maternal, neonatos, estresse, corticosterona.

Apoio Financeiro: CAPES e CNPq.

Efeitos de Diferentes Modelos de Estresse Crônico Sobre a Ansiedade e SOBRE o *Binding* de Receptores A1 de Adenosina em Hipocampo de Ratos Wistar

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Animais submetidos ao estresse crônico, tanto repetido (ECR) quanto variado (ECV) podem apresentar diferentes respostas comportamentais, por exemplo alterações em tarefas que avaliam comportamento ansioso como o labirinto em cruz elevado (LCE), e o teste do campo aberto (CA). Tais alterações podem ser devidas a diferentes efeitos sobre o sistema límbico, fundamental na expressão de comportamentos com caráter emocional. Partindo do exposto acima, nosso objetivo foi verificar os efeitos do ECR e do ECV sobre a ansiedade e sobre o *binding* de receptores A1 de adenosina em hipocampo.

Ratos Wistar adultos foram divididos em intactos (N=15), submetidos ao ECR (N=15) e submetidos ao ECV (N=15). O modelo escolhido de ECR foi o de contenção, os animais foram imobilizados durante 1h/dia, 5 dias/semana, por 40 dias. No ECV os animais foram submetidos a diferentes estressores em diferentes horários durante 40 dias. Vinte e quatro horas após a última sessão de estresse, todos os grupos foram submetidos aos testes do LCE e do CA. Após as tarefas comportamentais os animais foram sacrificados e seus hipocampus dissecados e congelados a -70° C até o dia da análise. O *binding* foi feito segundo Rebola, 2005*. Não houve diferença significativa entre os grupos nos parâmetros avaliados no LCE (tempo e entradas nos braços abertos e fechados) ($p > 0,05$), no entanto houve uma diminuição significativa nos grupos estressados cronicamente sobre o tempo nos quadrados centrais (Controle: $8 \pm 0,97$, ECR: $3,2 \pm 0,67$, ECV: $2,8 \pm 0,84$) no teste do CA ($p < 0,05$), além disso verificou-se um aumento no Bmax de receptores A1 no hipocampo desses animais (Controle: $477,9 \pm 46,35$, ECR: $613,36 \pm 30,41$, ECV: $573,73 \pm 46,99$) ($p < 0,05$). Os resultados foram expressos como média $\pm$ EPM, sendo utilizado uma ANOVA de 1 via seguida do teste post-hoc de Duncan.

O presente estudo mostrou que diferentes modelos de estresse modularam de maneira semelhante a resposta comportamental. Tanto os animais submetidos ao ECR quanto ao ECV apresentaram comportamento do tipo ansioso quando analisados na tarefa do CA e este comportamento pode estar em parte relacionado com um aumento de receptores A1 de adenosina no hipocampo desses animais.

*Referência: *Neuroscience*: 132: 893, 2005 **Palavras-Chave:** Estresse Crônico, ansiedade, adenosina, receptor A1

Apoio Financeiro: CNPq, FINEP/IBNnet

E.04.030

A Negative Correlation between peritraumatic tonic immobility and grey matter volume in prefrontal regions in PTSD patients: A Voxel Based Morphometry (VBM) Study

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Tonic immobility, the last-ditch defense under mortal threat, is characterized by reflexive profound motor inhibition and is known to increase the chances of surviving an attack, but also to have hazardous consequences. Psychometric measures have indicated the occurrence of peritraumatic tonic immobility in humans and its link with PTSD symptoms severity. This study employed VBM to detect brain anatomical differences in PTSD patients (n=16) relative to trauma-exposed controls (n=17) by analysing T1-weighted magnetic resonance images. The intensity of paralysis/immobility during trauma was assessed retrospectively. Direct group comparisons using t test showed reduced grey matter volume in prefrontal cortex, anterior cingulate, bilateral superior frontal cortex, right parietal cortex ($p < 0.001$, uncorrected) of PTSD patients. We observed an inverse correlation of grey matter volume and intensity of reported motor symptoms of peritraumatic tonic immobility in prefrontal cortex areas ($p < 0.0001$ $r = -0.85$). In conclusion, aside from a brain volume reduction in several regions of PTSD patients, these findings provide evidence that brain morphology in specific prefrontal regions is associated with peritraumatic tonic immobility. They could either reflect a predisposition to react with tonic immobility or a result of having exhibited this reaction.

Palavras-Chave: PTSD, MRI, Tonic Immobility

Apoio Financeiro: CNPq - Projeto Milênio; CAPES.

MEMÓRIA EPISÓDICA VERBAL E LÓGICA EM ESTUDANTES PRÉ-VESTIBULANDOS: EFEITOS DA TERAPIA MANUAL

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Introdução: Estudantes pré-vestibulandos são expostos a condições de estresse durante o ano do vestibular, sobretudo nos períodos de inscrição e provas. Tais condições podem resultar em prejuízos no desempenho cognitivo e justificam a investigação sobre estresse em pré-vestibulandos.

Objetivo: Avaliar o efeito de terapia manual sobre o índice de estresse e o desempenho de pré-vestibulandos em testes de memória episódica verbal e lógica.

Métodos: Foram avaliados 54 estudantes, de ambos os gêneros, com $19,0 \pm 1,8$ anos. O grupo propósito (n=26) recebeu terapia manual (drenagem linfática, mobilização de músculos e fâscias), de setembro a novembro (02 sessões/semana); e o grupo controle (n=28) não foi tratado. Foram utilizados: (a) Questionário de Estresse em Adolescentes (QEA), em setembro e novembro; (b) Teste de Aprendizagem Auditivo-verbal de Rey (*TAAVR*), com recordação imediata e após 30 min; (c) narrativa de história, com recordação imediata e após uma semana; e (c) teste de extensão de dígitos direto e indireto do WAIS-R. As avaliações ocorreram nas duas semanas que antecederam a primeira fase do vestibular, com as sessões gravadas em áudio para posterior análise. Os grupos foram comparados por teste de Mann-Whitney, com índice de significância de $p < 0,05$.

Resultados: Em setembro, não houve diferença no escore do QEA entre os grupos ($137,6 \pm 27,6$ vs. $154,5 \pm 34,3$, n=12 e 17). O grupo controle manteve o escore em novembro ($144,3 \pm 37,5$, $p=0,45$) e o grupo propósito mostrou redução ($139,9 \pm 39,8$, $p=0,02$). O grupo propósito apresentou desempenho significativamente melhor na evocação tardia do TAAVR ($p=0,049$) e recordação imediata da história ($p=0,014$). A evocação tardia da história indicou diferença entre grupos marginalmente significativa ($p=0,084$). Não houve diferença significativa entre os grupos no desempenho no teste de extensão de dígitos.

Conclusão: Observou-se efeito positivo da terapia manual sobre os índices de estresse e a memória verbal e uma utilidade potencial dos testes para analisar marcadores cognitivos de estresse. A investigação dos efeitos desse tratamento em indicadores fisiológicos de estresse pode esclarecer relações entre estresse e memória e contribuir para intervenções adequadas.

Palavras-Chave: estresse, memória, terapia manual

Relação entre os níveis basais dos hormônios sexuais e a resposta de díades de machos e fêmeas de sagüi (*Callithrix jacchus*) a estressores psicossociais.

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A relação entre o status social, geralmente associado a diferenças nos hormônios sexuais, e as alterações fisiológicas frente ao estresse se expressam diferentemente entre as espécies. O animal do posto social que recebe maior carga alostática apresenta maiores níveis de cortisol e está mais propenso a desenvolver patologias associadas ao estresse. Neste estudo investigamos se os níveis basais dos hormônios sexuais modulam a resposta ao estresse psicossocial no *Callithrix jacchus*. Seis díades com níveis dos hormônios sexuais basais significativamente diferentes (DH) (machos= 3 e fêmeas= 3) e 8 díades sem diferenças hormonais (SDH) (machos=3 fêmeas= 5) foram expostas a um ambiente novo, isoladas e reunidas (7 dias cada). Nos machos foram analisados os níveis de andrógenos e nas fêmeas a presença ou não de ciclicidade ovariana. Testes estatísticos paramétricos e não-paramétricos foram utilizados para investigar a resposta hormonal (cortisol fecal) e comportamental, respectivamente. O nível de significância considerado foi $p \leq 0,05$. A resposta foi sexualmente dimórfica. As fêmeas SDH apresentaram uma resposta mais agressiva que as DH. Pois, elevaram o cortisol e a marcação durante as fases de separação e reunião. E reduzem as interações sociais na reunião. Enquanto as DH intensificam os comportamentos sociais na reunião. Os machos apresentaram uma resposta mais afiliativa que as fêmeas. Os animais SDH e os dominantes das díades DH reduziram o cortisol na reunião. Todavia, as díades SDH reduziram comportamentos afiliativos e demonstram altos índices de deslocamento na reunião. Os animais dominantes de ambos os sexos apresentaram maiores frequências de piloereção e marcação ao longo do estudo. Estas respostas sexualmente dimórficas parecem ser um reflexo da organização social desta espécie. Estes resultados fortalecem as evidências de estratégias diferenciadas de machos e fêmeas de *C. jacchus*, ao mesmo tempo em que acrescenta informações indicando a importante participação dos hormônios sexuais como possíveis co-adjuvantes na expressão dos mecanismos de dominância social em machos e fêmeas, tornando-os diferentemente vulneráveis aos efeitos deletérios do estresse.

Palavras-Chave: Estresse, primatas, hormônios, comportamentos

Apoio Financeiro: CNPQ

CONSERVED CELLULAR FUNCTION AND STRESS-MEDIATED REGULATION AMONG MEMBERS OF THE PROTEOLIPID PROTEIN FAMILY.

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Chronic stress causes morphological alterations in the hippocampus including atrophy of CA3 dendrites and loss of synapses. The molecular mechanisms underlying these structural changes remain largely unknown. We have previously identified M6a as a stress responsive gene and shown that M6a is involved in filopodium/spine outgrowth and, likely, synapse formation. Therefore, M6a could be, at least in part, responsible for the plastic alterations observed in the hippocampus of chronically stressed animals. M6a belongs to the proteolipid protein (PLP) family. Other members of this family include M6b and PLP, with its splice variant DM20. No function has been described for M6b. In addition, M6b, PLP and DM20 have not been studied in relationship to the stress response. **Objectives:** to study the effect of chronic stress on the hippocampal expression levels of M6b, PLP and DM20 and to assess the capacity of these proteins to induce filopodium formation in hippocampal neurons. **Methods:** male C57BL/6 mice were subjected to chronic stress by daily immobilization, 4 hours a day, during 3 weeks. Then, the hippocampi were surgically removed and the expression level of the different members of the PLP family was assessed by reverse transcriptase (RT) real time polymerase chain reaction (PCR). To study the capacity of different members of the PLP family to induce filopodium formation, primary cultures of hippocampal neurons were transfected with plasmids coding for the different proteins, and the number of filopodial protrusions in the neuronal processes was quantified. **Results:** we found that chronic restraint stress in mice significantly reduces M6b (69 ± 2 % of control,) and DM20 (61 ± 7 % of control), but not PLP, mRNA levels in the hippocampus. In addition, M6b and DM20 (164 ± 6 % and 126 ± 4 % of control), but again not PLP, induce filopodium formation in primary cultures of hippocampal neurons. **Conclusions:** there is a conserved stress mediated regulation and function in filopodium formation among different members of the PLP family. Our results reveal a novel function for M6b and DM20 while providing new evidence for an involvement of PLPs in the stress response.

Palavras-Chave: chronic stress, proteolipid proteins, filopodium formation, hippocampus

Apoio Financeiro: International Research Scholars Grant from the Howard Hughes Medical Institute and by the National Research Council (CONICET, Buenos Aires).

E.04.034

ANÁLISES COGNITIVAS EM RATOS SEPARADOS DA MÃE NO PERÍODO NEONATAL E SUBMETIDOS A UM MODELO DO TRANSTORNO DE ESTRESSE PÓS-TRAUMÁTICO QUANDO ADULTOS

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Objetivos:

Pacientes com história de adversidades precoces apresentam maior frequência do Transtorno do Estresse Pós-Traumático (TEPT), que pode ocasionar prejuízo de memória e dificuldade de concentração, além de reação exagerada de medo. O objetivo deste estudo é verificar se a separação da mãe (SM) piora os efeitos de um modelo de TEPT em ratos na idade adulta sobre o desempenho em testes envolvendo memória [labirinto aquático de Morris (LAM), esQUIVA inibitória (EI), teste de reconhecimento de objetos (RO)] e resposta de medo condicionado [tempo de imobilidade (TI) na reexposição ao local usado para modelar o TEPT].

Métodos e Resultados:

Ninhadas foram divididas em intactas e SM (3h/dia, dias 1-10 pós-natal). Quando adultos, foram divididos em expostos ou não a um modelo de TEPT (exposição a choque inescapável; EC) e a 3 recordatórios situacionais (RS), quando foi medido o TI. Um mês após, os animais foram expostos às tarefas de RO, LAM e EI, com intervalos entre tarefas de 7 dias. Dados comparados por ANOVA de 2 vias (SM e choque), ou teste de Kruskal-Wallis (EI).

O choque aumentou em média em 900% o TI nos RS ($P < 0,001$). No RO houve diferença na exploração dos objetos familiar versus novo no teste, sem diferença entre grupos ($P < 0,005$). Na EI, não houve efeito significativo, enquanto no LAM os animais submetidos ao modelo de TEPT apresentaram aumento marginalmente significativo na latência do último dia de treino ($P = 0,065$), sem efeito no teste. Dados são média $\pm$ SEM ou, no caso da EI, mediana (interquartis), em segundos.

		LAM		EI		RO	
		Latência (último treino)	Quadrante alvo (teste)	Treino	Teste (24h)	Novo	Familiar
Intactos	Controle	29 $\pm$ 6,5	18 $\pm$ 5	8 (7-14)	35 (25-144)	9 $\pm$ 2,7	4 $\pm$ 1,3
	TEPT	47 $\pm$ 8,3	16 $\pm$ 7,6	23 (13-60)	180 (180-180)	5 $\pm$ 1	4,7 $\pm$ 1
SM	Controle	37 $\pm$ 6	16 $\pm$ 3,3	8,5 (5-9)	180 (41-180)	5,6 $\pm$ 1	4,6 $\pm$ 0,7
	TEPT	45 $\pm$ 4,4	16 $\pm$ 4	14 (7,3-19,3)	180 (145-180)	9,3 $\pm$ 1,7	5 $\pm$ 1

Conclusão:

Há uma ligeira redução no aprendizado da tarefa espacial em animais submetidos ao modelo de TEPT. Tanto a SM quanto o estresse na idade adulta causaram alteração no desempenho, especialmente em tarefas com conteúdo emocional, sugerindo-se nestas um aumento de comportamentos tipo ansioso, mas sem interação entre os tratamentos.

Palavras-Chave: TEPT (Transtorno de Estresse Pós-Traumático), Separação Materna, Análises Cognitivas

Apoio Financeiro: CAPES, CNPq

E.04.035

Participación de los mecanismos oxidativos celulares en las alteraciones conductuales inducidas por estrés durante las primeras etapas del desarrollo

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El efecto del estrés durante las primeras etapas del desarrollo provoca alteraciones tanto inmediatas como tardías que se reflejan en cambios en los sistemas cognitivo y neuroquímico.

El objetivo de este trabajo fue evaluar el rol de los mecanismos oxidativos celulares en las alteraciones conductuales en la adultez inducidas por estrés durante las primeras fases del neurodesarrollo.

Métodos: Se sometieron ratas Wistar preñadas desde el día 14 de gestación hasta la parición a estrés por inmovilización con tres ciclos diarios de 45 minutos. Se analizaron en los cerebelos e hipocampos de la descendencia: la actividad de Oxido Nítrico Sintasa (NOS), la expresión de RNA y el perfil proteico de nNOS mediante técnicas de RT-PCR y western-blot.

Resultados: Se observa que las ratas provenientes de madres estresadas presentaron un aumento significativo en la actividad de NOS total (pmol/gtej/30min) en distintas las edades PN7: C=3381,4 vs. E=5587,1 (*p<0.05); PN15: C=1742,8 vs. E=2284,7 (*p<0.05) y PN90: C=540,1 vs. E= 800,5 (*p<0.05). Asimismo se observó un aumento significativo en la producción de especies reactivas de oxígeno (ROS) al estimularse con NMDA en cerebelos PN15. En cerebelos e hipocampos provenientes de madres estresadas se observó un aumento en el perfil proteico de la isoformas NOS neuronal (n-NOS) (nNOS: DO estresado/control=2.39 a PN15; 1.41 a PN30; 3.83 a PN45 y 2.05 a PN90) junto a una alteración de la expresión de RNA.

Concluimos que el estrés en las primeras etapas del desarrollo induce alteraciones en los mecanismos oxidativos cerebelar e hipocampal. Teniendo en cuenta resultados previos de alteraciones conductuales suponemos a las alteraciones oxidativas como eventos primarios durante el desarrollo que se manifiestan en la adultez como cambios significativos en el comportamiento del animal.

Palavras-Chave: estres prenatal - NOS - cerebelo - hipocampo - neurodesarrollo - conducta

Apoio Financeiro: UBACyT M0826

E.04.036

EFEITO DA SEPARAÇÃO MATERNA ACOMPANHADA DE MANIPULAÇÃO NEONATAL SOBRE A INTEGRIDADE CELULAR DE ESTRUTURAS DO SISTEMA NERVOSO CENTRAL DE FILHOTES DE RATOS

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Considerando que a manipulação neonatal pode induzir alterações estruturais, com alteração da densidade celular no Sistema Nervoso Central (SNC) de ratos, decidimos verificar se essas são acompanhadas de lesão celular. O trabalho tem por objetivo analisar o efeito da separação materna breve, acompanhada de manipulação, sobre a integridade do DNA nuclear, das moléculas lipídicas e na formação de grupamentos sulfidril(SH) no bulbo olfatório e no hipocampo de filhotes de ratos.

Filhotes de ratos foram divididos em 3 grupos: manipulação repetida (filhotes manipulados diariamente durante os 10 primeiros dias após nascimento); manipulação única (uma manipulação no 10º dia de vida); e controle (não manipulados). A manipulação consiste em retirar os filhotes do ninho e tocá-los gentilmente durante 1 minuto. No 10º dia os animais foram sacrificados, tendo o hipocampo e o bulbo olfatório rapidamente retirados. As estruturas, dissociadas em PBS, foram submetidas ao Ensaio Cometa (*Exp. Cell Res.* 175:184, 1988), ensaio de lipoperoxidação (*Meth. Enzymol.* 186:421, 1990) e ao ensaio de formação de grupos SH.

A média de dano ao DNA do grupo manipulação repetida do bulbo olfatório ($n=7$; 130 ± 35) foi significativamente maior ($P=0,003$) do que nos grupos controle ($n=10$; 66 ± 37) e manipulação única ($n=10$; 60 ± 45). No entanto, não houve diferença entre as médias de dano entre os três grupos no hipocampo. Dados preliminares indicam não haver diferenças na lipoperoxidação e na formação de grupos sulfidril entre os grupos no bulbo olfatório enquanto que no hipocampo a manipulação parece ter um efeito protetor contra lipoperoxidação, sem alterar a formação de grupamento SH.

Os dados preliminares apontam para um efeito protetor do procedimento de separação materna seguida de manipulação no hipocampo, sem aumento de danos no bulbo olfatório. Quanto ao dano ao DNA evidenciado, propomos a idéia de que o aumento do cuidado materno favorece o desenvolvimento do SNC do filhote (*Nat.* 213:150, 1967; *Brit. J. Nutr.* 19:397, 1965), que se dá através de seleção de certas redes neurais com morte por apoptose de outras, justificando a presença de fragmentação do DNA genômico sem outros danos celulares.

Palavras-Chave: Manipulação Neonatal, Lipoperoxidação, Dano ao DNA, Grupamentos SH

Apoio Financeiro: BIC/UFRGS

HYPOTHALAMIC-PITUITARY-ADRENAL AXIS RESPONSES TO STRESS IN WISTAR AUDIOGENIC RATS.

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Audiogenic seizures (AS) are a model of generalized tonic-clonic seizures, evoked by high-intensity (110 dB) acoustic stimulation in susceptible animals such as Wistar Audiogenic Rats (WAR). AS has been shown to evoke neuroendocrine responses, however, hypothalamic-pituitary-adrenal activity in WAR is not established. In this study we evaluated plasma ACTH and corticosterone (B) levels under basal condition and after restraint. Adrenal responses to exogenous ACTH stimulus was also evaluated. WAR and Wistar rats were decapitated under basal condition or after restraint for 15 minutes for plasma B and ACTH measurements by specific radioimmunoassays. To verify the adrenal responsiveness to ACTH, rats were anesthetized with 2.5% tribromoethanol (1ml/100g, ip) and had a silastic cannula placed at right jugular vein. Next day, rats were pre-treated with dexamethasone (100ug/100g, sc) and 2 hours after they received an intravenous injection of ACTH (8 ng/rat) or 0.9% NaCl. Trunk blood samples were collected for plasma B determination by decapitation 15 minutes after the injection. Plasma B values in basal conditions and after restraint were, respectively, 1.4 ± 1.0 ug/dl and 30.0 ± 3.6 ug/dl in WAR and 0.6 ± 0.2 ug/dl and 32.0 ± 3.8 ug/dl in Wistar rats. Plasma ACTH values in basal conditions and after restraint were, respectively, 30.9 ± 13.8 pg/ml and 658.0 ± 154.3 pg/ml in WAR and 23.8 ± 7.9 pg/ml and 468.9 ± 75.5 pg/ml in Wistar rats. Compared to basal conditions, there was an increase of plasma B and ACTH levels after restraint. There was no difference in B responses to stress between both groups of rats, however plasma ACTH levels after stress were higher ($p < 0.01$) in WAR compared to Wistar rats. Plasma B values after ACTH stimulus were significantly higher in WAR (19 ± 8.7 ug/dl), compared to Wistar (9.2 ± 2.5 ug/dl) groups ($p < 0.05$). These data indicate that WARs show higher pituitary and adrenal responsiveness. The association of the increased responsiveness of hypothalamic-pituitary-adrenal axis and the susceptibility to seizure remains to be determined. **Palavras-Chave:** Corticosterone, ACTH, stress, Wistar Audiogenic rats.

Apoio Financeiro: FAPESP, FAPESP-Cinapce, CNPQ, PROEX-CAPES, FAEPA

E.04.038

Dynamic and selective association of arginylated calreticulin to stress granules in cells

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The post-translational arginylation of proteins is the covalent union of arginine to an acidic amino acid at the N-terminal position. We characterized calreticulin (CRT) as substrate of this modification. This modified protein (R-CRT) showed a sub-cellular localization in the cytosol different from that known for CRT in the endoplasmic reticulum (JBC, 282, 8237-45). In neurons and fibroblasts, we determined that the decrease in cytosolic calcium concentration increases the production of R-CRT, which forms aggregates that are associated with stress granules (SGs).

Furthermore, we found R-CRT associated to SGs in cells exposed to oxidative stress and heat shock, similar to the calcium stress condition. However, in cells treated with UV radiation, R-CRT does not aggregate with SGs. In correlation with these observations, cytosolic calcium decreases in both oxidative stress and heat shock conditions, whereas after the UV radiation calcium levels are unchanged. After withdrawal of the stressor, SGs identified with TIA-1 (a SGs marker) rapidly disappear but the R-CRT remains aggregated after 2 h. Thus, there are different rates of disassociation between TIA-1-labeled SGs and R-CRT aggregates after the environmental stress has ended. These changes correlate with the restoration of calcium levels, which in itself depend on the magnitude of the stress.

In conclusion, intracellular calcium changes promote the selective association of R-CRT to SGs under particular stress conditions. Taking into consideration that SGs are highly dynamic structures whose interaction constantly switches between the polysomes and the mRNAs degradation system, and considering the binding capacity to mRNA described for CRT, our results suggest that R-CRT could selectively modulate the specific translation of certain mRNAs according to the environmental stress conditions.

Palavras-Chave: Arginylation, Calcium, Calreticulin

Apoio Financeiro: Supported by SECyT-UNC, CONICET, ANPCyT-PICT.

Comparison of the Effects of Predictable versus Unpredictable Stress on Skilled Walking after Ischemic Stroke in Rats

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Introduction: Cerebrovascular injury, such as stroke, is a leading cause of disability. A major consequence of stroke is severe motor impairment. The severity of functional impairment and subsequent recovery can be influenced by stress and stress hormones. Previous studies have reported that stress impairs normal motor function and diminishes recovery of skilled movement after ischemic injury involving motor cortex. It has been suggested that the effects of stress strongly depend on the stressor's type and strength.

Objectives: The purpose of the present study was to compare the effects of chronic predictable restraint stress and chronic unpredictable variable stress on skilled walking in a rodent model of stroke.

Materials and Methods: Adult male rats were trained to cross the horizontal ladder rung walking task. Testing of skilled walking in this task occurred at three time points during the experiment; baseline, prior to lesion and post-lesion. Animals were assigned to daily treatments of either restraint stress or variable stress starting one week prior to lesion up to three weeks post-lesion. One group served as lesion only control.

Results: The results revealed a distinct pattern of recovery and compensation of skilled walking. Analysis revealed that both restraint and variable stress treatment exaggerated disruption of movement patterns used for skilled walking as compared to lesion control. Animals exposed to daily restraint stress and lesion controls made significantly more errors while crossing the ladder beam during the post-lesion testing time point with their contralateral limbs. Furthermore, the variable stress did not cause increases in the number of errors made while crossing the ladder rung walking task.

Conclusions: The findings indicate that both chronic predictable restraint stress and unpredictable variable stress influence the course of recovery from stroke, however, chronic restraint stress might affect stroke recovery through a different route than chronic variable stress.

Palavras-Chave: stress, stroke model, skilled walking

Apoio Financeiro: Canadian Institutes of Health Research, Canadian Stroke Network, National Sciences and Engineering Research Council of Canada, and the Alberta Heritage Foundation for Medical Research.

E.04.040

EFEITO DA ROTINA DE MANEJO NOS NÍVEIS FECAIS DE CORTISOL DE GRUPOS SOCIAIS DE SAGÜI, *Callithrix jacchus*.

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Mudanças ambientais relacionados à rotina de manejo em cativeiro, como a oferta de alimento, a limpeza de recintos, a pesagem e medicação dos animais, a presença de intrusos e de ruído parecem influenciar a atividade do eixo Hipotálamo-Hipófise-Adrenal (HHA) pela variação nos níveis de cortisol. O objetivo desse estudo foi investigar o efeito dessas mudanças ambientais em quatro grupos familiares de sagüi (*Callithrix jacchus*) mantidos no Núcleo de Primatologia da UFRN, Natal-RN, pela comparação dos níveis fecais de cortisol apresentados pelos animais em duas condições ambientais: semana (S: dia não calmo) e final de semana (FS: dia calmo). As diferenças entre as condições foram: a) colocação do alimento (S: 2 vezes/dia; FS: 1 vez/dia); b) limpeza das gaiolas-viveiro e pesagem dos animais (apenas durante a semana) e c) captura e transferência dos animais (principalmente durante a semana). Os animais foram mantidos em gaiolas-viveiro medindo 2x2x3m, submetidos ao ciclo claro-escuro ambiental e amostras de fezes dos pares reprodutores e suas proles foram coletadas por 2 meses. O perfil de excreção de cortisol dos pares reprodutores e suas proles apresentou diferença significativa entre as duas condições testadas, com os maiores níveis do hormônio sendo verificado durante a semana. Além disso, as fêmeas foram mais responsivas à essas mudanças. Assim, variação no ambiente relacionada às atividades de manejo na colônia influenciou a resposta hormonal dos pares reprodutores e suas proles de sagüi, sugerindo que a variação na atividade do eixo HHA parece ter um caráter adaptativo no ajuste do indivíduo ao seu ambiente e que o perfil hormonal mais elevado nas fêmeas parece refletir a dinâmica social do estabelecimento do posto reprodutivo.

Palavras-Chave: Rotina de manejo, Dia da semana, eixo HHA, cortisol, *Callithrix jacchus*

Apoio Financeiro: CAPES, ANAP e FINEP

Chronic caffeine consumption prevents neuroplasticity and behavioural alterations in mice subject to chronic unpredictable stress.

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Caffeine (a non-selective adenosine receptor antagonist at non-toxic doses) is the most widely consumed psychoactive drug. Its chronic consumption prevents memory deficits upon aging or Alzheimer's disease, an effect attributed to the antagonism of A_{2A} receptors. We previously found that the selective blockade of A_{2A} receptors affords protection against sub-chronic restrain stress (Cunha et al., 2006, *Neuroscience* 141:1775). The aim of the present study was test if caffeine affords neuroprotection against neurochemical and behavioural modifications induced by chronic unpredictable stress (CUS), a behavioural model linked to the development of depression-like states in rodents and closely related to the human situation. We focused on modifications present in the hippocampus since the application of long lasting stress in mice causes cognitive impairments accompanied by changes in limbic brain structure such as synaptic loss (McEwen et al., 2000, *Biol. Psychiatry* 48:721). The CUS protocol was applied for 21 days in adult male C57B6 mice (25-30g) and includes uncontrollable daily stressors (tilted cage, food/water deprivation, foot shock, paired caging, continuous light, wet bedding). The exposure of mice to the CUS produced a significant ($P<0.05$, $n=8$) increase in the plasma corticosterone levels ($13.15\pm5.92\%$) and a lower gain of weight (control: 1.49 ± 0.20 g, CUS: 0.37 ± 0.12 g). Chronic caffeine consumption (1g/L in the drinking water starting 4 weeks before the CUS) prevented stress-induced alterations in the behavioural profile, such as depressive-like effect in the forced swimming test and cognitive deficits in the Y-maze test, without affecting locomotion in the open-field arena or the anxiogenic-like effect of CUS (elevated plus maze). In addition, CUS decreased the immunoreactivity of synaptic proteins such as SNAP-25 and syntaxin (Western blot analysis) in the hippocampus, an effect prevented by caffeine consumption. Caffeine was also able to prevent the CUS-induced increase in GFAP immunoreactivity (Western blot and immunohistochemistry) of hippocampal CA1 and CA3 regions. These results support the notion that chronic consumption of caffeine, possibly acting through A_{2A} receptor blockade, confers a robust protection against neurochemical and behavioural alterations induced by CUS.

Palavras-Chave: Caffeine, A2A receptors, stress, neuroprotection

Apoio Financeiro: CAPES-Brazil and FCT

Expression of the *fos* gene in the brain of *Apis mellifera*: preliminary results

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The *fos* gene expressed in neurons is being considered an important metabolic marker of structures of the nervous system, and has been used to evaluate alterations caused by various stimuli in the central nervous system. The experiments carried out for the evaluation of many composites of the expression of *fos* gene use rodents as the experimental model, most of the time. From the discovery of the expression of the *fos* gene in insects, new possibilities of experimental models and new applications of this marker had become viable. So that insects can be used as experimental models of the *fos* gene expression, a mapping of the basal expression through immuno-histochemical studies is necessary, that is being done with *Apis mellifera* bees. The preliminary results were obtained with workers 21 days of age. When they reach this phase, inside the characteristic age polyethism of the social organization of *Apis mellifera*, the workers start to carry out activities outside the colony. The Ab-5 anti-*fos* antibody (Calbiochem) positively marked neuron nuclei of specific regions of the brain. In the antennal lobes the marking was positive in the peripheral region, where neuron bodies are concentrated, being negative in the neuropile region where, almost exclusively, the cellular prolongations are found. In the optical lobes, the positive marking was observed in the region of the connection of the lamina with the ommatidia and in the cells that compose them. These two regions with a positive marking receive intense stimulation when the worker starts foraging. These stimulations can be external, such as variations in the intensity of light waves and contact with new types of chemical substances present in the environment, or physiological, because the circulating juvenile hormone levels are higher when the workers are foraging instead of the ones performing intracolony activities. **Palavras-Chave:** *fos* gene, *fos* protein, bee brain, immunohistochemistry

Apoio Financeiro: FAPES, CNPq

Chronic stress decreases the expression of sympathetic markers in the pineal gland and increases plasma melatonin concentration in rats.

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Introduction: Chronic stress affects brain areas involved in learning and emotional responses. Although most studies have concentrated on the effect of stress on limbic-related brain structures, in this study we investigated whether chronic stress might induce impairments in diencephalic structures associated with limbic components of the stress response.

Materials and Methods: Specifically, we analyzed the effect of chronic immobilization stress on the expression of sympathetic markers in the rat epithalamic pineal gland by immunohistochemistry and western blot, whereas the plasma melatonin concentration was determined by radioimmunoassay.

Results: We found that chronic stress decreased the expression of three sympathetic markers in the pineal gland, tyrosine hydroxylase, the p75 neurotrophin receptor and α -tubulin, while the same treatment did not affect the expression of the non-specific sympathetic markers Erk1 and Erk2, and glyceraldehyde-3-phosphate dehydrogenase. Furthermore, these results were correlated with a significant increase in plasma melatonin concentration in stressed rats when compared with control animals (23.00 h, stress: 365 ± 73 pg/mL; control: 149 ± 24 pg/mL, $p < 0.05$).

Conclusions: Our findings indicate that stress may impair pineal sympathetic inputs, leading to an abnormal melatonin release that may contribute to environmental maladaptation. In addition, we propose that the pineal gland is a target of glucocorticoid damage during stress.

Palavras-Chave: Depression, pineal gland, stress, epithalamus, melatonin

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Restrain stress impairs auditory avoidance conditioning but not affect the startle responses: fear processing implications

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Stress impairs areas of the limbic system affecting learning and emotional responses. In this study we investigated whether restrain stress affects the processing of auditory conditioned (80 dB) and unconditioned (90 dB) stimuli using active avoidance conditioning and acoustic startle tests respectively. In addition, we analyzed the stress effects on the neuronal morphology of the medial geniculate nucleus (MG, subserving auditory information) and the posterior thalamic nucleus (PO, subserving somatosensory information) by Golgi preparations. We found that stressed rats showed a stronger impairment in the acoustic conditioning ($p < 0.001$) and a significant dendritic atrophy in the MG (control: 808 ± 120 mm and stress: 407 ± 55 mm; 49% difference; $p < 0.01$), both respect to control animals. In contrast, stress did not affect the acoustic startle responses, prepulse inhibition and the neuronal morphology of the PO. Our results suggest that the auditory conditioned stimuli became processed in part as auditory unconditioned stimuli in the stressed animals, and it could decrease the level of the fear threshold activation in the brain. Comparable alterations could be induced by psychosocial stress in humans and have a role in the development of depressive disorders.

Palavras-Chave: Stress, depression, auditory system, medial geniculate

Apoio Financeiro: Anillo de Ciencia y Tecnología N° ADI-09 (Programa Bicentenario-CONICYT) and DGIP N° 10301217 (Universidad Católica del Norte) grants

Pseudo cereal *Quinoa* improves memory and decreases anxiety of stressed rats: behavioral and molecular approaches

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Chronic stress affects the limbic system impairing memory and increasing anxiety. In addition, stress induces dendritic atrophy in the inferior colliculus (IC, a main component of the auditory system), which was correlated with a decrease of auditory conditioning. The aim of this study was to investigate the effects of *quinoa*, an original food plant of Latin America, on memory (Y-Maze) and anxiety (light/dark box maze) of controls and stressed rats (*Sprague Dawley*). We used the following experimental groups: 1) control, 2) restrain stress (2 hrs/day, 10 days), 3) control + *quinoa* and 4) restrain stress + *quinoa*. After restrain stress protocol, groups 3) and 4) were fed during 7 days with a mix between 25 % *quinoa* and 75 % food rat. Groups 1) and 2) were fed only with food rat during the whole experiment. Stressed rats of group 4 had a significant improvement of memory (group 2: -27.8 ± 18.9 ; group 4: 1.5 ± 3.9 ; $p < 0.01$) and a decrease of anxiety respect to group 2. Moreover, stress decreases Brain-derived neurotrophic factor (BDNF, 21.3 ± 1.8 %, $p < 0.05$) and glucocorticoid receptor (46.9 ± 0.8 %, $p < 0.01$) expression, and increases (66.8 ± 1.3 %, $p < 0.001$) mineralocorticoid receptor expression in the IC respect to control animals (RT-PCR studies). These gene expression alterations could be part of the molecular mechanism through stress induce neuronal atrophy in the IC. *Quinoa* could reverse the adverse effects induced by psychosocial stress in human and have a therapeutic role in patients with major depression.

Palavras-Chave: Stress, Memory, Anxiety

Apoio Financeiro: Anillo de Ciencia y Tecnología N° ADI-09 (Programa Bicentenario-CONICYT) and DGIP N° 10301217 (Universidad Católica del Norte) grants

Peripheral metabolic responses to microinjection of bicuculline into the hypothalamic paraventricular nucleus

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Objective: The paraventricular hypothalamic nucleus (PVN) has a important role in autonomic and endocrine regulation. The aim of the present study was to investigate the status of jejunal absorption and peripheral metabolism of glucose in rats after PVN disinhibition induced by the GABA_A antagonist bicuculline methiodide (BMI) microinjection.

Methods: Rats Wistar male (n=7) (270-290g) were anesthetized (tribromoethanol 250 mg/Kg) and received a guide cannulas implant into PVN. After 3 days, under anesthetized the animals received a femoral artery catheter. After 24h, the animals received a microinjection (100 nL) of vehicle (saline 0,9%) or BMI (10 pmol). Cardiovascular parameters were recorded for 15 min. after the microinjection. Then the animals were anesthetized (pentobarbital sodium 40 mg/kg) and the jejunal loop was isolated and infused (0,5 mL min⁻¹) with a tyrod solution. Samples were taken 10 min. intervals over a 40 min. period. At the end of the experiment, samples of liver and gastrocnemius muscle were taken to measure the levels of glycogen and intermediate metabolite of glycolytic pathway (glucose-6-phosphate, fructose-6-phosphate) and ATP. BMI in PVN increased significantly the blood pressure (0,3±1,2 vs. 5,0±1 mmHg) (p<0,01) and heart rate (0,6±1 vs. 18±3 bpm) (p<0,001) and glucose absorption in the time 10 (2,0±0,3 vs 3,0± 0,2 mg/dL) (p<0,05), 20' (1,5±0,2 vs. 3,7±0,3 mg/dL) (p<0,001), 30' (1,9±0,2 vs. 3,3±0,2 mg/dL) (p<0,001) e 40' (2,0±0,3 vs. 3,0±0,2 mg/dL) (p<0,01) when compared with the controls. We observed a decrease in the muscle content of glucose-6-phosphate (0,396±0,054 vs. 0,262±0,019 mmol/g) and ATP (0,157±0,054 vs. 0,041±0,013mmol/g) in rats submitted to BMI injection.

Conclusion: According to the present results, glucose jejunal absorption was altered by disinhibition of PVN and this effect was accompanied by glycolytic pathway activation.

Palavras-Chave: Jejunal absorption, peripheral metabolism, PVN, stress

Apoio Financeiro: Supported by CAPES

Electroconvulsive Therapy Further Exacerbates Heightened Acoustic Startle in Animal Model of Traumatic Stress

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Objectives: Given the frequent comorbidity of post-traumatic stress disorder (PTSD) and major depressive disorder (MDD) along with the effectiveness of electroconvulsive therapy (ECT) for treatment of MDD alone, recent interest has been shown in using ECT for comorbid MDD and PTSD and potentially for PTSD alone. Initial investigation indicates some possible benefit of ECT for PTSD, though it may only help with certain symptoms (1). We sought to investigate the effect of ECT on one major symptom of PTSD, heightened acoustic startle, using a rat model of traumatic stress (2).

Methods: Using 64 adult, male Sprague-Dawley rats in a 2 x 2 design (stress/control x ECT/control), we investigated the effects of ECT on the heightened acoustic startle response (ASR) associated with traumatic stress. The animals were subject to a restraint/tailshock paradigm and then treated with ECT. The ASR tests were conducted at 1, 4, 7 and 10 days after ECT treatment. These results were compared to baseline ASR recordings taken before treatment and analyzed using repeated measures ANOVAs.

Results: Animals treated with ECT following with exposure to traumatic stress showed significant increases in ASR on days 1 ($M = 725.1 \pm 164.4$), 4 ($M = 974.5 \pm 172.5$), 7 ($M = 834.0 \pm 148.2$) and 10 ($M = 902.4 \pm 144.9$) compared to both untreated ($M = 525.7 \pm 86.8$; 416.8 ± 46.8 ; 412.5 ± 97.6 ; 394.2 ± 33.5 on days 1, 4, 7 and 10 respectively) and control groups ($M = 589.4 \pm 43.6$; 584.1 ± 61.1 ; 421.4 ± 63.3 ; 545.8 ± 38.7 on days 1, 4, 7 and 10 respectively).

Conclusions: While ECT may prove to be an effective treatment for comorbid MDD/PTSD or PTSD alone in general, it may exacerbate heightened arousal associated with PTSD. Study should be undertaken to determine if this is the case clinically, and necessary pharmacological treatment for this symptom should be considered when using ECT to treat PTSD.

References: 1- J ECT 93-95, 2007; 2- Biol. Psychiatry 539-546, 1995.

Palavras-Chave: electroconvulsive therapy, PTSD, traumatic stress, acoustic startle, animal model

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Cuidado Maternal, Estresse e Ocitocina em Ratos Neonatos.

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Objetivos:

As primeiras semanas de vida dos ratos é um período no qual o animal está muito sujeito às influências ambientais que podem ter um efeito persistente por toda a vida, tal como o próprio cuidado maternal (CM). Ratos que receberam diferentes níveis de CM podem apresentar diferenças na ansiedade e nas respostas ao estresse (E) quando adultos. Neste trabalho, investigamos se as variações de CM influenciam as respostas da ocitocina (OT), um dos hormônios envolvidos no E, em ratos no período neonatal submetidos a um estressor.

Métodos:

Foram utilizadas 60 ratas Wistar prenhas e no final do dia 0 – dia do nascimento, cada ninhada foi padronizada em 8 filhotes. A partir do dia 1, foi realizado o registro do CM até o dia 10, o qual é avaliado principalmente pela frequência de lambidas (FL) da mãe nos filhotes. Foram realizados 4 registros/dia, 72 min cada, nos horários: 9:30, 12:30, 15:30 e 18:30 h. A média de FL da população foi $5,52 \pm 0,1863$. Foram selecionadas as ratas que apresentam maior e menor FL, definidas como Muito-Cuidadoras (MC, média > 6,991, n=10), Pouco-Cuidadoras (PC, média < 4,1814, n=12), respectivamente. No dia 13 pós-parto, foram utilizados 3 machos (M) e 3 fêmeas (F) por ninhada, 2 casais submetidos a um E por frio (0°C, 6 min), sendo que um casal foi sacrificado 15 min (M15 e F15) e outro 30 min pós-E (M30 e F30). O casal não submetido ao E foi usado como controle (M0 e F0) e sacrificados no início do experimento. O sangue do tronco foi coletado para análise da OT plasmática (ng/ml) por radioimunoensaio.

Resultados:

A ANOVA de três vias mostrou uma diferença apenas de gênero na OT (pg/ml), aumentada nos filhotes fêmeas ($P < 0,05$).

Mãe	MC						PC					
Filhote	M0	M15	M30	F0	F15	F30	M0	M15	M30	F0	F15	F30
Média	24,8	17,6	11,6	18,8	28,7	29,7	9,1	15,4	7,3	20,2	19,3	28,7
Erro-padrão	9,7	7,0	4,6	6,0	8,2	7,1	2,2	5,5	2,1	11,4	5,6	9,0

Conclusões:

Ao contrário da literatura com adultos, não houve resposta da OT ao E. O sistema hipotalâmico neurohipofiseal pode não estar maduro e a OT não ser tão responsiva ao frio – os principais E que liberam OT são o hiperosmótico e por contenção. A OT aumentada nas F pode ser devido a maior atividade da OT do que em M, tal como maior expressão de seus receptores.

Palavras-Chave: cuidado maternal, neonatos, estresse, ocitocina.

Apoio Financeiro: CAPES e CNPq

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Whole brain pattern classification of PTSD patients using gray matter volume

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Introduction: Recently, multivariate pattern recognition methods (e.g. Support Vector Machines, SVM) have been applied to fMRI data (1) and structural MRI data (2). The main advantage of these approaches is that they allow evaluation of whole brain as a spatial pattern, taking into account correlations between regions. In these applications the scans are treated as spatial patterns and statistical learning methods are used to identify statistical properties of the data that discriminate between groups of subjects (e.g. patients and controls).

Methods: We used SVM to classify grey matter segmented from T1-weighted MR scans from 16 PTSD patients and 16 healthy controls. We applied a leave-one out cross validation test.

Results: Preliminary findings showed that PTSD patients were correctly assigned in 56.0% of trials (sensitivity 56.0%, specificity 69.0%). Middle frontal gyrus, lateral occipital gyrus and anterior cingulate were regions prominent in achieving this classification.

Conclusion: The SVM was able to discriminate PTSD patients from healthy subjects with accuracy above the chance level and was able to find the most discriminating regions between the groups. We believe these results can be significantly improved with larger sample sizes. Our results show that pattern recognition methods have the potential to play an important role for computer based diagnostic image analysis for clinical practice.

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Palavras-Chave: Pattern Recognition, Support Vector Machine, Post-Traumatic Stress Disorder

Apoio Financeiro: CAPES, CNPq

ESTUDO DA MEMÓRIA ESPACIAL E POSSÍVEIS DANOS AO ADN CELULAR EM RATOS MACHOS ESTRESSADOS CRONICAMENTE E SUBMETIDOS À UMA DIETA HIPERPALATÁVEL

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Objetivo: O estresse crônico e/ou níveis aumentados de glicocorticóides podem produzir efeitos deletérios. A ingestão de alimentos palatáveis pode reduzir a liberação de glicocorticóides, mas a ingestão excessiva de alimentos gordurosos pode levar a dano ao ADN celular. Nesse trabalho, avaliamos os efeitos da exposição ao estresse crônico na vigência (ou não) de uma dieta altamente palatável sobre o desempenho no labirinto aquático de Morris e sobre os danos ao ADN no hipocampo (estrutura mais vulnerável ao estresse e relacionada com a memória espacial).

Métodos: Ratos machos Wistar foram divididos em 2 grupos, segundo a dieta [ração apenas e chocolate mais ração *ad libitum*], e subdivididos em estressados e controles. Usou-se estresse por contenção (1h/dia, 5 dias/semana, por 40 dias). Após, os animais foram submetidos ao labirinto aquático para verificar memória espacial de referência: cinco dias de treino (quatro tentativas/dia) e um dia de teste, sem a plataforma. Para análise do dano ao ADN foi realizado o Ensaio Cometa. Os dados foram analisados por ANOVA (2 vias, dieta e estresse como fatores) ou de medidas repetidas. Houve efeito facilitador da dieta (chocolate) durante as sessões de treino ($F_{1,32}=7,21$), e também uma interação entre dieta e tempo ($F_{4,128}=3,28$); no entanto, após os vários dias de treino, todos os grupos apresentaram, no teste, desempenho semelhante. O estresse ($F_{1,28}=186,35$) e o chocolate ($F_{1,28}=9,17$) aumentaram o dano ao ADN, havendo interação entre esses fatores ($F_{1,28}=11,20$) ($P \leq 0,01$ em todos os casos). Os principais achados deste trabalho estão mostrados na tabela (média $\pm$ EPM) abaixo.

Conclusão: Apesar do aumento na lesão ao ADN hipocampal nesses animais, não houve prejuízo na memória espacial. Com a dieta altamente palatável houve, inclusive, um aprendizado mais rápido durante o treino.

		Labirinto aquático (dias de treino) latência (s)			Índice de dano ao ADN
		1	2	3	
Controle	Dieta normal	49,2 $\pm$ 3,2	45,2 $\pm$ 5,1	42,2 $\pm$ 4,1	24,125 $\pm$ 1,0
	Ração + chocolate	49,7 $\pm$ 3,0	29,4 $\pm$ 3,1	34,1 $\pm$ 5,2	39,125 $\pm$ 1,9
Estressados	Dieta normal	45,0 $\pm$ 3,7	43,9 $\pm$ 4,2	39,4 $\pm$ 3,7	64,125 $\pm$ 2,3
	Ração +	48,6 $\pm$ 3,3	32,7 $\pm$ 5,2	24,6 $\pm$ 4,0	63,375 $\pm$ 3,5

	chocolate				
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**Palavras-
Chave:** Estresse
Crônico,Chocolate e

memória

Apoio Financeiro: CAPES, CNPq, rede IBNnet, FAPERGS/PRONEX

E.04.051

Effect of voluntary physical activity on oxidative stress parameters in mice brain

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Physical activity and exercise have beneficial effects on brain health and cognition. A current frontier in the neurodegenerative disorders concerns the role of physical activity for promoting brain health through protective, regenerative and adaptive neural processes. However, physical activity can cause oxidative stress by increasing the production of reactive oxygen species (ROS). Then, the purpose of this study was to evaluate the effect of physical activity in the ROS production in cerebral cortex and cerebellum of mice submitted to a voluntary wheel-running activity. Adult male Swiss mice (35-45g) were randomly divided into two groups: sedentary and running. The running group had free access to a home cage running wheel. After 21 days of physical activity, the cerebral cortex and cerebellum were removed and the parameters of oxidative stress were measured by thiobarbituric acid reactive substances (TBARS), glutathione (GSH) levels and the activity of the antioxidant enzymes glutathione peroxidase (GPx) and glutathione reductase (GR). The results showed that voluntary wheel-running activity did not induce neither lipoperoxidation, measured by TBARS nor alterations on glutathione (GSH) levels in the cerebral cortex and cerebellum of mice. Nevertheless, we observed a significant decrease in the cerebral cortex activity of GPx and GR in running group ($81.7 \pm 4.6\%$ and $59.2 \pm 9.5\%$ when compared to the sedentary control group = 100%) ($p < 0.05$). The activity of antioxidant enzymes were not modified by physical activity in cerebellum. These results indicate that, whereas physical activity may decrease the activity of antioxidant enzymes in cerebral cortex, the lipoperoxidation was not induced.

Palavras-Chave: Oxidative stress, Physical activity, lipoperoxidation.

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Oxidative stress and high concentration of glutamate induce neuronal death and change in the phosphorylation state of proteins in the rat brain primary cortical neuronal cultures.

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Using primary culture of cortical neurons we investigated the involvement of phosphoproteins from both systems the cytoskeletal (microtubule associated protein *tau*) and the cell signaling pathways (MAPKs and GSK3) in the mechanism of action of two neurotoxic factors: i) high concentration of glutamate and, ii) oxidative stress caused by hydrogen peroxide. The phosphoproteins were separated by gel electrophoresis and identified with antibodies, by immunofluorescence, through the Western blot technique. The quantitative analyses were done using densitometric assay. A significant decrease ($p<0,01$) in the viability of cortical neurons was observed for both neurotoxic factors. In addition, these effects were verified to be dependent on incubation time. An incubation time of one minute with hydrogen peroxide (1000 μ M) lead to a significant ($p<0,01$) effect on cellular viability (40%), while a smaller effect was observed for a longer incubation time. The next step was to verify if the high concentration of glutamate or hydrogen peroxide affects the level of phosphorylation and/or total concentration of the following classes of phosphoproteins: tyrosine kinase, isoforms of *tau*, and sub-classes of MAPK and GSK. Both treatments hydrogen peroxide and high glutamate concentration caused significant increase in the dephosphorylated form of *tau* and significant changes in the phosphorylation levels of different sub-classes of MAPKs. In addition, for both treatments a significant main effect of the incubation time on phosphorylation levels of MAPK and GSK was observed. In conclusion, the results indicate that both neurotoxic factors induce dysfunctions in components of the phosphorylation systems related with conformational changes from the active/inactive to inactive/active state, respectively. These changes might be involved in the mechanism of cell death caused by the oxidative stress or high glutamate concentration. **Palavras-Chave:** neuronal cultures, Oxidative stress, glutamate, phosphorylation

Apoio Financeiro: CNPq

CHRONIC STRESS IMPAIRS REELIN EXPRESSION OF FRONTAL ASSOCIATION CORTEX, HIPPOCAMPUS AND AMYGDALA IN RAT

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Objectives: The study of stress response has taken great relevance in medical and scientific research. The present lifestyle of modern societies, has increased dramatically the prevalence of stress, major depression and several other mental diseases 1,2,3. It is well known that stress affects key brain areas involved in learning and emotional responses, like prefrontal cortex, hippocampus and amygdala in humans and animals models. Recently, Reelin a glycoprotein of extracellular matrix, crucial in the positioning of neurons during development and involved in neural plasticity in the adult, has been discovered as a marker for major depression, an other diseases like schizophrenia and Alzheimer 4,5,6. In the present study, we show that Reelin expression is impaired on these key areas: Frontal association cortex (FrA), Hippocampus (Hip), and Amygdala (Am) of the rat's brain and settles basis to relate chronic stress and several mental disease prevalence increases.

Methods: Male rats (Sprague-Dawley, 3 months old) were stressed under a restraint movement protocol. Animals were killed under deep anesthesia with sodium pentobarbital. All procedures were approved by the Institutional Animal Ethics Committee. The brains were removed and grouped for Immunohistochemical Studies and Westernblot analysis using a polyclonal antibody specific for rat Reelin⁵ (1:1000 and 1:500 dilutions respectively, Mouse-Ab 142, Chemicon). For qualitatives and semiquantitative studies, the intensity of Reelin Images taken and bands was measured by densitometry comparing stressed (n=6) and control groups (n=6) of brains for each experiment. Significance of data sets were analyzed using a t-student test.

Results: we obtained in all cases a significant reduction in immunoreactivity in **FrA** (37.5%, p<0,01), **Hip** (46.4%, p<0,05) and **Am** (64.2%, p<0,01). Western blotting showed significant downregulation in protein products for Reelin 410 and Reelin 180 kDa (p<0,05 in all cases).

Conclusions: Chronic stress, reduce the Reelin protein levels in Frontal association cortex, hippocampus and amygdala. Future studies are required to understand the association of stress and the emergence of mental diseases related to stress and Reelin signaling process.

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Apoio Financeiro: Dr. Francisco Aboitiz

E.05.001

The lateral parabrachial nucleus controls hypertonic, not isotonic, NaCl intake.

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Preference for NaCl solutions reaches its peak at isotonic concentrations in rats. The inverted "U" shape of the preference curve is similar for hydrated and sodium-depleted rats suggesting that mechanisms that control sodium intake act changing the amplitude, not the shape of the curve. However, it is possible that some inhibitory mechanisms, like those from the lateral parabrachial nucleus (LPBN), act preferentially on the hypertonic side of the curve. The present work tested this hypothesis by either pharmacological intervention or immunohistochemistry for immediate early gene (c-fos) expression in the LPBN using two different protocols, systemic furosemide combined with low dose of captopril (FURO/CAP) and sodium depletion induced by peritoneal dialysis (PD). Methysergide (serotonin antagonist) injected into the LPBN (n=7/group) had no effect on 0.15 M NaCl intake (methysergide: 19±5.2; vehicle: 19.3±4.2, ml/2 h), but it enhanced 0.3 M NaCl intake (methysergide: 16.6±3.5; vehicle: 6.6±1.5, ml/2 h) induced by FURO/CAP without changing FURO/CAP-induced thirst. The number of positive cells for c-fos protein in the LPBN of rats made hypovolemic by PD enhanced when they ingested hypertonic (42±10 vs. hydrated: 18±1), but not isotonic (27±10 vs. hydrated: 20±8) NaCl. The results suggest that the LPBN has a preferential control over hypertonic rather than isotonic NaCl intake.

Palavras-Chave: sodium appetite, thirst, serotonin, preference

Apoio Financeiro: CNPq, CONICET, FAPESP, PROSUL

E.05.002

“Early activation of the tuberomammillary nucleus is a common factor of the appetitive phase in sex and drinking behavior in rats”

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Histamine neurons of the tuberomammillary nucleus show an earlier activation during the appetitive phase of feeding, compared to the other arousal system nuclei. To test if in different appetitive behaviors also these histaminergic neurons become active first, we studied changes in Fos-ir in arousal nuclei during sexual and drinking behavior. Male rats were exposed to sexually receptive or to non-receptive female rats, allowing sensory but not sexual contact. Receptive females elicited increased sniffing time which positively correlated with Fos-ir in the dorsal raphe, laterodorsal tegmental nucleus, orexin hypothalamic neurons and tuberomammillary nucleus. Non receptive females induced less sniffing and no Fos-ir increase in the males. Other male rats were deprived of water for 48 h and presented with an empty water bottle to induce appetitive behavior, which induced increased approaches to the bottle while they tried to drink. While water deprivation *per se* increased Fos-ir in the dorsal raphe and the locus coeruleus, the presentation of the bottle increased Fos-ir in the tuberomammillary nucleus and induced a further Fos-ir increase in the locus coeruleus. To evaluate the importance of the histaminergic neurons in the appetitive phase of these motivated behavior, we lesioned the tuberomammillary nucleus using saporin conjugated to hypocretin 2. The histaminergic neurons lesion blunted the appetitive phase in both motivated behaviors studied, without affecting general motor capacities. Taken together our results indicate that the histaminergic neurons become active at the onset of different motivated behaviors and that they are key players in motivated arousal. Other arousal nuclei may contribute to motivated arousal depending on the particular behavior.

Palavras-Chave: tuberomammillary nucleus, arousal system, appetitive behaviors

Apoio Financeiro: Fondecyt 1060476

E.05.003

Study of MCH neurons during predatory behavior and the normal peak food ingestion

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The hypothalamic neuropeptide known as melanin-concentrating hormone (MCH) has been shown to play a key role in driving feeding likely to be mediated, at least in part, by enhancing arousal and locomotor activity. In a recent study, we have shown increased Fos levels, in the region of the lateral hypothalamic area containing the MCH cells, in response to predatory hunting, as well as to the peak of food ingestion observed during the onset of the dark period. In the present study, combining Fos and MCH immunostaining, we first investigated how the population of MCH cells is mobilized during these experimental conditions. Our results revealed that both experimental conditions significantly increased Fos levels in the population of MCH cells. Next, using the *in situ* hybridization methodology, we determined the expression of MCH mRNA during the same experimental conditions. Both experimental conditions induced significant increase of MCH mRNA levels. Overall, the present results corroborate the idea the MCH system participates in the control of feeding behavior, and further functional studies are obviously needed to determine which response related to this behavior is mostly related to the MCH control.

Palavras-Chave: Feeding, Lateral Hypothalamic Area, Predatory Behavior, MCH

Apoio Financeiro: FAPESP 04/13849-5

E.05.004

Efecto de la serotonina en la dinámica de ingestión de la hormiga nectívora *Camponotus mus*

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Las aminas biogénicas están involucradas en el control y la modulación de diversos comportamientos en insectos. Particularmente, la serotonina (5-HT) regula comportamientos asociados con la alimentación.

La hormiga *Camponotus mus* es capaz de modular la tasa de ingestión variando la frecuencia de contracción de la bomba de succión, manteniendo constantes las propiedades de la solución y la morfometría del insecto, y dependiendo únicamente del estado interno del individuo el cual varía de acuerdo al ayuno de la colonia (*J. Comp. Physiol. A* 194:491, 2008). Nuestro objetivo fue estudiar si la serotonina interviene en la regulación del comportamiento alimentario en la hormiga *C. mus*. Para ello, dos grupos de hormigas provenientes de la misma colonia fueron sometidos a una dieta de solución de sacarosa sola (grupo CTR) o con serotonina (grupo 5HT). Luego, se registró el comportamiento y la actividad eléctrica de la bomba de succión durante la ingestión de solución de sacarosa 30% p/p. Esto se repitió en dos nidos independientes (N1 y N2). El análisis comportamental de las recolectoras en la fuente de alimento no mostró variaciones en el tiempo de ingestión (N1, $H_{(1,24)}=2.02$, $p=0.15$; N2, $H_{(1,17)}=0.02$, $p=0.88$; K-W). No obstante, el grupo 5HT mostró una tendencia a rechazar el alimento (CTRs, 100% de aceptación; 5HT, N1:92%, N2:67%) y a disminuir la carga del buche relativa (N1, 5HT:0.49±0.06 CTR:0.6±0.06; N2, 5HT:0.43±0.05 CTR:0.57±0.07; microl/mg de hormiga). Mediante el análisis de los registros eléctricos se observaron cambios en la dinámica de ingestión. Recolectoras alimentadas con 5-HT ingirieron a una velocidad significativamente menor (N1, 5HT:6.6±0.9 CTR:10±0.9; N2, 5HT:8.5±1 CTR:13.3±1; nl/seg*mg hormiga). Dicho descenso en la tasa de ingestión se debió no sólo a una disminución en las frecuencias de bombeo (N1_{Frec Ini}, 5HT:5.75±0.16 CTR:6.43±0.09; N2_{Frec}, 5HT:5.63±0.12 CTR:6.13±0.17), sino también al decremento del volumen incorporado en cada bombeo (N1, 5HT:1.4±0.1 CTR:2±0.2; N2, 5HT:1.4±0.1 CTR:2±0.1; nl/bombeo*mg hormiga). En conjunto, estos resultados indicarían un efecto depresor de la 5-HT en el comportamiento alimentario de la hormiga *Camponotus mus*.

Palavras-Chave: succión de néctar, tasa de ingestión, hormigas, Serotonina

Apoio Financeiro: CONICET PIP 5986; IBRO Travel Grant

E.05.005

Central inhibition of Sirt1 decreases food intake

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OBJECTIVES: Sirt1 is a deacetylase of the sirtuin family proteins that plays a role in many metabolic pathways. We hypothesize Sirt1 might be implicated in the regulation of food intake, a key homeostatic function that is centrally regulated by hypothalamus. In this work, we investigate the expression of Sirt1 in the hypothalamus, the variation of Sirt1 levels during the feeding cycle and the possible effect of Sirt1 central regulation on food intake. **METHODS:** to detect hypothalamic Sirt1 content, male C57Bl/6J mice hypothalamus (n=5) were submitted to immunohistochemistry and western blotting techniques during different metabolic status (fast, fed, refeed). To block selectively Sirt1 activity *in vivo* and to investigate its effects over food intake, mice (n=6-8) received an i.p. (10 up to 1000 mg/Kg) and i.c.v. (30 and 300 mcg) injection of nicotinamide, a Sirt1 inhibitor. Similarly, we used a Sirt1-specific inhibitor, EX-527 (1 and 10 mg/Kg, i.p.; 200mM, i.c.v.), to confirm if Sirt1 was the main pathway involved in the nicotinamide food intake effects. **RESULTS:** Sirt1 was expressed in the hypothalamus and its levels did not change during normal feeding cycle (light and dark) neither after fasting. A robust inhibition of food intake over the dark phase was observed after nicotinamide 333 mg/Kg i.p. compared to vehicle group (2.71 ± 0.14 g vs 3.72 ± 0.23 g, respectively, $p < 0.001$) and 30 mcg i.c.v. (0.41 ± 0.19 g vs 1.25 ± 0.42 g, respectively, $p < 0.01$). Both peripheral and central EX-527 administration inhibited food intake over the dark phase compared to vehicle group (0.82 ± 0.43 g vs 2.25 ± 0.47 g, respectively, $p = 0.01$, related to EX-527 10 mg/kg i.p. vs vehicle). **CONCLUSIONS:** we showed that Sirt1 was largely found in the hypothalamus, the levels of Sirt1 did not change during feeding cycle or after fasting. Additionally, Sirt1-specific and non-specific inhibitors decreased food intake along the dark phase when peripherally or centrally administered. Our study provides evidences that Sirt1 has a role in the regulation of feeding behavior, but the precise mechanisms of its action remain unclear.

Palavras-Chave: Sirt1, metabolism, food intake.

Apoio Financeiro: CNPq, CAPES, IBN-Net, NIH.

E.05.006

Efeitos comportamentais da injeção central de serotonina em pombos (*Columba livia*): participação de receptores 5-HT₁₋₂

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Objetivos: Em mamíferos, a atividade de neurônios serotoninérgicos controla de forma importante a expressão de comportamentos ingestivos (água, alimentos) e de sono. Neste trabalho, examinamos os efeitos comportamentais e ingestivos agudos e de longo prazo de injeções intracerebroventriculares (icv) de serotonina (5-HT) e do pré-tratamento com metergolina (MET, icv) em pombos (*Columba livia*).

Métodos: Foram utilizados pombos adultos (300-400g) portando cânulas-guia implantadas cronicamente no ventrículo lateral direito, com água e alimento *ad libitum*, e tratados com 5-HT (veículo, 50, 150 e 300 nmol, em 2 µl). Um grupo de 8 animais recebeu injeções de MET (veículo, 50, 150 nmol em 2 µl) 20 min antes da injeção de 5-HT (150 nmol ou veículo). Durante a 1ª h após a injeção foram registradas a latência para o 1º episódio, a frequência e a duração de comportamentos ingestivos (água e alimento) e não-ingestivos (auto-limpeza, locomoção, imobilidade alerta, exploratório e de sono). A quantidade de alimento e água ingeridos foi registrada 1, 2, 3 e 24 horas após os tratamentos. **Resultados:** A 5-HT provocou intensos efeitos dipsogênicos (150 e 300 nmol na 1ª h e 24ª h), hipofágicos (150 nmol na 2ª h) e hipnogênicos (150 e 300nmol, na 1ª h). O efeito hipofágico foi acompanhado por diminuição da duração e aumento da latência do primeiro episódio ingestivo. O efeito dipsogênico foi acompanhado por aumento na duração e frequência, além de diminuição da latência de beber. O efeito hipnogênico foi acompanhado pelo aumento da duração de sono e diminuição da latência para o primeiro evento. O pré-tratamento com MET bloqueou os efeitos hipofágicos e dipsogênicos da 5-HT, sem afetar o efeito hipnogênico. A MET aumentou, *per se*, a quantidade de comida e de água ingeridas, sem afetar o sono. **Conclusão:** Estes dados sugerem a participação de circuitos serotoninérgicos no controle dos comportamentos ingestivos e de sono em pombos. Os efeitos dipsogênico e hipofágico podem ser mediados por receptores 5-HT₁₋₂, e envolvem ação inibitória tônica sobre a ingestão. Já os efeitos hipnogênicos da 5-HT podem ser mediados por receptores 5-HTérgicos ainda a serem determinados.

Palavras-Chave: EFEITOS COMPORTAMENTAIS; SEROTONINA; POMBOS

Apoio Financeiro: Capes, CNPq, FAPESC

EFEITOS INGESTIVOS DA INJEÇÃO DE METERGOLINA (MET) NO NÚCLEO ARQUEADO (ARC) OU NO HIPOTÁLAMO LATERAL (HL) DE RATAS EM DIFERENTES FASES DO CICLO ESTRAL

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Objetivos: Determinar o tipo de influência serotonérgica, fásica ou tônica, exercida sobre neurônios do ARC ou do HL envolvidos no controle do consumo de alimento e verificar uma possível influência das variações hormonais ao longo do ciclo estral sobre os efeitos ingestivos induzidos pela MET. **Métodos:** Foram utilizados ratos adultos, *Wistar*, fêmeas, peso corporal entre 200 e 300 g, portando cânulas-guia implantadas 2 mm acima do ARC ou do HL. Após 5 dias de recuperação cirúrgica, os animais foram habituados a ingerir uma dieta rica em sacarose 10%, durante 1h por 3 dias consecutivos. Após esse período, os animais foram tratados com 0,2ml de MET (0, 2 ou 20nmol) no ARC ou no HL, sendo imediatamente transferidos para a caixa de registro comportamental para avaliação, por 1h, do consumo de água e de alimento. Também foram mensurados os comportamentos não ingestivos de locomoção, exploração vertical, auto-limpeza e imobilidade. Os experimentos foram realizados durante a fase de diestro ou de estro porque são fases precedidas, respectivamente, por níveis baixos ou elevados de estradiol no sangue. **Resultados:** A ingestão de alimento permaneceu inalterada após tratamento com MET no ARC em ambas as fases do ciclo estral. No entanto, ocorreu uma redução na ingestão de alimento após a injeção de ambas as doses de MET no HL durante o estro bem como o diestro. A duração da ingestão de alimento diminuiu apenas após a injeção da dose mais elevada de MET no HL de ratas em estro. A resposta dipsogênica e demais comportamentos não ingestivos mantiveram-se inalterados após a injeção de MET nos sítios hipotalâmicos investigados, exceto pela duração do comportamento de imobilidade que aumentou após a injeção da menor dose de MET no HL, na fase de estro. **Conclusão:** Esses dados sugerem que impulsos serotonérgicos inibitórios tônicos não atuam sobre os neurônios do ARC ou do HL. Além disto, parece que os hormônios esteróides ovarianos intensificam a eficiência de sinais de saciedade desencadeada pela ativação de receptores 5-HT_{1/2} encontrados no ARC ou HL antecipando a finalização da ingestão de alimento.

Palavras-Chave: hipotálamo, serotonina, ciclo estral, ingestão de alimento

Apoio Financeiro: Capes e CNPq

VARIAÇÕES HORMONAIS DURANTE A FOME E A SACIEDADE EM MULHERES OBESAS COM COMPULSÃO ALIMENTAR PERIÓDICA.

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Objetivos: O aumento da prevalência da obesidade tem estimulado várias pesquisas voltadas ao estudo dos mecanismos envolvidos na modulação do processo de fome e saciedade. Em nosso estudo avaliamos especificamente os hormônios envolvidos nestes processos em mulheres adultas (32 a 50 anos).

Métodos: O estudo experimental foi constituído de 3 grupos de mulheres: Eutrófico (GE), Obeso (GO) e Obesas com Compulsão Alimentar Periódica (CAP). Foram dosados os seguintes hormônios: insulina, peptídeo YY₃₋₃₆ (PYY₃₋₃₆), grelina, adiponectina e orexina séricos. As concentrações hormonais foram analisadas nos tempos de 1 hora antes de refeição fornecida e 15, 45, 60 e 90 minutos após. As medidas repetidas foram analisadas entre os grupos e ao longo do tempo, excluindo a variável índice de massa corporal (IMC), n=25. As refeições ingeridas foram controladas em 55% carboidratos, 15% proteínas, 30% lipídios. Os dados foram analisados com os valores médios por grupo e expressos como média ± desvio-padrão.

Resultados: As concentrações de insulina de GO (140,4±80,3) são maiores (p=0,01) que os de CAP (99,4±43,2) e GE (67,1±27,4) e ao longo do tempo (p<0,0001). A adiponectina do grupo CAP (29,8±12,7) apresentou menor concentração (p=0,01) que GO (36,1±20,0) e GE (46,7±15,6) e não foram encontradas variações ao longo do tempo (p= 0,88). As concentrações de grelina de CAP (740,8±215,6) foram menores (p=0,002) que os de GO (945,2±421,6) e GE (976,6±281,0) e, não foram encontradas variações ao longo do tempo (p=0,35). As concentrações de orexina de CAP (2,4±0,35) apresentaram menor concentração (p<0,0001) ao longo do tempo que GO (2,3±0,45) e GE (2,3±0,37), porém, não foi encontrada diferença estatística entre grupos (p=0,73). Já as concentrações de PYY do grupo CAP (78,5±32) foram menores (p=0,02) que os de GO (115,5±33,2) e GE (97,3±41,9) e, diferentes ao longo do tempo (p=0,03).

Conclusão: Os resultados apresentados mostram que as diferenças hormonais da compulsão alimentar em obesas associam-se às alterações dos hormônios sendo que o PYY em particular é um específico marcador desta patologia. **Palavras-Chave:** Peptídeo YY, Obesidade, Compulsão Alimentar Periódica, Fome e Saciedade

Apoio Financeiro: Ministério da Saúde/CNPq, NIH/Harvard, FAPERJ.

EFEITO DO JEJUM NO COMPORTAMENTO ALIMENTAR DE RATOS NORMAIS E COM SOBREPESO

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Objetivo: Diversos estudos sobre o comportamento alimentar em animais de laboratório envolvem a submissão dos espécimes a prévio jejum de 12h anterior aos registros. Neste estudo avalia-se o comportamento alimentar de animais adultos normais e com sobrepeso durante este período.

Métodos: Cinco pares de ratos Wistar adultos, controle (C) e com sobrepeso mínimo de 30% (S), foram filmados das 18h00min às 07h00min, sem jejum (CN e SN) e após jejum de 12h (CJ e SJ). A ração dos comedouros foi pesada no início e fim das gravações. Registrou-se a hora do início e fim de cada evento de alimentação. Agrupamos eventos com intervalo inferior a 30 minutos. Registrou-se o consumo de ração, a frequência e duração de refeições, e o intervalo entre elas, pelas médias de três réplicas por experimento. Usamos o teste de Mann-Whitney para a comparação entre os grupos e a Média $\pm$ Erro Padrão como estatística descritiva.

Resultados: Embora tenhamos encontrado diferença no consumo da ração entre animais controle ($p < 0,01$; CN=64,4 $\pm$ 3,44g vs SN=90,8 $\pm$ 7,56g), e submetidos ao jejum ($p < 0,01$; CJ=70,6 $\pm$ 5,39g vs SJ=92,8 $\pm$ 5,51g), não detectamos uma alteração comportamental quanto à frequência de refeições ($p > 0,4$; CN=5,1 $\pm$ 0,25 vs SN=4,7 $\pm$ 0,12 e $p > 0,22$; CJ=5,2 $\pm$ 0,06 vs SJ=5,0 $\pm$ 0,10) ou ao intervalo entre elas ($p > 0,6$; CN=91,8 $\pm$ 10,20min. vs SN=91,0 $\pm$ 4,97min. e $p > 0,5$; CJ=97,8 $\pm$ 6,77min. vs SJ=97,0 $\pm$ 12,93min.). Encontramos diferença na duração das refeições dos animais com alimentação normal ($p < 0,01$; CN=64,4 $\pm$ 3,44min. vs SN=90,8 $\pm$ 7,51min.) e submetidos ao jejum ($p > 0,01$; CJ=70,6 $\pm$ 5,39min. vs SJ=92,8 $\pm$ 5,51min.), porém, não encontramos a mesma significância quando comparamos animais submetidos e não submetidos ao jejum ($p > 0,3$; CN vs CJ e $p > 0,3$; SN vs SJ).

Conclusão: O aumento de consumo de ração observado nos animais SN e SJ em relação aos animais CN e CJ não resulta da variação da frequência, porém do aumento na duração das refeições, e o jejum de 12h não alterou de maneira significativa, os parâmetros estudados na avaliação do comportamento alimentar dos ratos Wistar adultos.

Palavras-Chave: Food Intake Behavior, Metabolic Imprinting, Obesity, Fast

Apoio Financeiro: NIH, PIBIC-UERJ, CNPq, CAPES.

E.05.010

EFEITOS DA ADMINISTRAÇÃO CRÔNICA E AGUDA DE CAFEÍNA NO COMPORTAMENTO ALIMENTAR DE RATAS.

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Objetivos: Alguns estudos demonstram que a administração de cafeína poderia exercer efeito anorético tanto em roedores como em humanos. Contudo, esses efeitos ainda são controversos na literatura. Assim, o presente estudo avaliou o efeito da administração crônica e aguda de cafeína no comportamento alimentar de ratas.

Metodos: Para o experimento 1 foram usadas 21 ratas Wistar, divididas em 2 grupos: controle (água) e cafeína 1.0 g/L (administrada na água) durante 40 dias. Após esse período os animais foram submetidos a 5 dias de habituação com Froot Loops®. No 6º dia foi realizado o teste de comportamento alimentar. Durante a habituação as ratas continuaram recebendo cafeína na água. Para o experimento 2 mais 20 ratas foram habituadas a esse alimento e foram excluídas as ratas que não consumiram nenhuma unidade de Froot Loops®. Após a habituação, 9 ratas receberam cafeína na água e o teste foi realizado 24 horas, 7 e 15 dias após o início da administração de cafeína.

Resultados: A administração crônica da cafeína diminui o consumo de Froot Loops® [$t(20) = 2,52$, $p < 0,05$]. Já a administração da cafeína, após os animais conhecerem o tipo de alimento, não influenciou o consumo do alimento doce nos tempos testados [$F(3,39) = 1,76$, $p > 0,05$]. Os resultados dos dois experimentos estão expressos nas tabelas abaixo como média $\pm$ erro padrão.

Conclusão: Essas observações sugerem que o consumo crônico de cafeína diminui o consumo de alimento doce quando os animais são habituados ao alimento mediante o consumo de cafeína, ou após um período longo de administração. Um maior tempo de consumo da droga após o animal conhecer esse tipo de alimento será necessário para que se exclua a hipótese de que a cafeína apenas diminui o consumo de alimento doce quando o animal conhece o alimento sob efeito dessa substância. Além disso mais estudos são necessários para a elucidação do mecanismo desse efeito.

Experimento1

Controle	2,04 $\pm$ 0,34
Cafeína	0,65 $\pm$ 0,42

Experimento2

Controle	2,19 $\pm$ 0,25
Cafeína 24 hs	2,82 $\pm$ 0,21
Cafeína 7 dias	2,92 $\pm$ 0,26
Cafeína 15 dias	2,55 $\pm$ 0,30

Palavras-Chave: Comportamento Alimentar, Cafeína e Fêmeas.

Apoio Financeiro: CNPQ, FAPERGS, PIBIC/CNPQ e CAPES.

ROL DEL AREA PREÓPTICA DEL HIPOTÁLAMO EN EL CICLO SUEÑO-VIGILIA

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Introducción. El área preóptica del hipotálamo (POA) es crítica en la generación y mantenimiento del sueño lento o no-REM (NREM). Dadas las características del sueño del gato, consideramos a este animal un modelo ideal para la utilización de la técnica de la proteína Fos (índice de actividad neuronal) en el estudio de los mecanismos de sueño.

Objetivos. El objetivo del presente trabajo fue estudiar la participación del POA del gato en los mecanismos del sueño y la vigilia.

Métodos. Gatos adultos implantados para estudios polisomnográficos fueron mantenidos por 60-120 minutos previo a la eutanasia en vigilia (W), NREM o sueño REM (REM). Posteriormente, se realizó la técnica de inmunohistoquímica para detectar Fos. El número y distribución de las neuronas inmunomarcadas (Fos+) fueron analizadas. En otro grupo experimental, se analizaron los efectos de microinyecciones en el núcleo mediano del POA (MnPN) de agonistas y antagonistas GABAérgicos sobre la W y el sueño. Resultados. Se detectó un aumento en el número de neuronas Fos+ en el núcleo supraquiasmático durante NREM (83.4 ± 25.6) en comparación con W (5.1 ± 1.3 , $P < 0.05$). En el MnPN el número de neuronas Fos+ fue mayor durante NREM (39.5 ± 6.1) en comparación con W (13.5 ± 1.4 , $P < 0.05$) y REM (16.1 ± 2.0 , $P < 0.05$). La inhibición del MnPN produjo un aumento significativo de la W ($79.0 \pm 1.7\%$) y una disminución del NREM ($19.7 \pm 2.0\%$) en comparación con los controles (53.1 ± 2.2 y 39.4 ± 2.1 , respectivamente; $P < 0.01$). Por otro lado, la desinhibición del MnPN promueve el NREM ($37.2 \pm 13.3\%$) en comparación con los controles (27.7 ± 14.9 ; $P < 0.05$).

Conclusiones. Demostramos que el MnPN del gato está involucrado en el control de NREM. Sugerimos que en el resto del POA, la red neuronal responsable de la generación del NREM está dispersa y entremezclada con las neuronas relacionadas con la W.

La hormona concentradora de melanina regula la generación del sueño a nivel del núcleo dorsal del rafe

Devera A.¹; LAGOS, P.¹; CHASE, M. H.²; TORTEROLO, P.¹ - ¹FACULTAD DE MEDICINA - FISIOLÓGIA; ²WEBSCIENCES INTERNATIONAL and UCLA School of Medicine - Websciences International

Objetivo. La hormona concentradora de melanina (MCH) es un neuropéptido que se ha postulado como factor hipnagógico, ya que facilita especialmente la generación de sueño REM. En este trabajo estudiamos la presencia y distribución de fibras y receptores MCHérgicos en el núcleo dorsal del rafe (NDR), así como el efecto de la aplicación local de MCH en la vigilia y el sueño del gato. **Métodos.** Encéfalos de tres gatos adultos fueron preparados para realizar técnicas inmunohistoquímicas con el fin de detectar: MCH, receptores MCHérgicos tipo 1 y serotonina. Además, 3 gatos adultos (G1, G2 y G3) previamente implantados para registro polisomnográfico fueron adaptados para ser registrados en condiciones semirestringidas. En éstos se realizaron series de microinyecciones de MCH (1 µg en 0.2 µl) y vehículo (salina 0.2 µl) en la región caudal (G1 y G3) y rostral (en G2) del NDR. Luego se analizó el porcentaje de tiempo en que los animales permanecieron en el estado de vigilia, sueño lento y sueño REM, así como la latencia a cada estado comportamental. **Resultados.** El NDR resultó ser la región más inervada por fibras MCHérgicas del tronco del gato; aposiciones entre fibras MCHérgicas y neuronas serotoninérgicas fueron observadas. Observaciones preliminares también sugieren la presencia del receptor tipo-1 para la MCH. Las microinyecciones de MCH en el NDR produjeron una disminución significativa de la latencia al sueño REM ($57.56 \pm 20.58\%$ vs. $16.84 \pm 5.7\%$; $p < 0.05$). En las microinyecciones más caudales (G1 y G3) la MCH determinó un aumento significativo del sueño REM en relación con los controles ($2.9 \pm 1.5\%$ vs. $19.53 \pm 4.2\%$; $p < 0.05$). En las más rostrales (G2) se observó un aumento significativo del sueño lento ($31.22 \pm 3.94\%$ vs $57.12 \pm 4.9\%$; $p < 0.01$). **Conclusiones.** Datos anatómicos y funcionales sugieren que las neuronas MCHérgicas regulan la vigilia y el sueño modulando la actividad de las neuronas serotoninérgicas del NDR. *Parcialmente financiado por PDT 76/36.*

Palavras-Chave: hormona concentradora de melanina (MCH), núcleo dorsal del rafe (NDR), serotonina, sueño REM

Apoio Financeiro: Parcialmente financiado por PDT 76/36.

E.06.003

O ciclo vigília-sono antes e após intervenção na rotina de idosos institucionalizados

Machado, A. V. S.¹; Andrade, M. M. M.¹ - ¹Unesp - Faculdade de Ciências e Letras de Assis - Departamento de Ciências Biológicas

Nesse estudo analisamos a organização temporal das atividades de 5 idosos institucionalizados (60-77 anos) e a ocorrência de cochilos a partir da observação da rotina diária: comparamos número, duração e horário dos cochilos e o padrão temporal da temperatura auricular antes e após modificação da rotina. Foi introduzida a atividade física moderada das 18h às 18h40min, e atividade cognitiva e conscientização sobre higiene do sono entre 15h e 17h. Houve redução da duração do sono diurno (de $151,83\text{min} \pm 90,13$ para $73,89\text{min} \pm 42,16$, teste de Wilcoxon: $T=0$ e $p=0,04$), principalmente entre 15h e 19h, e aumento da temperatura das 19h às 21h na semana pós-intervenção (de $35,93 \pm 0,32$ °C para $36,50 \pm 0,19$ °C, $T=0$ e $p=0,04$), provavelmente pela atividade mantida por mais tempo no dia. A atividade realizada na maior parte do tempo era a intelectual, seguida das atividades social, física e manual, devido a organização asilar que privilegiava as atividades dos funcionários, não havendo programações que estimulassem o aprimoramento intelectual e físico dos idosos. Após a intervenção, houve um aumento da duração da atividade intelectual no período das 11h às 15h (de $96,37\text{min} \pm 24,71$ para $108,16\text{min} \pm 27,40$, $T=0$ e $p=0,04$) possivelmente relacionado à pequena redução no tempo antes dedicado aos cochilos. A intervenção cognitiva e física resultou no aumento das atividades social ($67,04\text{min} \pm 22,34$ vs $119,92\text{min} \pm 18,83$) e física ($37,58\text{min} \pm 15,67$ vs $86,07\text{min} \pm 15,67$) no período das 15h às 19h ($T=0$ e $p=0,04$). Não foi observada modificação no padrão do sono noturno, podendo estar associado ao pouco tempo de intervenção, aos critérios de avaliação subjetiva ou à dificuldade em recordar por parte dos idosos. O estudo mostrou que através do emprego de atividades simples, foi possível reduzir a quantidade de cochilos e prolongar o tempo de atividade dos idosos devido ao relativo aumento das atividades manuais e físicas e conseqüente aumento da temperatura à noite. Propõe-se como complementação à estratégia adotada, estudar formas de incentivar e viabilizar a participação dos idosos nas atividades tendo em vista limitações de infra-estrutura e recursos humanos da instituição.

Palavras-Chave: ciclo vigília-sono, envelhecimento, intervenção não-farmacológica, ritmo de temperatura

E.06.004

El bloqueo farmacológico de las sinapsis eléctricas origina una disminución del sueño paradójico de la rata.

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Las sinapsis eléctricas están constituidas por conexiones, los cuales permiten el libre flujo de electrolitos y la sincronización eléctrica entre varios conjuntos neuronales (Nature Rev. 6:191, 2005). Dichas sinapsis eléctricas han sido descritas en células colinérgicas involucradas en la regulación del sueño paradójico (Sleep 30:1403, 2007).

Objetivo. Describir los efectos del bloqueo específico de los conexiones mediante el uso de quinina sobre los parámetros de sueño de la rata.

Métodos. Ratas Wistar adultas fueron implantadas con electrodos bipolares en la corteza sensoriomotora y con una cánula guía para microinyección en el ventrículo lateral. Siete días después de la implantación, se comparo un grupo control ($n = 8$) administrado con vehículo (dimetilsulfoxido al 5%) con un grupo experimental ($n=6$) tratado con quinina (500 nM). Dicha comparación se realizo mediante la valoración electrográfica y conductual de los diferentes estadios del sueño (vigilia, sueño de ondas lentas y sueño paradójico). Los resultados fueron evaluados mediante un análisis estadístico utilizando prueba *t student*.

Resultados. Nuestros resultados indican que la microinyección de quinina significativamente provoca una disminución del tiempo total de sueño paradójico durante las primeras 12 horas (fase de luz) (control 64.91 ± 5.71 minutos vs experimentales 34.5 ± 10.12 minutos) y un incremento en el tiempo de aparición del primer episodio de sueño paradójico (control 57.04 ± 8.93 minutos vs experimentales 310.17 ± 21.54 minutos). La posición de la cánula guía en el ventrículo lateral fue exitosamente confirmada mediante cortes coronales en el 100% de las ratas utilizadas en este estudio.

Conclusiones. Estos resultados sugieren que las sinapsis eléctricas podrían estar participando en la modulación del sueño paradójico.

Palavras-Chave: sinapsis eléctricas, ciclo sueño vigilia, sueño paradójico, quinina

Apoio Financeiro: CONACYT

GETTING THERE ON TIME: NO/cGMP SIGNAL TRANSDUCTION AND CIRCADIAN ENTRAINMENT

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The NO/GC/cGMP/PKG pathway is essential for light sincronization of the circadian clock. Phosphodiesterases (PDEs) are key regulators of intracellular cyclic nucleotide concentrations. To study the regulation of cGMP levels in the hamster SCN, we have determined by RT-PCR the presence of PDE5 in this model. In hamsters receiving specific PDE5 inhibitors (sildenafil, vardenafil or taladafil), reentrainment to a 6h phase-advance of the LD cycle took significantly shorter than controls. PDE5 inhibitors also elicited an increase in light-induced phase advances when injected 45 min before light stimulation at CT18. No differences were observed in either reentrainment rates after a delay in the LD cycle, or light-induced phase delays after a light pulse at CT14.

We have studied the role of nitric oxide (NO) in the intercellular communication within the dorsal and ventral portions of the SCN. Administration of the NO scavenger 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO) blocked photic phase advances in a dose-dependent manner and inhibited light-induced cFos-ir, without affecting phase delays. In addition, preliminary results show an inhibition in the non-parametric entrainment to 23.5 h cycle (L:1 D:22.5) in hamsters receiving a single dose of PTIO before light stimulation.

These results demonstrate that pharmacological inhibition of PDE5 affects photic entrainment, indicating a potential benefit for circadian disorders which require an increase in light signaling to the clock. These findings could serve as a basis for pharmacological treatment for optimizing circadian adaptation to environmental changes, including transmeridian flight schedules (jet-lag). Also, a role for extracellular NO in the intra-SCN communication is suggested.

Palavras-Chave: Circadian - cGMP - Nitric Oxide - PDE - Jet Lag

Apoio Financeiro: ANPCyT - CONICET - UNQ

Clock Gene Expression and Cellular Localization in The Golden Hamster Retina.

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Departamento de Ciencia y Tecnología

Objectives: The mammalian retina contains single or multiple intrinsic circadian oscillators that can be entrained by light cycles. The aim of the present work was to investigate circadian variations of two clock-gene proteins (BMAL1 and CLOCK), their localization, and the influence of the suprachiasmatic nuclei (SCN) on these rhythms. **Methods:** Male Golden hamsters were kept under a photoperiod of 14h of light-10h of darkness (LD), with free access to food and water. In some experiments, animals were kept in constant darkness (DD) for 48h. In one group of animals, bilateral thermic lesions of the SCN were performed. After surgery, SCN-lesioned animals were exposed to a light-dark cycle for 15 days, and then transferred to constant darkness for 24h. BMAL1 and CLOCK levels were assessed by *Western blotting* of nuclear and cytosolic fractions from hamster retinas excised at midday, midnight, subjective midday or subjective midnight. BMAL1 and CLOCK localization was examined by immunohistochemistry. **Results:** Nuclear levels of BMAL1 and CLOCK were significantly higher at midday (BMAL1: 19897±181, CLOCK: 514±89) than at midnight (BMAL1: 13410±205, CLOCK: 171±41) ($p<0.01$), while in the cytosolic fraction no changes were observed. A similar profile was observed in animals kept in constant darkness (BMAL1: 12h, 16833±344; 24h, 9246±1068. CLOCK: 12h, 371±64; 24h, 91±53) as well as in SCN-lesioned animals (BMAL1: 12h, 17455±371; 24h, 9571±566. CLOCK: 12h, 405±53; 24h, 113±47). In both cases, nuclear levels of these proteins were significantly higher at subjective midday than at subjective midnight ($p<0.01$). BMAL1 and CLOCK were localized in ganglion cell layer. **Conclusions:** These results indicate significant circadian variations of BMAL1 and CLOCK nuclear levels in the hamster retina, which are regulated by a local circadian clock, presumably located in retinal ganglion cells. **Palavras-Chave:** Clock genes, circadian rhythms, Clock, Bmal

Apoio Financeiro: Agencia Nacional de Promoción Científica y Tecnológica, CONICET

¿Un reloj en los gusanos? Ritmos circadianos en *Caenorhabditis elegans*

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El reloj circadiano tiene un período de aproximadamente 24 horas y su componente principal en mamíferos es el núcleo supraquiasmático (NSQ) del hipotálamo. Una importante propiedad de los ritmos circadianos es su plasticidad ante la presencia de un sincronizador externo ("zeitgeber", o dador del tiempo), siendo el sincronizador ambiental más importante el ciclo de luz-oscuridad. Estos ritmos son endógenos, es decir que se mantienen en condiciones constantes aún en ausencia de factores externos.

El nematodo *C. elegans* es capaz de sincronizar su reloj circadiano a ciclos de luz:oscuridad (LD). Sin embargo, los mecanismos involucrados en la fotorrecepción aún no han sido dilucidados. Este nematodo no posee *ocelli*, y la presencia de melanopsina u otros fotopigmentos no ha sido demostrada.

Utilizando un dispositivo capaz de registrar actividad locomotora en este organismo hemos determinado los periodos circadianos de actividad de nematodos de la cepa N2 (*wild type*), siendo $24,2 \pm 2,2$ h ($n=25$, $p<0,05$) para luz:oscuridad (LD) 12:12 y $23,1 \pm 2,4$ h ($n=36$, $p<0,05$) para oscuridad constante (DD).

Para determinar si *C. elegans* pueden sentir la luz de diferentes longitudes de onda, estudiamos la respuesta fototáctica de los mismos utilizando filtros para luz verde (520nm) y luz roja (>600nm). Nuestros resultados preliminares muestran que los nematodos tienen una preferencia hacia la luz verde. Esta observación nos llevó a realizar estudios de sincronización con luz roja y luz verde. Así se determinó que los nematodos no son capaces de sincronizar su reloj en condiciones de luz roja:oscuridad.

A fin de evaluar las moléculas involucradas en la sincronización y propagación de los ritmos biológicos en el nematodo, se estudió la actividad de la enzima aaNAT, la cual está involucrada en la síntesis de melatonina. Nuestros resultados muestran que, bajo condiciones de LD, la enzima aaNAT posee un ritmo en su expresión con un pico a ZT 12 (ANOVA, $p<0,01$). Para determinar si *C. elegans* produce melatonina se midió el contenido de la misma mediante radioinmunoensayo (RIA), encontrándose un pico a ZT 18 (ANOVA, $p=0,0257$). Para determinar si estos ritmos son endógenos, actualmente nos encontramos estudiando la actividad de la enzima aaNAT en condiciones de oscuridad constante (DD).

En resumen, nuestros resultados demuestran la capacidad fotorreceptiva en *C. elegans*, y abren las posibilidades de estudiar los mecanismos de sincronización circadiana en esta especie.

Palavras-Chave: *Caenorhabditis elegans*, circadiano, aaNAT, melatonina

Apoio Financeiro: ANPCyT / U.N.Q

E.06.008

Relação entre a aptidão cardiorrespiratória e as variáveis qualidade e eficiência do sono de pacientes com insônia crônica primária.

Passos, G. S.¹; Poyares, D.¹; Santana, M. G.¹; Guilherme, J. P. L. F.¹; Tufik, S.¹; De Mello, M. T.¹ -

¹UNIFESP - EPM - Psicobiologia

- 0,31

- 0,45

- 0,11

- 0.07

Índice Geral IQSP

0,41*

0,38*

0,23

0,12

Tempo Total de Sono IQSP

0,41 *

0,51*

0,29

- 0,01

Eficiência do Sono IQSP (%)

- 0,54 *

- 0,33

- 0,41*

- 0,20

Latência do sono IQSP (min)

NAFH

(escore total)

NAFH (Atividade de Lazer)

NAFH (Atividade Esportiva)

NAFH (Ocupacional)

Variáveis

- 0,31

- 0,45

- 0,11

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Variáveis

Introdução e Objetivo: Diversos estudos têm ressaltado os benefícios do exercício físico na qualidade do sono. Em um estudo epidemiológico, De Mello (2000) sugere que pessoas fisicamente ativas têm menos queixas de insônia que pessoas sedentárias. Nesse sentido, o objetivo deste estudo foi verificar a possível relação entre a aptidão cardiorrespiratória (VO_{2max}) e as variáveis qualidade e eficiência do sono de pacientes com insônia crônica primária.

Métodos: Após a aprovação do Comitê de Ética em Pesquisa da UNIFESP (CEP 1404/05), foram selecionados dezoito voluntários (14 mulheres e 4 homens - idade 46 ± 8) com insônia crônica primária. Para determinar a aptidão cardiorrespiratória (consumo máximo de oxigênio – VO_{2max}) foi utilizado um teste incremental de velocidade, realizado em esteira rolante (3 min de aquecimento a 4 km/h e incrementos de 0,5 km/h a cada 1 min). Para a avaliação da eficiência do sono foi utilizado um exame polissonográfico – PSG e para avaliação da qualidade subjetiva do sono foi aplicado o Índice de Qualidade de Sono de Pittsburg - IQSP. Para a análise estatística dos dados foi utilizado o teste de *Correlação de Pearson* e o nível de significância adotado foi de 5%.

Resultados: Foi observada uma correlação significativa entre o VO_{2max} e a eficiência do sono (0,52) avaliada por PSG. Além disso, o VO_{2max} também correlacionou-se significativamente com o escore do IQSP (-0,53).

Conclusão: Estes dados sugerem que quanto menor o nível de aptidão cardiorrespiratória, menor é a eficiência do sono de pacientes com insônia crônica primária. Além disso, a qualidade subjetiva do sono também parece ser menor em insônes com baixa aptidão cardiorrespiratória.

Referencias: Psychiatry Research. 28: 193, 1989; Rev Bras Med Esporte. 6: 119, 2000.

Palavras-Chave: qualidade do sono, aptidão cardiorrespiratória, insônia.

Apoio Financeiro: FAPESP, AFIP, CEPE, CNPq e CAPES.

E.06.009

Efeitos comportamentais e eletrográficos após administração sistêmica de 8-OH-DPAT em pombos (*Columba livia*)

Hoeller, A. A.¹; dos Santos, M. M.¹; Meneghelli, C.¹; Santos, T. S.¹; Dallazen, A. R.¹; Bruxel, R. R.¹; André, E. S.²; Marino-Neto, J.³ - ¹Universidade Federal de Santa Catarina - Departamento de Ciências Fisiológicas; ²Universidade Regional de Blumenau - Departamento de Fisioterapia; ³Universidade Federal de Santa Catarina - Departamento de Ciências Fisiológicas e Instituto de Engenharia Biomédica

Receptores 5-HT_{1A} têm sido associados ao controle de comportamentos de defesa, ingestão de alimentos/água e estados de sono e vigília (W) em mamíferos, mas são escassos os dados sobre o papel destes receptores em aves. Dados do nosso laboratório indicam que a injeção central de serotonina (5-HT) provoca intenso efeito hipnagênico em pombos. Este trabalho investigou os efeitos de injeções sistêmicas de 8-OH-DPAT (8-OH, agonista de receptores 5-HT_{1A}) sobre o comportamento de sono (ou “sleep-like behavior”, SLB) e seus correlatos eletrográficos (atividade EEGráfica hipocampal), registrados simultaneamente durante 1 hora após o tratamento em pombos.

Foram utilizados 22 pombos adultos, (ambos os sexos, 400-450 g), acondicionados em gaiolas individuais (ciclo de 12:12 h, a 22-24° C), com livre acesso a ração e água, tratados com 8-OH (0.06, 0.2, 0.6 e 2.0 mg/kg; G8OH) ou veículo (ac. ascórbico 5%, controles, GC), 1 ml/kg via intramuscular. Os pombos receberam todos os tratamentos, havendo intervalo mínimo de 5 dias entre os experimentos.

A administração sistêmica de 8-OH diminuiu significativamente a latência para o início de SLB em todas as doses utilizadas durante o período matutino (M), mas apenas nas 3 doses maiores durante à tarde (V). A duração de SLB aumentou (F[4,70]=19.6, p<0.0001) em todas as doses utilizadas, nos períodos M e V. As duas doses maiores de 8-OH (0.6 e 2 mg/kg) diminuíram a duração acumulada de W quando comparados ao GC. A latência para o primeiro episódio de sonolência (D) não foi afetada, mas ocorreu diminuição da duração de D nos animais G8OH em relação ao GC. Após administração da maior dose de 8-OH houve redução da latência para o primeiro episódio de sono de ondas lentas (SWS) aumentando de forma dose-dependente a duração deste estado. Não observamos episódios de sono paradoxal neste estudo.

Estes dados sugerem que: 1) ao contrário de mamíferos, a injeção sistêmica de 8-OH é capaz de diminuir o tempo de W e aumentar a duração de SWS; 2) pode existir um importante envolvimento de receptores 5-HT_{1A} em mecanismos de controle de sono em pombos; 3) os efeitos do 8-OH foram mais intensos ao final da tarde que pela manhã, sugerindo uma importante modulação circadiana na atividade de receptores 5-HT_{1A} associados ao controle do ciclo vigília-sono em pombos.

Palavras-Chave: Sono, receptores 5-HT_{1A}, hipocampo, aves

Apoio Financeiro: CNPq

E.06.010

HÁ UMA RELAÇÃO ENTRE OS ÍNDICES DE APTIDÃO CARDIORRESPIRATÓRIA E O SONO DE IDOSOS?

Santana, M. G.¹; Viana, VAR¹; Yoshida, C. H.¹; Silva, A. H. O¹; Passos, G. S.¹; Tufik, S.¹; De Mello, M. T.¹ - ¹UNIFESP - EPM - Psicobiologia

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(escore total)

NAFH (Atividade de Lazer)

NAFH (Atividade Esportiva)

NAFH (Ocupacional)

Variáveis

Objetivo: O objetivo deste estudo foi relacionar índices de aptidão cardiorrespiratória com parâmetros do sono de idosos.

Métodos: Após a aprovação do Comitê de Ética em Pesquisa da UNIFESP, foram selecionados trinta e quatro idosos do sexo masculino. A aptidão cardiorrespiratória foi determinada a partir de dois testes: a) teste incremental de cargas realizado até a exaustão voluntária (velocidade inicial de 1.6 Km/h e incrementos de 0.8 Km/h e 1% de inclinação a cada dois minutos) e b) teste de caminhada de seis minutos (TC_{6min}). Estes testes forneceram os seguintes parâmetros: o Consumo Máximo de Oxigênio (VO_{2max}), o Limiar Ventilatório (LV), o Consumo de Oxigênio Final avaliado na TC_{6min} (VO_{2 6min}) e a distância realizada no TC_{6min} (D_{6min}). Para a avaliação dos parâmetros do sono foi utilizado um exame polissonográfico (PSG). Para a análise estatística dos dados foi utilizado o teste de Correlação de Pearson e o nível de significância adotado foi de 5%.

Resultados: Foram observadas correlações significativas da latência do início do sono com o VO_{2max} (r = - 0,39), o LV (r = - 0,42), a D_{6min} (r = - 0,40) e o VO_{2 6min} (r = - 0,47). Além disso, Houve uma correlação significativa da eficiência do sono com o VO_{2max} (r = 0,37) e o VO_{2 6min} (r = 0,38). Em relação aos estágios do sono houve somente uma correlação significativa entre o estágio 3 e o VO_{2max}.

Conclusão: Estes dados sugerem que há uma relação significativa entre os índices da aptidão cardiorrespiratória e a latência do sono, o que sugere uma associação da aptidão cardiorrespiratória com o início do sono. A eficiência do sono parece estar relacionada com os índices submáximos e máximos da aptidão cardiorrespiratória. Além disso, a aptidão aeróbia parece estar relacionada com uma maior quantidade estágio 3 do sono, estágio este relacionado com a restauração corporal.

Referências: Med. Sci. Sports Exerc. 35:169, 2003; Sleep Medicine Reviews. 4: 387, 2000; Clin Sports Med. 24: 355, 2005.

Palavras-Chave: aptidão cardiorrespiratória, sono, idosos

Apoio Financeiro: FAPESP, AFIP, CEPE, CNPq e CAPES.

E.06.011

Ação da prostaglandina na hipertermia e no déficit de memória desencadeado pela privação de sono

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Objetivo - O presente trabalho teve como objetivo avaliar o envolvimento da prostaglandina (PG) no prejuízo de memória e na hipertermia desencadeada pela privação de sono (PS). **Métodos** – Ratos machos *Wistar* (3 meses de idade) foram privados de sono pelo método da plataforma múltipla por um período de 96 horas. Concomitante à privação de sono, foi administrado Celecoxibe (10mg/Kg), um inibidor seletivo da enzima ciclooxigenase-2, responsável pela síntese de prostaglandinas. A administração foi realizada diariamente, por gavagem, durante os 4 dias de privação de sono, nos quais também foi realizada a medição diária da temperatura colônica dos animais. Imediatamente após o término da privação de sono, os animais foram submetidos ao treino da esQUIVA inibitória (choque de 0,8 mA com 1s de duração), e 24 horas depois, período em que foi permitido aos animais que dormissem livremente, os mesmos foram submetidos ao teste da esQUIVA inibitória. Todos os dados foram analisados através da ANOVA de 2 vias (grupo x tempo) com medidas repetidas no fator tempo seguido pelo teste de Tukey. O nível de significância adotado foi de $p < 0,05$. Previamente ao início do experimento, com o intuito de minimizar o efeito estressante da manipulação, os animais do grupo PS foram adaptados ao método da privação de sono (2 dias - 1 hora/dia) e todos os animais foram adaptados à técnica da medição da temperatura por um período de 5 dias. **Resultados** – Os dados referentes à temperatura indicam que, embora a administração de Celecoxibe não tenha sido capaz de impedir o aumento da temperatura nas primeiras 24 horas de PS, diminuiu significativamente a hipertermia no 4º dia de PS ($37,82^{\circ}\text{C} \pm 0,22$) quando comparado à sua medida verificada na 24ª hora de PS ($38,45^{\circ}\text{C} \pm 0,13$). No entanto, não foi observada reversão do prejuízo de memória desencadeada pela PS com a administração de Celecoxibe. **Conclusão** – Com base nos dados obtidos, acredita-se que a PG esteja envolvida na hipertermia desencadeada pela privação de sono, no entanto, o mesmo não pode ser afirmado em relação ao prejuízo de memória com a dose utilizada de Celecoxibe. **Palavras-Chave:** Prostaglandinas, Ciclooxigenase-2, Celecoxibe, Privação de sono, Hipertermia, Memória.

Apoio Financeiro: AFIP, CNPq, CEPID

Ação do óxido nítrico na hipertermia e no déficit de memória desencadeado pela privação de sono

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Objetivo – O presente trabalho teve como objetivo avaliar a participação do óxido nítrico (NO) no prejuízo de memória e na hipertermia desencadeados pela privação de sono (PS). **Métodos** – Ratos machos *Wistar* (3 meses de idade) foram privados de sono pelo método da plataforma múltipla por um período de 96h. Concomitante à privação de sono, foi administrado o inibidor da síntese de NO N-nitro-L-arginina metil éster (L-NAME - 50mg/Kg) via i.p., diariamente, durante os 4 dias de privação de sono, no qual também foi realizada a medição diária da temperatura colônica dos animais. Imediatamente após o término da privação de sono, os animais foram submetidos ao treino da esQUIVA inibitória (choque de 0,8 mA com 1s de duração), e 24 horas depois, período em que foi permitido aos animais que dormissem livremente, os mesmos foram submetidos ao teste da esQUIVA inibitória. Todos os dados foram analisados através da ANOVA de 2 vias (grupo x tempo) com medidas repetidas no fator tempo seguido pelo teste de Tukey. O nível de significância adotado foi de $p < 0,05$. Previamente ao início do experimento, com o intuito de minimizar o efeito estressante da manipulação, os animais do grupo privação de sono foram adaptados ao método da privação de sono (2 dias - 1 hora/dia) e todos os animais foram adaptados à técnica da medição da temperatura por um período de 5 dias. **Resultados** – Os dados referentes à temperatura indicam que a administração de L-NAME reverteu a hipertermia a partir de 48 horas de PS ($37,63^{\circ}\text{C} \pm 0,09$) quando comparada à sua medida basal ($37,33^{\circ}\text{C} \pm 0,08$), e se manteve até o final do experimento. No entanto, a dose utilizada não foi capaz de impedir o aumento da temperatura nas primeiras 24 horas de privação ($38,04^{\circ}\text{C} \pm 0,10$). Em relação ao prejuízo de memória desencadeado pela privação de sono, não foi observada reversão pelo L-NAME. **Conclusão** – Com base nos dados obtidos, acredita-se que o NO tenha participação na hipertermia desencadeada pela privação de sono, no entanto, o mesmo não pode ser afirmado em relação ao prejuízo de memória com a dose utilizada de L-NAME. **Palavras-Chave:** Óxido Nítrico, NOS, L-NAME, Privação de Sono, Hipertermia, Memória.

Apoio Financeiro: AFIP, CNPq, CEPID.

E.06.013

Intermittent Hypoxia and Sleep Restriction: Motor, Cognitive and Neurochemical Alterations in Rats

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Objectives: The present study evaluated the effects of intermittent hypoxia (IH) and sleep restriction (SR) upon motor and cognitive function in rats. Also evaluated were catecholamine concentrations and tyrosine hydroxylase (TH) protein expression in different regions of the forebrain.

Methods: Wistar Hannover rats were submitted to IH for 4 days or 21 days (2 min room air - 2 min 10% O₂ for 1000-1600h), followed by SR for 18h (1600-1000h). Rats were randomly assigned into four experimental groups: 1) control 2) IH 3) SR and 4) IH-SR. In the inhibitory avoidance task, an additional group of rats was submitted to paradoxical sleep deprivation (PSD) for 96 consecutive hours.

Results: Results showed that SR induced an increase in motor activity without modifying catecholaminergic turnover in the frontal cortex and striatum. The increase in exploratory activity in SR rats could be the result of impaired habituation. Neither SR periods induced cognitive deficits in the inhibitory avoidance task after 5 or 21 days. However, 96h of PSD impaired acquisition/retention in rats. Exposure to IH did not affect motor and cognitive function but IH was associated with SR in increased motor activity. After 21 days, IH and IH-SR reduced striatal norepinephrine concentration although neither SR nor IH affected TH protein expression.

Conclusions: The results presented here suggest that hypoxia and sleep loss exert distinct deleterious effects upon the central nervous system.

Palavras-Chave: hypoxia, sleep deprivation, motor, memory, norepinephrine, rat

Apoio Financeiro: AFIP, FAPESP (#04/04008-7), CEPID (#98/14303-3) and CNPq

E.06.014

PREFERÊNCIA CLARO-ESCURO EM *Danio rerio* COLETADA EM DIFERENTES PERÍODOS DO DIA.

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Objetivo:

Testar a influência da hora de coleta no comportamento de preferência claro/escuro em *Danio rerio* no aquário branco/preto.

Método:

Dezesseis animais comprados em pet shops locais de sexo e idade indeterminados e mantidos por sete dias em regime de 14 horas de luz por 10 horas de escuro (início 05h) e divididos em grupos (n=4) segundo período da coleta (manhã - 6-12 h, tarde - 12-18 h, noite - 18-24 h e madrugada - 24-06 h) e submetidos a uma única sessão de teste em um aquário branco/preto (45 cm comprimento x 15 cm altura x 10 cm largura), contendo um espaço central de 5 cm, separado por duas comportas. Cada animal foi submetido à adaptação por 5 minutos na área central, após esse período as comportas são retiradas e o animal pode explorar livremente por 15 minutos. O tempo total, número de passagens de um lado para outro, e a primeira latência para a exploração foram computados como medidas relevantes.

Resultados:

Os dados obtidos indicaram diferenças no tempo de permanência entre os grupos, mantendo a preferência pelo lado escuro (Tempo total no lado escuro - Manhã: 803,00±36,01; Tarde: 523,67±23,29; Noite: 349,00±314,84; Madrugada: 794,50±29,58), alteração no número de alternâncias (Manhã: 39,00±19,66; Tarde: 75,00±33,81; Noite: 59,50±68,90; Madrugada: 37,50±15,15), e primeira latência (Manhã: 384,00±211,14; Tarde: 78,00±128,17; Noite: 279,00±415,79; Madrugada: 210,75±214,52). A análise estatística (ANOVA de uma via de Kruskal-Wallis) indicou significância no tempo total no lado escuro [$H(3)=10,787$, $p=0,013$], sendo que o pós teste (Dunn) indicou diferenças entre os grupos manhã e noite. A análise das alternâncias e da primeira latência não mostrou diferenças significativas.

Conclusão:

O teste de preferência claro escuro é sensível ao horário de coleta no seu parâmetro de permanência, sem efeitos significativos na motricidade e na latência de exploração na espécie estudada.

Palavras-Chave: *Danio rerio*, Comportamento, Ritmo Circadiano, Preferência.

Apoio Financeiro: Capes

Sleep pattern in an experimental model of osteoarthritis

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Osteoarthritis (OA) is a major healthcare burden of increasing prevalence. It has been demonstrated that the relationship between pain and sleep produces changes in sleep patterns and pain perception. However, electrophysiological studies in animal models of pain are limited. The current study examined the effect of chronic articular pain on sleep patterns in an experimental model of OA. Rats were implanted with electrodes for electrocorticogram and electromyogram recording. OA was induced in these rats by the intra-articular administration of monosodium iodoacetate into the left knee joint. Sleep recordings were monitored during light and dark periods lasting 12 h each and were evaluated at baseline (prior to administration), and on day 1, 10, 15, 20 and 28 after the iodoacetate injection or assignment to a control group. Pain threshold was also assessed by hot plate test in other groups of rats at the same time-points. Results demonstrated that OA induced a significant reduction in thermal pain threshold from day 10 until the end of experiment, as measured by paw withdrawal latency. Analysis of the recordings showed that OA rats had sleep alterations such as reduced sleep efficiency, slow wave sleep, paradoxical sleep and an increased number of microarousals during the light period compared with the baseline and the control group. These changes in sleep patterns occurred mostly between day 10 and day 28. In the dark period, sleep disturbances were also observed by decreased sleep efficiency, slow wave sleep, and sleep paradoxical although sleep was not fragmented. Our results suggest that pain associated with the rat OA model causes alterations in rat sleep architecture mainly in the light period.

Palavras-Chave: sleep, pain, osteoarthritis, knee joint, EEG, hot plate, rats.

Apoio Financeiro: FAPESP 07/56620-6, CEPID #98/14303-3 , AFIP, CNPQ

RELAÇÃO ENTRE OS PARÂMETROS DO SONO COM A TAXA METABÓLICA BASAL E A COMPOSIÇÃO CORPORAL EM HOMENS COM IDADE ACIMA DE 65 ANOS

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Objetivo: Verificar a associação entre os parâmetros do sono, a taxa metabólica basal (TMB) e a de repouso (TMR) e as variáveis da composição corporal em indivíduos adultos sedentários com idade acima de 65 anos.

Métodos: A amostra foi composta por 60 indivíduos do gênero masculino sedentários com média de idade $68 \pm 3,77$ anos. O estudo foi aprovado pelo comitê de ética da UNIFESP sob o protocolo 1107/06. Os valores da composição corporal foram obtidos por meio da pletismografia do Sistema Bod Pod®. Para a avaliação das taxas metabólicas (repouso e basal) realizada antes e após a polissonografia foi utilizado o analisador de gases Fitmate – Cosmed. A avaliação do sono ocorreu por meio do exame polissonográfico (Embla) realizado no Instituto do Sono. Para a análise estatística dos dados utilizou-se a correlação de Pearson e o nível de significância foi fixado em 5%.

Resultados: Todos os parâmetros da polissonografia foram correlacionados com as variáveis da composição corporal (massa corporal, índice de massa corporal – IMC, massa magra e massa gorda), a TMB e a TMR. Os resultados da TMB apresentaram correlações positivas e significantes com a eficiência do sono ($r=0,31$) e o sono REM ($r=0,36$) e correlações negativas com a latência de REM ($r=-0,27$), o tempo acordado ($r=-0,33$) e o estágio 1 ($r=-0,37$). O estágio 1 também se correlacionou negativamente com a TMR ($r=-0,33$).

Conclusão: Houve associação entre alguns parâmetros referentes à qualidade do sono com a TMB em indivíduos sedentários com idade acima dos 65 anos. Assim, sugere-se que as alterações no padrão e na qualidade do sono estão relacionadas à TMB do dia seguinte ao exame polissonográfico refletindo uma associação importante entre os dois fatores fisiológicos estudados.

Referências: Arch Gen Psychiat, 18:230-50, 1968; J Nutr, 122:2057-65, 1992; Nature, 437(27):1264-71, 2005.

Palavras-Chave: Sono, Taxa Metabólica Basal, Composição Corporal e Idosos

Apoio Financeiro: FAPESP, CEPE, CNPq, AFIP.

E.06.017

RITMOS DE PROLIFERACIÓN DE CÉLULAS PROGENITORAS EN LA RETINA DE PEZ CEBRA

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La retina de los peces teleósteos, a diferencia de los mamíferos, crece durante la vida adulta adicionando nuevas células. Posee un anillo tisular periférico o zona proliferativa marginal, con células progenitoras (CP) capaces de originar todos los tipos celulares retinianos. Estas células se diferencian a través del borde proliferativo y hacia el centro del tejido que representa la retina madura.

Hasta el momento, no se describieron variaciones diarias de proliferación de las CP en la retina de pez cebra y si el estímulo fótico regula este proceso.

Objetivo: determinar si existe un ritmo diario de proliferación y describir los posibles factores involucrados en la regulación del mismo.

Métodos: Se utilizaron peces cebra mantenidos con un ciclo de luz/oscuridad de 14/10 horas. Estos fueron separados en dos grupos con idénticas condiciones de temperatura y alimentación. A un grupo se le invirtió el ciclo de luz/oscuridad (O) tal que la etapa de oscuridad ocurría durante la fase de luz del otro grupo (L). Ambos grupos recibieron una inyección intravítrea en la mitad de la fase de luz (grupo L-14 h) o de oscuridad (grupo O-15 h) con 5-bromo-2'-deoxiuridina (BrdU) y luego de dos horas fueron sacrificados. Se realizó inmunofluorescencia, utilizando un anticuerpo contra BrdU. Se cuantificó el número promedio de células positivas para BrdU por margen proliferativo en cortes de 15 µm.

Resultados: Se encontraron diferencias significativas ($P < 0.0001$) entre el grupo L-14 h (4.79 ± 0.94) y el grupo O-15 h (2.66 ± 0.46). Se realizó un control (grupo O-03 h, en la mitad del día) para descartar que las diferencias observadas se debieran a condiciones no controladas entre ambos grupos. No se encontraron diferencias entre O-03 h y L-14 h. En cambio, O-03 h (4.71 ± 0.15) y O-15 h (2.66 ± 0.46) exhibieron diferencias significativas ($P < 0.0001$).

Conclusión: Estos resultados sugieren la presencia de variaciones diarias en el número de células mitóticamente activas en la retina del pez cebra.

Palavras-Chave: retina, ritmo, células progenitoras, pez cebra

Apoio Financeiro: ANPCyT, CONICET

EFEITOS DA PRIVAÇÃO DE SONO PARADOXAL NA NOCICEPÇÃO, ANALGESIA INDUZIDA POR MORFINA E NA IMUNOREATIVIDADE DA ENZIMA TIROSINA HIDROXILASE.

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Objetivos: A privação de sono paradoxal (PSP) leva a diversas alterações neuroquímicas e comportamentais, como alterações na sensibilidade à dor. Na substância cinzenta periaquedutal (PAG), neurônios GABAérgicos, serotoninérgicos e dopaminérgicos participam no controle da dor e da analgesia induzida por drogas opióides. Este trabalho tem por objetivo avaliar os efeitos da PSP e da recuperação da privação, na resposta nociceptiva a estímulos térmicos, na analgesia induzida pela morfina e na funcionalidade dopaminérgica na PAG através da imunoreatividade da enzima Tirosina Hidroxilase (TH).

Métodos: Ratos Wistar machos foram privados de sono paradoxal (PSP) por 96h, e recuperados da privação por 24h. Após esse período os animais receberam veículo ou morfina (5,0 e 10,0 mg/Kg) intraperitonealmente e foram submetidos ao teste nociceptivo da placa quente (46°C e 52°C) uma hora após. A técnica de imunohistoquímica foi utilizada para TH na região da PAG lateral para avaliação da funcionalidade do sistema dopaminérgico nessa região.

Resultados: Quando submetidos a estímulos de 52°C os animais PSP apresentaram redução na latência de retirada da pata (-53%, $p < 0,05$) e esse efeito persistiu mesmo após 24 horas de recuperação (-45%, $p < 0,01$). A administração de 5 ou 10mg/Kg de morfina não modificou a latência de retirada da pata nos animais controles, não evidenciando assim seu efeito analgésico característico. Com estímulos térmicos mais baixos (46°C) a diferença entre animais PSP e controle ainda é observada (-73%, $p < 0,05$), porém 24 horas de recuperação são suficientes para restabelecer a resposta álgica desses animais. O efeito analgésico da morfina foi observado nos grupos controle (+105%, $p < 0,05$) e recuperado (+140%, $p < 0,05$) na dose de 5mg/kg, porém não foi capaz de reverter a hiperalgesia induzida pela PSP. A contagem dos corpos celulares marcados para TH mostrou uma redução (-48%, $p < 0,05$) em animais PSP quando comparados aos controles.

Conclusões: A PSP aumenta a sensibilidade a estímulos dolorosos e altera a resposta nociceptiva induzida pela morfina e esta alteração pode estar relacionada à redução nos níveis de TH na PAG. **Palavras-Chave:** Privação de sono paradoxal, dor, Tirosina Hidroxilase

Apoio Financeiro: CAPES, CNPQ

LONGITUDINAL FOLLOW UP OF BIOLOGICAL RHYTHMS IN PRETERM BABIES

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Introduction: Rhythmicity can be seen in some variables in preterm babies, evolving from an ultradian to a circadian pattern. However there are doubts about the functional status of this system in the first months of life and how environmental factors can influence the rhythmic expression in neonates.

Objectives: This study aims to analyze the evolution of rhythmicity in sleep/wake, rest/activity, temperature and feeding cycles in preterm babies in the neonatal care unit (NCU) and their posterior adaptation to home environment until six months of age. We intend to identify hospital and home factors that can influence rhythmic expression during neonatal life.

Material e methods: Three preterm babies were evaluated in the University Hospital of USP since the second week of life. Sleep/wake, feeding and procedures data were collected continuously in the NCU through diaries. The rest/activity behavior was monitored by actigraphy and temperature was recorded by a skin thermistor. After delivering home one baby continued data registration for 15 days every month until six months of age.

All data obtained were analyzed by the Lomb Scargle method for spectral analyses of frequencies. Temperature was also analyzed by the Cosinor method.

Results: A non-24-hour circadian rhythm in temperature and activity/rest behavior is present since the 31^a week of post-conceptional age. This rhythm evolves in a waxing and waning pattern also in relation to the period of the circadian rhythm, which nears a 24-hour rhythm mainly after delivering home. Procedures, sleep/wake and feeding behavior have an ultradian pattern in the NCU. After the second month of life feeding behavior becomes arrhythmic.

Discussion and Conclusions: We identified an endogenously generated circadian rhythm since the first weeks of life. The environment in the NCU exerts a masking effect through procedures and feeding schedule. After going home the temporization system synchronizes to a 24-hour zeitgeber.

Palavras-Chave: biological rhythms, preterm, babies

Apoio Financeiro: FAPESP

Caracterização de processos moleculares rítmicos em retinas *in vitro*

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A retina de mamíferos contém um relógio circadiano endógeno que controla e regula diversas funções bioquímicas, fisiológicas e moleculares. Devido à morfologia complexa da retina o entendimento do funcionamento e localização desse relógio nas diversas camadas da retina ainda não foi definitivamente elucidado. O objetivo desse trabalho foi investigar, *in vitro*, ao longo de 72 horas, o padrão de expressão dos genes relógio (*Period1*, *Period2*, *Cryptochrome1*, *Cryptochrome2*, *Clock*, *Rev-Erba* e *Bmal1*), dos genes que controlam as funções fotorreceptoras [*Rodopsina*, *Crx* (*Cone-Rod Homeobox*), *Nrl* (*Neural Retina Leucine*) e *Recoverina*] e dos genes que são controlados pelos genes relógios os ccg - *clock controlled genes* – [*AC1* (*Adenilato ciclase1*), *Aanat* (*Aril- Alquilamina N-Acetiltransferase*) e *c-Fos*], em retinas mantidas em escuro constante (livre curso). Além disso, foram analisados aspectos fisiológicos considerados rítmicos como síntese e liberação do hormônio melatonina, assim como a expressão protéica da enzima passo limitante da via de síntese da melatonina, a AANAT. As retinas obtidas de ratos machos Wistar com 5 semanas sincronizados ao ciclo claro/escuro 12:12, foram dissecadas, colocadas em cultura e mantidas escuro constante. O material foi coletado a cada 4 horas durante 72 horas. As expressões gênicas foram determinadas por PCR em tempo real, a dosagem hormonal foi feita por Radioimunoensaio (RIA) e a expressão protéica foi determinada por *Western blot*. Nossos dados indicam que, apesar da ausência do hormônio melatonina e da enzima AANAT, os níveis do RNA mensageiro dos genes relógio variam circadianamente ao longo do período de 72 horas em cultura, corroborando com a existência desse relógio circadiano na retina, e mostrando que é possível, apesar das diversas camadas e diferentes tipos celulares, detectar ritmicidade em estudos com retinas intactas. **Palavras-Chave:** Retina, genes relógio, melatonina

Apoio Financeiro: FAPESP 04/06767-2 ; CNPq 210011/2006-8, 141071/2005-2

E.06.021

Efeitos do alcoolismo sobre a expressão dos receptores de melatonina em estruturas do sistema nervoso central (SNC)

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Objetivos: Pacientes alcoólatras freqüentemente têm distúrbios de sono, aprendizagem e memória. A melatonina participa destes processos, o que levanta a questão do impacto do alcoolismo sobre a síntese de melatonina pineal e na expressão dos seu receptores no SNC, assim como dos genes relógio no núcleo supraquiasmático (NSQ).

Métodos e Resultados: Para induzir o alcoolismo, ratos machos Wistar tiveram suas garrafas de água substituídas por outras com solução de etanol 10 % e foram mantidos por 35 dias. Em paralelo, havia animais controles ingerindo água. A melatonina pineal foi dosada por cromatografia HPLC revelando que os animais alcoólatras apresentavam uma produção noturna de melatonina menor. Experimentos de RT-PCR no NSQ nos revelaram que a expressão do receptor de melatonina MT1 é significativamente menor nos animais alcoólatras, mas não do receptor MT2. Alguns dos genes relógios analisados apresentaram uma expressão bastante modificada em decorrência do alcoolismo. No hipocampo os dois receptores tiveram sua expressão reduzida. No cerebelo a expressão de MT2 não foi alterada, contudo a de MT1 apresentou um aumento, além de um adiantamento de fase.

Conclusão: Sabe-se que interação da melatonina com o receptor MT1 no NSQ inibe disparos nesta estrutura, marcando, para o relógio, o início da noite e, portanto, período de repouso e sono para os seres humanos. A baixa de melatonina acoplada a essa reduzida expressão de MT1 no NSQ poderia ser uma das razões da insônia em pacientes alcoólatras. A dessincronização dos genes relógio, provavelmente decorrentes da menor melatonina disponível, poderia ser responsável por eventuais alterações rítmicas circadianas. As alterações da expressão dos receptores de melatonina no hipocampo e no cerebelo, associados à queda da melatonina circulante, poderiam participar das alterações de processos de aprendizagem e memória e das alterações motoras e de equilíbrio apresentados pelos pacientes alcoólatras.

Palavras-Chave: Melatonina, Alcoolismo, Núcleo supraquiasmático, cerebelo, hipocampo

Apoio Financeiro: FAPESP

EFFECTS OF CONSPECIFIC VOCALIZATIONS ON CIRCADIAN ACTIVITY RHYTHM IN MARMOSETS (*Callithrix jacchus*)

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Objective: To evaluate the effects of conspecific vocalizations on circadian activity rhythm (CAR) in the diurnal primate *Callithrix jacchus*.

Methods and Results: Motor activity of 5 adult males, in individual cages, was continuously registered. The animals were isolated in a room under constant dim light (~2 lux), with controlled temperature ($26,8 \pm 0,2^{\circ}\text{C}$) and humidity ($81,6 \pm 3,6\%$), and partial acoustic isolation. Pulses of conspecific vocalizations were applied in total darkness at 19:00 h (external time), during 1h for 30 consecutive days on attempt to synchronize the CAR. The record was composed by “phee”, “twitter”, “peep” and “tsik”, which were randomly reproduced. None animal was synchronized by these daily pulses, although oscillations in endogenous period were observed for all. This result may be due to habituation. Other possibility is the absence of social significance of the vocalizations for the animals due to random reproduction, since each vocalization has a function that could be lost by a mixture of sounds. Besides that, marmosets CAR showed oscillations in endogenous period and active phase duration influenced by vocalizations from animals outside the experimental room, which could have interfered on the phase responses to daily pulses. Furthermore, social masking and relative coordination with colony were observed.

Conclusion: Conspecific vocalizations induce social masking and relative coordination in marmosets CAR, acting as weak zeitgeber.

Palavras-Chave: circadian activity rhythm; social cues; social entrainment; marmosets; *Callithrix jacchus*; circadian rhythms

Apoio Financeiro: Coordenadoria de Aperfeiçoamento de Pessoal e do Ensino Superior (CAPES) e Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

O polimorfismo de repetição do gene *Per3* não está associado com a sonolência excessiva diurna em uma amostra da população de São Paulo.

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Em humanos o sono é regulado por dois componentes: circadiano e homeostático. Hoje se sabe que a essência da oscilação circadiana reside em um mecanismo molecular que pode ser gerado em cada célula do organismo. Os genes relógio (*Clock*, *Bmal1*, *Per1*, *Per2*, *Per3*) têm um importante papel neste mecanismo. O gene *Per3* possui um VNTR (“*Variable Number of Tandem Repeat*”) que é caracterizado por 4 ou 5 seqüências repetitivas de 54pb. Existem trabalhos mostrando que este VNTR está associado com a regulação circadiana do ciclo sono-vigília (EMBO Rep. 2: 342, 2001; Sleep. 26: 413, 2003; Sleep. 28: 29, 2005). Um trabalho publicado em 2007 (Curr Biol. 17: 613, 2007) sugere que o VNTR esteja também relacionado com a regulação homeostática do sono, pois os indivíduos homozigotos 5 repetições apresentaram uma maior pressão de sono. Assim, o objetivo do nosso trabalho foi buscar uma associação entre o VNTR do gene *Per3* e a sonolência excessiva diurna. Foram selecionados 1042 indivíduos da grande São Paulo. A escala de sonolência Epworth foi aplicada em todos os indivíduos do estudo e foram feitas coletas de sangue para extração de DNA. Dos 1042 indivíduos selecionados, 1006 indivíduos foram genotipados para o VNTR do gene *Per3* por meio de PCR (“*Polymerase Chain Reaction*”). Os indivíduos foram divididos em dois grupos: os que não tem sonolência excessiva diurna (menos de 10 pontos na escala Epworth) e aqueles que apresentam sonolência excessiva diurna (mais de 10 pontos na escala Epworth). Por meio do teste do X² nenhuma diferença estatisticamente significativa foi encontrada. Segundo o estudo publicado em 2007 (Curr. Biol. 17: 613, 2007), esperaríamos que os indivíduos homozigotos 5 repetições tivessem maior tendência a apresentar sonolência excessiva diurna. O trabalho de Viola & col. (Curr. Biol. 17: 613, 2007) foi feito em condições de luminosidade totalmente controladas. Em nosso estudo os indivíduos estão em condições ambientais normais e com sua própria rotina, portanto a condição ambiental em que vivemos é fundamental para sincronização e é bem provável que seja importante também para o processo homeostático do sono.

Palavras-Chave: Gene *Per3*, Sono, Sonolência Excessiva Diurna

Apoio Financeiro: FAPESP (Cepid) e AFIP

EFEITO DA PINEALECTOMIA NA EXPRESSÃO DOS GENES RELÓGIO NA MUSCULATURA ESTRIADA ESQUELÉTICA

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Objetivo: A glândula pineal participa da regulação circadiana de muitos processos fisiológicos através da síntese e liberação de melatonina. Sendo que os *clock genes* são necessários para a geração e regulação dos ritmos circadianos nas células individuais e por todo o organismo, o objetivo do estudo é examinar o papel da melatonina na organização temporal diária da expressão dos clock genes no músculo sóleo.

Método: Ratos Wistar machos jovens, mantidos em biotério com ciclo de luz e temperatura controlados, foram submetidos à pinealectomia (PINX). Animais controle (CTL) foram colocados sob as mesmas condições ambientais. Cerca de 30 dias após a cirurgia os animais foram sacrificados circadianamente e o músculo sóleo foi extraído para análise da expressão dos *clock genes* pelo RT-PCR. Os dados foram submetidos à análise de variância e análise ritmométrica usando-se o procedimento do ajuste co-senoidal do Cosinor para o período de 24h.

Resultados: A análise de variância bifatorial mostrou que os grupos CTL e PINX possuem ritmo circadiano para Cry1 (CTL, P=0.0003; PINX, P=0.0024), Bmal (CTL, P<0.0001; PINX, P<0.0001) e Per2 (CTL, P=0.0007; PINX, P=0.0044). Esses ritmos são representáveis pelo Cosinor. O grupo PINX do Per1 (P<0.0001) e CTL do Cry2 (P=0.0025) possuem ritmo, mas não são representáveis pelo Cosinor. CTL e PINX variaram entre si no Bmal no ZT6 (P<0.05) e no ZT21 (P<0.001); no Per1 no ZT3 (P<0.01), ZT9 (P<0.001) e ZT 21 (P<0.05); Cry2 no ZT3 (P<0.05); Per2 no ZT21 (P<0.001). A análise ritmométrica mostrou diferenças no mesor entre os grupos CTL e PINX do Cry1 (P=0.0061) e nas acrofases desses grupos no Bmal (P<0.0001) e no Per2 (P=0.0019).

Conclusões: Os resultados demonstram que Cry1, Bmal e Per2 possuem ritmos circadianos que não são afetados pela pinealectomia. A pinealectomia gerou ritmo no Per1 e aboliu no Cry2. Esse procedimento também gerou variações pontuais nas expressões dos genes Bmal (ZT6 e 21), Per1 (ZT3, 9 e 21), Cry2 (ZT3) e Per2 (ZT21).

Palavras-Chave: clock genes; melatonina, pinealectomia; sóleo

Apoio Financeiro: FAPESP (04/06767-2) e CAPES

OS EFEITOS DA RESTRIÇÃO DE SONO CRÔNICA SOBRE O DESEMPENHO DE RATOS NA TAREFA DE ESQUIVA INIBITÓRIA

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A restrição de sono de maneira crônica é uma condição observada por parte da população em geral e esta condição crônica é regida por mecanismos reguladores fisiológicos do sono (processo homeostático e circadiano). Recentemente, verificamos que ratos submetidos ao modelo experimental de restrição de sono, descrita por Machado et al. ([Behav Brain Res.](#) 160(2):356, 2005), e que tiveram a oportunidade de sono (OP) tanto na fase clara, fase escura e em fases alternadas não apresentaram prejuízo no desempenho na tarefa de esquiwa inibitória (EI). Dessa forma, o objetivo do presente trabalho foi avaliar se reduções na duração da OP no modelo de restrição crônica de sono prejudicaria o desempenho dos animais na tarefa de esquiwa inibitória (EI). Utilizamos ratos Wistar machos, com idade 3-4 meses. Foram realizados dois experimentos: Experimento 01: OP 4h (fase clara e fase escura) e 20h de restrição de sono durante 14 dias; Experimento 02: OP 3h (fase clara) e 21h de restrição de sono durante 14 dias. O método de privação de sono adotado foi o das plataformas múltiplas. No final de cada experimento os animais foram treinados e testados na tarefa EI.

Resultados:

experimento	grupo	latência treino		latência teste	
		media	erro	media	erro
1	fase clara PS	11,08	± 1,89	96,91	± 42,07
	fase clara CTL	10,5	± 1,78	173,17	± 47,16
	fase escura PS	9,25	± 1,71	24,16	± 8,88 *
	fase escura CTL	16,27	± 3,57	119,17	± 45,980
2	PS	9,58	± 1,52	48,83	± 16,26 *
	CTL	15,27	± 2,39	231,45	± 46,370

Teste ANOVA de medidas repetidas, seguida de teste a posteriori de Tukey. *diferente do grupo controle na mesma fase, p<0,05

A oportunidade de sono de 4 horas após a restrição de sono somente previne o prejuízo na EI quando ocorre durante a fase clara, ou seja, durante a fase, predominantemente, de repouso dos ratos. Entretanto, quando os animais têm a OP de 3h na mesma fase (fase clara), verificamos que o desempenho dos animais na EI está prejudicado. Desta forma, podemos concluir que a prevenção do déficit de memória na tarefa da esquiwa inibitória, no modelo de restrição crônica somente ocorre quando há coincidência entre os fatores homeostáticos (devido OP de 4 horas) e a fase circadiana de propensão ao sono (a fase clara ou de repouso), e que 3h de oportunidade de sono não é suficiente para prevenir este déficit.

Palavras-Chave: restrição cronica de sono, memoria, mecanismos reguladores do sono, privação de sono

Apoio Financeiro: AFIP, FAPESP, CNPq, CEPID

O polimorfismo C111G não está associado com a preferência diurna em uma amostra da população de São Paulo.

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A maioria dos seres vivos apresenta ritmicidade circadiana. Esta ritmicidade é controlada por osciladores biológicos endógenos. Em mamíferos, o principal oscilador endógeno circadiano é o núcleo supraquiasmático. Nele são expressos os chamados genes relógio que desempenham um importante papel no mecanismo de regulação circadiana: traduzem proteínas que estabelecem uma oscilação estável num período de 24h, sinalizando essa temporalidade para todo o organismo. Alterações e/ou polimorfismos nesses genes podem modular o ritmo circadiano em roedores e drosófilas e, em humanos, podem levar a uma expressão alterada do comportamento circadiano, fora de um padrão pré-estabelecido socialmente. Toh *et al*, (Science 291:1040, 2001) estudaram uma família que apresentava um comportamento de Fase Avançada do Sono. Esse comportamento foi associado com o polimorfismo S662G do gene *Per 2*. Xu *et al* (Cell 128:59, 2007) introduziram este polimorfismo humano em camundongos e estes apresentaram o mesmo fenótipo. Estudos recentes realizados na Inglaterra (J. Sleep Res. 14: 293,2005), revelam que o polimorfismo C111G no gene *Per2* está associado com a matutuidade (preferência diurna). Esse polimorfismo está localizado na região 5' não codificante, podendo alterar a estrutura secundária do transcrito. Em uma amostra representativa da grande São Paulo, composta por 1042 indivíduos, selecionamos 264 indivíduos extremos, sendo 80 extremamente vespertinos e 145 extremamente matutinos. Estes indivíduos foram genotipados para o polimorfismo C111G. Foi realizado o teste de X^2 e não foi encontrada associação entre o alelo e a preferência diurna. Não encontramos nenhum indivíduo 311G homozigoto. As frequências alélicas encontradas de forma geral na amostra foram 94% para o alelo C e 6% para o alelo G. Ao contrário do resultado descrito por Carpen, não replicamos a associação entre o polimorfismo C311G e a preferência diurna na população brasileira. Pode ser devido à formação étnica da população estudada ou pela diferente latitude que proporciona níveis de insolação bem maiores, modulando os cronotipos diferentemente.

Palavras-Chave: Genes Relógio, Ritmo Circadiano, Preferência Diurna, *Per 2*.

Apoio Financeiro: FAPESP E AFIP

Amitriptyline do not prevent the hyperalgesia induced by paradoxical sleep deprivation

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Objectives: Sleep deprivation may lead to behavioral alterations and it has been associated with a hyperalgesic state in human beings and animal models.

The tricyclic antidepressant amitriptyline can be used as an analgesic drug in patients and in chronic pain animal models that are not improved with classical analgesics, such as spinal nerve injury induced model of peripheral neuropathy.

The pain hypersensitivity following both paradoxical sleep deprivation (PSD) and peripheral nerve injury shares common spinal mechanisms, which involve at least the glutamate receptors and nitric oxide. In this way, we evaluated the effects of amitriptyline pretreatment in the thermal hyperalgesia observed in paradoxical sleep deprived rats.

Methods: Amitriptyline (10 and 30 mg/Kg) or saline were administered i.p. during 11 days to male Wistar rats (n = 7/group, 250 – 350 g). In the last 3 or 4 days of treatment the animals were submitted to 72 or 96 hours of PSD, respectively, or remained in home cages, being subsequently evaluated for their thermal sensitivity on a hot plate test (52°C or 46°C), 1 or 24 hours after the last drug administration.

Results: The results demonstrated that paw withdrawal latency response to 52°C was significantly lower in paradoxical sleep deprived rats than controls (-37%, p<0.05). This hyperalgesic effect was also detected in animals pretreated with 10 mg/kg (-41%, p<0.05) or 30 mg/Kg (-53%, p<0.05) of amitriptyline. When the animals were allowed to recover for 24h from sleep deprivation, the pretreatment with amitriptyline (10 mg/Kg) was not able to prevent the hyperalgesic state (-60%, p<0.05).

Even with lower thermal stimulus (46°C) and sleep deprivation period (72h), the difference between control and sleep deprived animals could still be detected (-40%, p<0.05), with no changes after an amitriptyline 10 mg/Kg pre-treatment (-43%, p<0.05).

Conclusions: Overall, these findings highlight that thermal pain hypersensitivity induced by PSD was not prevented by amitriptyline pretreatment, as observed in other models of inductive pain. **Palavras-Chave:** sleep deprivation, pain, amitriptyline, antidepressants, analgesia

Apoio Financeiro: CNPq

SLEEP DEPRIVATION: CAN LOSS OF SLEEP BE RECOVERED?

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Objectives: One of the most frequent repercussions inherent to societal development is the restriction/sleep deprivation (SD). Ever so often individuals who work double shifts or who sleep ever less as they yield to social and economic pressure get less hours of sleep than they would require during the week. These people are deprived of the last half of the night's sleep, thus losing most of REM sleep. In the majority of cases, the lack of sleep is compensated on weekends. Thus, the purpose of the present study was to evaluate the pattern of sleep recovery after total SD or REM SD.

Methods: This study was conducted at the Universidade Federal de São Paulo (UNIFESP) with the approval of the local Ethical Committee (#1676/06). Twenty two normal male subjects were randomized in three distinct protocols (7 non-SD, 8 total SD or 7 REM SD). All volunteers underwent a polysomnography recording before (baseline) and during the recovery period (3 nights). The REM SD group was suppressed of this phase for 4 nights by calling the name of the subject over an intercom each time the graph elements that characterize REM appeared in a 30 s recording interval. The total SD group was kept wake during the 2 days of sleep deprivation under the observation of at least two experimenters.

Results: After 2 nights of total SD, the latency of slow wave sleep (SWS) was decreased by 70% (29.3 ± 13.7 vs 8.4 ± 5.5 , $p < 0.01$) and the percentage of this phase was increased in 50% (20 ± 6.1 vs 31.1 ± 5.3 , $p < 0.01$). REM sleep latency was increased in 80% (66.5 ± 8.1 vs 123.5 ± 68.7 , $p < 0.05$) and the percentage of that phase was decreased in 25% (25.1 ± 4.8 vs 19.1 ± 4.6 , $p < 0.01$). After 4 nights of REM SD the percentage of REM sleep was increased in 50% (18.4 ± 5.9 vs 26.5 ± 4.5 , $p < 0.05$) and did not return to normal values even after 3 nights of sleep recovery.

Conclusions: Selective REM SD for 4 days has longer lasting effects on sleep pattern during the recovery period than the total SD condition.

Palavras-Chave: Privação de Sono, Rebote de Sono

Apoio Financeiro: AFIP, CNPq and FAPESP (#06/58276-8 to R.C.S.M. and CEPID #98/14303-3 to S.T.).

EFEITOS INVERSOS SOBRE PÂNICO E ANSIEDADE GERADOS PELA PRIVAÇÃO DE SONO PARADOXAL EM RATOS

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O objetivo do estudo foi testar os efeitos da privação de sono paradoxal (PSP) sobre as manifestações de pânico e ansiedade utilizando métodos convencionais aplicáveis em ratos de laboratório. Inicialmente, ratos adultos machos Wistar foram submetidos à PSP por 120 horas pelo método da plataforma isolada e testados quanto à exibição de lutas defensivas induzidas em grupos de cinco animais, por 60 minutos, como forma de avaliar a propensão à manifestação de pânico. Na sequência, determinou-se o nível de ansiedade dos sujeitos através do teste do labirinto em cruz elevado (LCE). No dia seguinte, com os animais ainda submetidos à PSP (total de 144 horas), as lutas defensivas foram novamente registradas e, na sequência, realizou-se o teste do campo aberto (CA). O grupo controle foi submetido aos mesmos procedimentos, exceto pela PSP. A média de brigas defensivas/indivíduo foi de zero no grupo controle, $14,8 \pm 5,7$ (EPM) no grupo com PSP de 120 horas e de $19,7 \pm 6,9$ no grupo com PSP de 144 horas. Houve diferença estatisticamente significativa entre os dois tempos de privação (Teste de Wilcoxon, $p < 0,05$). A permanência (% de tempo) nos braços abertos do LCE do grupo privado ($33,83 \pm 5,59$) foi maior (teste t; $p < 0,05$) em relação aos controles ($4,82 \pm 1,62$). O número total de entradas nos braços do LCE do grupo privado (mediana = 32, intervalo interquartis de 28 a 42) foi maior (teste de Mann-Whitney; $p < 0,05$) em comparação com os controles (mediana = 8; intervalo interquartis de 6 a 13). O número de transições no CA foi menor para o grupo controle (mediana = 39; intervalo interquartis de 32 a 42) em relação aos privados (mediana = 100; intervalo interquartis de 89 a 113). Quanto à permanência nos quadrantes centrais, o grupo controle apresentou média de $3,06\% \pm 0,97$, sendo estatisticamente inferior ($p < 0,05$) em relação aos privados ($10,67\% \pm 1,73$). Os resultados mostraram que a PSP facilita comportamentos de pânico (lutas defensivas) de forma tempo-dependente. Ratos privados mostraram-se mais ativos nos testes de LCE e CA, mas com índices de redução de ansiedade. Pode-se concluir que a PSP possui efeitos inversos sobre comportamentos de pânico e ansiedade.

Palavras-Chave: pânico, ansiedade, privação do sono paradoxal

Apoio Financeiro: FAPESP

ALTERED EXPRESSION OF CERULOPLASMIN IN CAVERNOSAL TISSUE FROM PARADOXICALLY SLEEP DEPRIVED RATS

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Paradoxical sleep deprivation (PSD) markedly promotes an increase of penile erection and influences sexual motivation in rats. Nitric oxide (NO) appears to play a role in the vasodilatation and relaxation of corpus cavernosum, which will ultimately lead to erection. It has been shown that Ceruloplasmin (Cp), a multi-copper oxidase, affects the NO homeostasis and nitrite synthesis, as well as vascular response. This study was designed to evaluate the mRNA expression of two Cp splice variants (CpGPI; membrane anchored and Cp serum) in cavernosal tissue from animals exposed to selective PSD. Adult male Wistar rats were assigned into three groups (6-7/group): home-cage (control), PSD (96h) or rebound (24h). After the experimental period, the rats were killed for blood and tissue collection. Real time RT-PCR was performed on an ABI PRISM 7500 using the SYBR® Green I chemistry. The comparative CT method was used for the relative quantification of expression with beta-actin as the housekeeping gene. The analyses demonstrated a similar increase in the expression of both Cp serum (1.7 fold) and Cp-GPI isoforms (1.8 fold) in sleep deprived rats for 96 hours. Moreover, the elevated mRNA expression returned toward control levels during the sleep recovery period in the rebound group. The results suggest that the expression of Ceruloplasmin gene in cavernosum corpus of rats is influenced by sleep deprivation. Given the well-established association between PSD and increase penile erection, we speculate that Cp might influence normal erectile function through the modulation of bioavailability of NO.

Palavras-Chave: gene expression, sleep deprivation, cavernosal tissue

Apoio Financeiro: AFIP, FAPESP (CEPID #98/14303-3 to ST and 06/58274-5 to T.A.).

ANÁLISE DOS PROCESSOS EPILEPTOGÊNICOS EM RATOS SUBMETIDOS A PRIVAÇÃO DE SONO PARADOXAL (MODELO DA PILOCARPINA).

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OBJETIVO: Avaliar os efeitos da privação de sono paradoxal (PSP) sobre a evolução de crises epilépticas límbicas induzidas por Pilocarpina (*Behav Brain Res* 9(3):315,1983).

METODO: Ratos Wistar, machos adultos (N=140), foram divididos em dois grupos: GI) Ratos PSP (N=70): Animais submetidos à PSP na plataforma única (*Pharmacol Biochem Behav* 2(4):553,1974) por 96 hs e GII) Ratos Controle (CTL) (N=70): Animais, sem privação, porém mantidos em caixas individuais por 96 hs. Após esse período, os animais de ambos os grupos (PSP e CTL) foram tratados com Pilocarpina (PILO) (360 mg/kg) para a indução de crises límbicas. Os seguintes eventos epileptogênicos foram avaliados: 1) Resistência à PILO (RP), avaliada pelo número de suplementações necessárias para desencadear crises límbicas; 2) Número de animais que entraram em *Status Epilepticus* (SE); 3) Sensibilidade à PILO após a PSP, avaliada pela ocorrência de óbitos, logo após a injeção (O/TC), no início do SE (O/SE1) e 10 dias após de SE (O/SE10). As latências para a primeira crise tônico-clônica (TC) e início do SE foram analisadas pelo teste de Mann-Whitney, com nível de significância $p < 0.05$.

RESULTADOS: As análises iniciais revelaram: 1) (RP): O número de suplementações foi menor no grupo PSP (2,85%) em relação ao grupo CTL (17,14%); 2) O número de animais que entraram em SE foi ligeiramente maior em animais PSP (60%) em relação aos CTL (50%). 3a) O/TC: O grupos PSP e CTL apresentaram equivalência quanto ao número de óbitos devido à crises TC, 42,85% e 46%, respectivamente; 3b) O/SE1: A ocorrência de óbitos durante o SE foi maior no grupo PSP (50%) em relação ao grupo CTL (32%); 3c) O/SE10: O grupo CTL apresentou maior número de óbitos (22%) no decorrer dos dias comparado ao grupo PSP (7,14%); 4) Não se observou diferença estatística entre os grupos para a latência da primeira TC ($p=0,61$) e para o início do SE ($p=0,54$).

CONCLUSÃO: Os resultados acima descritos sugerem que ratos submetidos à PSP são mais susceptíveis ao desenvolvimento do SE por PILO, assim como, mais sensíveis as crises límbicas que precedem o SE. A médio prazo, as análises sugerem um efeito protetor da PSP sobre a mortalidade de animais após o *status epilepticus*.

Palavras-Chave: privação de sono paradoxal, pilocarpina, crises límbicas, epilepsia do lobo temporal

Apoio Financeiro: CAPES, FFM, FMUSP

Does swimming lesson under morning sunlight twice a week influence the sleep-wake cycle of adolescents?

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Objective: To evaluate the effect of swimming lesson under morning sunlight on sleep-wake cycle (SWC) of adolescents.

Methods: 160 high school students were exposed to: lesson in usual classroom (Group C), lesson in swimming pool under sunlight, half of them swimming (Group EE) and the other resting (Group EL). The SWC was evaluated one week before and during the intervention (swimming lesson under morning sunlight on Monday and Wednesday between 7:45 and 8:30 AM) by sleep logs, Karolinska Sleepiness Scale KSS) and Psychomotor Vigilance Test (PVT). In the baseline the SWC was characterized by the questionnaire "Health and Sleep" and chronotype (H&O).

Results: During the intervention, the wake-up time on the weekend delayed. The time in bed showed a trend to increase for all groups on week. At weekend, only the groups EE and EL increased time in bed. Bedtime, sleep schedules irregularity and nap variables did not change (Wilcoxon, $p < 0.05$). Sleepiness showed a daily pattern characterized by lower levels at 11:30 AM and higher levels at bedtime and wake-up time on week, but lower levels on weekend ($F_{(30, 4575)} = 3.4$, $p < 0.05$). In the intervention days, sleepiness increased at 11:30 AM on groups EE and EL (t test, $p < 0.05$), possibly due to a relaxing effect of pool water. In addition, the group EE showed higher alert levels at 14:30 PM on Monday ($t_{(36)} = 1.97$, $p \leq 0.05$) and at 8:30 AM in the Wednesday ($t_{(49)} = 2.37$, $p \leq 0.05$), possibly caused by an exercise arousal effect. The reaction time assessed through the PVT did not vary between the stages (t test, $p < 0.05$). The sleep quality improved in the three groups (t test, $p < 0.05$), making impossible the evaluation of intervention effect. However, the sleep quality increased on Monday ($Z_{(47)} = 2.11$, $p < 0.05$) and Tuesday ($Z_{(46)} = 2.39$, $p < 0.05$) only on the group EE.

Conclusion: The swimming lesson under sunlight promoted effects on the sleepiness at some hours of the day, but no effects on the other SWC variables, possibly due to a large SWC irregularity on weekends. Thus, the evaluation of higher weekly frequency EF is necessary, since only two days were insufficient to promote greater effect on adolescents SWC.

Palavras-Chave: adolescent, exercise, sleep-wake cycle, sleep deprivation, school schedules.

Apoio Financeiro: CAPES e UFRN

E.06.033

Phase changes in sleep-wake cycle at adolescence: a longitudinal study of the motor activity rhythm in marmosets during puberty.

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Objective

This study intended to characterize the circadian activity rhythm and evaluate the influence of social interactions during puberty in common marmosets (*Callithrix jacchus*) under semi-natural conditions.

Methodology

Three dyads (one with two females and two with female and male) with 5-12 months old twins were kept within family group in outdoor cages (Natal, Brazil - 05°46'S 35°12'W). Motor activity was continuous registered by Actiwatch (MiniMitter Inc). Since the social factor influences the behavior of this species, behavioral observations were made twice a week in 30 minutes windows to evaluate the influence of social dynamic interactions. The puberty onset was detected by measures of fecal progesterone and estrogens in females, and androgens in males.

Results

According to the multiple regression analysis the total activity was the only parameter influenced by age ($R^2=0,18$; $\beta=-0,28$), the activity onset was the parameter more explained by the model ($R^2=0,60$), and sunrise was the factor that most influenced it ($\beta=0,69$). The activity parameters showed differences when considering subject age or time of the year (Anova, $p<0,05$). After the puberty onset, 2 dyads showed activity onset advance (t test, $p<0,05$). The total activity decreased in one dyad and increased in two (t test, $p<0,05$). This increase may be related to the birth of infants in these families. The circadian component of motor activity rhythm stabilized later in one dyad, coinciding with the puberty onset of the animals (Lomb-Scargle). The bimodal profile that is consequence of the 8 h component showed seasonal modulation. It was not possible to evaluate the agonistic behavior due to the reduced number of events. The affiliative behavior analysis detected changes in contact for one dyad ($F_{(6)}=12,68$; $p<0,05$), in made-grooming for one animal ($F_{(6)}=16,65$; $p<0,05$) and in received-grooming for two animals ($F_{(6)}=14,63$ and $F_{(6)}=15,80$; $p<0,05$).

Conclusion

Although there is evidence of puberty influence on the activity motor rhythm, the photoperiodic fluctuations that also influenced the rhythm do not yet make possible to confirm if the puberty modulates the activity rhythm in marmosets.

Palavras-Chave: circadian, sleep-wake cycle, seasonality, marmosets

Apoio Financeiro: CAPES, CTINFRA and UFRN

High-frequency oscillations in the cortical up states in vitro and in computo

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Objectives: High-frequency oscillations in cortical networks have been linked to a variety of cognitive and perceptual processes. The synchronization of neurons at these frequencies can be mediated by mechanisms in the local cortical circuit, as evidenced in *in vitro* slice preparations. However, *in vitro* experiments have hitherto necessitated of strong neuromodulators or non-physiological stimulation to generate robust synchronized activity in the beta/gamma range. Our objective here was to find if the cortical microcircuitry contains all the mechanisms for the spontaneous generation of beta and gamma oscillations in the absence of neuromodulators or externally applied synaptic agonists.

Methods: Intracellular recordings and local field potentials were recorded from ferret cortical slices in a preparation that engages in the slow oscillatory activity characteristic of slow-wave sleep (Sanchez-Vives & McCormick, Nat Neurosci 3:1027,2000). Multi-taper spectral estimates were used to compute power spectra and cross-spectra.

Results: Beta and gamma synchronization appeared during the up states of the slow oscillation. Simultaneous intracellular and local field potential recordings from slow oscillatory activity revealed synchronization between the timing of incoming synaptic events and population activity. We show that this rhythm is mechanistically similar to other pharmacologically induced gamma rhythms, as it also includes sparse, irregular firing of neurons in the population oscillation, predominant involvement of inhibitory neurons, and oscillation frequency decrease after barbiturate application. We find fast oscillations to be increased by cholinergic agonists and their peak frequency modulated by a thiopental, a barbituric known to increase time course of GABA_A. Finally, we show in a computer model how a synaptic loop between excitatory and inhibitory neurons is sufficient to explain the emergence of both the slow oscillation and the gamma rhythm in the neocortical network.

Conclusion: We therefore conclude that oscillations in the beta/gamma range that share mechanisms with activity reported *in vivo* or in pharmacologically activated *in vitro* preparations can be generated during slow oscillatory activity in the local cortical circuit without the presence of any neuromodulator.

Palavras-Chave: up states, brain rhythms, oscillations, cortical dynamics

Apoio Financeiro: Ministerio de Ciencia e Innovación de España and IDIBAPS.

Restrição de sono crônica com janela de sono na fase clara e na fase escura: efeito sobre a atenção em ratos.

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Objetivo: Avaliar em ratos se o desempenho atencional difere quando o sono é cronicamente reduzido em relação à sua duração normal e quando o sono é forçadamente deslocado para o período de atividade dos animais (fase escura).

Métodos: Foi usada uma caixa operante contendo e, um lado 5 aberturas dotadas de luzes de estímulo e sensores para detecção de resposta e uma abertura com um dispensador de recompensa no lado oposto. Ratos Wistar machos foram treinados para responder à luz apresentada (1s) ao acaso em uma das 5 aberturas para obter a recompensa. Foi medida a porcentagem de acertos (%AC), omissões (%OM) e respostas prematuras (%PRE) e perseverativas (PER) e as latências para respostas corretas (LATAC), incorretas (LATER) e para recolher a recompensa (LATREF). Atingida a estabilidade no desempenho pela %AC, os ratos foram submetidos à restrição de sono pelo método das plataformas múltiplas e testados diariamente na tarefa ao longo de 25 dias de restrição e 6 de recuperação.

Resultados: Foi feita uma por meio de análise de variância tendo como fatores os Grupos (JDIA, ratos restritos forçados a dormir na fase clara; JNOITE, ratos restritos forçados a dormir na fase escura; CON, não restritos de sono) e Dias (21 dias de restrição ou 6 de recuperação). Na restrição, não houve diferença entre os grupos na %AC (JNOITE 79,5±1,8; CON 82,1±1,5; JDIA 81,8±1,9), %OM (7,0±1,3; CON 6,6±1,1; JDIA 7,1±1), LATREF (JNOITE 1,3±0,09; CON 1,2±0,07; JDIA 1,1±0,09) e PER (JNOITE 3,7±0,8; CON 4,3±0,7; JDIA 3,9±0,9). Houve aumento nas PRE no grupo JDIA em comparação ao CON (JNOITE 9,1±1,4; CON 6,2±1,2; DIA 12,4±1,5) e redução na LATER nos dois grupos restritos em relação ao controle (JNOITE 1,4±0,09; CON 1,7±0,08; JDIA 1,3±0,1). Não houve diferença entre os grupos na recuperação, com exceção da %PRE que se manteve mais alta no grupo JDIA (JNOITE 11,0±2,1; CON 6,0±1,8; JDIA 14,8±2,3).

Conclusão: Apesar da acurácia de resposta não ter sido afetada, houve aumento da impulsividade especificamente no grupo JDIA mostrando que a restrição de sono tem efeitos diferentes se for permitido ao animal dormir no seu horário normal de repouso ou no seu horário de atividade.

Palavras-Chave: Sono; Atenção; Rato; Privação de sono; Restrição de sono

Apoio Financeiro: FAPESP; CEPID; AFIP

Influence of restricted feeding on reentrainment of the motor activity rhythm to a 6-h light-dark advance in rats

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Objectives: Evaluate the effect of a restricted feeding (RF) schedule on reentrainment of the motor activity (MA) rhythm in rats submitted to a 6-h light-dark (LD) cycle advance.

Methods: MA rhythms of 13 rats under a 12:12 LD cycle were monitored for 22 days. On day 23 the LD cycle was advanced in 6 h. On the first day of the new LD, seven animals were submitted to a RF schedule (RF group) with food available between ZT 17 and ZT 19 for 14 days. The other 6 animals were the control (Ad lib group). Actograms and serial analyses were performed using El Temps software (A. Díez Noguera, Universitat de Barcelona, 1999). The phase-advance of the ON and OFF active phase, and on the acrophase of the rhythm after LD shift were compared through t-tests for independent groups. The means of the number of days needed for a complete 6-h phase-advance of the rhythm were compared using the acrophase value, and t-test for independent groups.

Results: After the LD shift, the RF group showed a greater phase-advance in the ON activity at days 2 (2.36 ± 0.35 [S.E] h vs. 1.32 ± 0.23 h [Ad lib], $P < 0.04$) and 3 (3.36 ± 0.72 h vs. 1.34 ± 0.40 h, $P < 0.04$). For the OFF activity, the RF group presented a larger phase-advance on the first day of LD shift (6.55 ± 0.52 h vs. 2.57 ± 1.62 h, $P < 0.04$). The phase-advance in acrophase was larger for RF group on days 1-4 (5.02 ± 0.34 h vs. 2.56 ± 0.97 h; 5.75 ± 0.32 vs. 2.71 ± 0.84 h; 5.25 ± 0.22 h vs. 3.16 ± 0.69 h; 5.55 ± 0.28 h vs. 4.24 ± 0.37 h; $P < 0.03$ for all comparisons), 6 and 7 (7.59 ± 0.45 h vs. 5.29 ± 0.30 h; 6.59 ± 0.24 h vs. 4.99 ± 0.39 h; $P < 0.005$ for both), 9 (6.74 ± 0.24 h vs. 5.31 ± 0.58 h, $P < 0.04$) and 13 (7.66 ± 0.26 h vs. 6.12 ± 0.46 h, $P < 0.02$). The number of days needed for a complete 6-h phase-advance in acrophase also differed between groups (4.42 ± 0.90 vs. 7.83 ± 1.24 , RF and Ad lib, $P < 0.04$).

Conclusions: The restricted feeding schedule had an effect on reentrainment of the motor activity rhythm. This could be seen mainly on the first days after the LD shift.

References: 1-J Biol Rhythms 17:284, 2002; 2-Neuroscience 132:245, 2005.

Palavras-Chave: Motor activity rhythm, reentrainment, restricted feeding, rat

Apoio Financeiro: CNPq, FAPERN

E.06.038

Expressão de *c-fos* no núcleo pré-geniculado do sagüi (*Callithrix jacchus*) após estimulação não-fótica.

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OBJETIVOS: Utilizando técnicas imunohistoquímicas nosso objetivo foi caracterizar a atividade do núcleo pré-geniculado (NPG) do sagüi *Callithrix jacchus* após a apresentação de estímulos não-fóticos. Medindo a expressão protéica do proto-oncogene *c-fos*.

MÉTODOS: Utilizamos seis sagüis, machos, adultos jovens; do Núcleo de Primatologia da Universidade Federal do Rio Grande do Norte (autorização do IBAMA nº 1/24/92/0039-00). Mantidos, inicialmente sob ciclo claro escuro com 12 horas de claro e 12 horas de escuro, para que sincronizassem. Após esse período os animais foram submetidos à claro constante de 250 lux, para que expressassem seu período endógeno. A atividade foi monitorada por um sistema de aquisição de dados (Software Aschoff), através de um sensor infravermelho localizado no topo da gaiola do animal e conectado a uma placa de aquisição de dados, e totalizada a cada 5 minutos. Para análise da atividade, foi utilizado o software El Temps. Após a estabilização do período endógeno dos animais, foram aplicados quatro pulsos de escuro com duração de 45 minutos. Os pulsos foram planejados para atingir as horas circadianas 4, 10, 16 e 22; pontos escolhidos por ter respostas bem caracterizadas nas curvas de respostas dependentes de fase para pulsos de escuro. Trinta minutos após o fim do pulso, um sagüi foi capturado, vendado e anestesiado, perfundido transcardiacamente, teve o encéfalo removido, submetido à microtomia e processado imunohistoquimicamente contra *c-fos*. O grupo controle foi submetido aos mesmos processos, à exceção dos pulsos de escuro.

RESULTADOS: Não houve aumento na atividade locomotora no dia do pulso, comparando-o aos dias imediatamente anteriores ou posteriores, e não há incremento da atividade na hora atingida pelo pulso quando comparado com a mesma hora nos dias sem estimulação. As duas lâminas do NPG, do grupo estimulado, mostraram aumento na expressão de *c-fos* quando comparado ao grupo controle. A lâmina externa mostra uma maior ativação que a lâmina interna, tanto dentro do mesmo grupo, quanto entre os grupos.

CONCLUSÕES: O pulso de escuro modifica a expressão de *c-fos* no NPG, entretanto não há correlação com a atividade locomotora. **Palavras-Chave:** NPG, sagüi, estímulo não-fótico

Apoio Financeiro: CNPq

E.06.039

Comparação entre necessidade e tempo de sono relatado nos diferentes turnos de trabalho

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Objetivos: Comparar a necessidade subjetiva de sono do trabalhador de turno com o tempo subjetivo de sono referido, nos diferentes turnos (manhã, tarde e noite) e nas folgas.

Métodos: Participaram deste estudo 309 trabalhadores de turnos que realizavam escalas em três turnos – tarde (15:00 às 23:00hs), manhã (7:00 às 15:00 hs) e noite (23:00 às 07:00), geralmente 06 dias no turno, seguido de 03 ou 06 dias de folga. Os trabalhadores responderam o Questionário de UNIFESP do Sono (Pires et al, 2007). Foram comparados os dados sobre necessidade de sono e o tempo relatado de sono durante os turnos da manhã, tarde, noite e, também nos dias de folga dos trabalhadores em turno das duas usinas nucleares brasileiras. A média de idade 38.6 anos (DP=8,7).

Resultados: A média ($\pm$ DP) da necessidade de sono encontrada foi de: 7.3 ± 1.2 , já o tempo de sono relatada pelos trabalhadores nas folgas e após o turno da manhã, tarde e noite foram respectivamente: $8,2 \pm 1,3$ h; $6,5 \pm 1,3$ h; $7,3 \pm 1,3$ h e $5,6 \pm 1,6$ h. Foram encontradas diferenças significativas entre a necessidade de sono e os tempos referidos de dormir nas folgas, no turno da manhã e da noite ($p < 0.001$). O teste estatístico adotado foi ANOVA de medidas repetidas e o nível de significância adotado foi de 5%.

Conclusões: A necessidade e o tempo subjetivo de sono referido dos trabalhadores foram iguais apenas quando os trabalhadores estavam no turno da tarde. Esse resultado é interessante uma vez que nem mesmo nos dias de folga, em que eles de fato, relatam um tempo maior de sono, esses trabalhadores não conseguem ter um sono reparador.

Palavras-Chave: Trabalhadores de turnos, Sono, queixas de sono

Apoio Financeiro: AFIP, FAPESP/CEPID (98/14303-3), CEMSA, CNPQ, CAPES.

E.06.040

MODELO IN VITRO PARA EL ESTUDIO DEL PAPEL DE LA UNIÓN MESOPONTINA EN LA GENERACIÓN DEL SUEÑO DE MOVIMIENTOS OCULARES RÁPIDOS (REM).

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La unión mesopontina contiene los núcleos tegmentales latero-dorsal y pedúnculo-pontino (LDT-PPT) y el núcleo pontis oralis (NPO), consideradas estructuras centrales para la generación del sueño de movimientos oculares rápidos (REM). La aplicación de agonistas colinérgicos en el NPO del gato induce un estado comportamental indistinguible del REM, en tanto que agonistas GABAérgicos lo suprimen. Pese al gran caudal de información que existe acerca de los procesos involucrados en la generación del REM, los mecanismos celulares y sinápticos responsables no han sido aún dilucidados. Para profundizar el conocimiento de estos mecanismos, hemos desarrollado un modelo de REM *in vitro*. Éste consiste en una rodaja de tronco encefálico de rata que incluye al LDT-PPT y NPO, así como al núcleo motor del trigémino (MV) cuyas motoneuronas son típicamente inhibidas durante el REM.

En nuestra rodaja, se realizaron dos series experimentales: marcaje retrógrado mediante inyección de neurobiotina en el NPO, y registro intracelular de neuronas del MV ante la aplicación en el NPO, por presión (20 psi, 100 ms), del agonista colinérgico carbacol (CCh; 10 mM) o el agonista GABAérgico muscimol (10 μ M).

El análisis inmunohistoquímico de las rodajas marcadas, reveló que células colinérgicas y no colinérgicas del LDT proyectan al NPO. Las aplicaciones de CCh o muscimol modificaron transitoriamente las propiedades de las neuronas del MV (latencia $\sim$ 2 min, efecto máximo $\sim$ 10 min, resolución $\sim$ 20 min). CCh provocó la reducción de la descarga provocada por pulsos de corriente ($48.9 \pm 27.2\%$) y de la resistencia de entrada (R_{in} ; $9.7 \pm 4.8\%$) así como el bloqueo de la invasión somática de la espiga antidrómica. Muscimol, en cambio, provocó incrementos de la descarga provocada ($159.0 \pm 58.0\%$) y de la R_{in} ($12.4 \pm 3.5\%$).

Los datos indican que los circuitos esenciales para el control del REM se encuentran operativos en la rodaja mesopontina. Por tanto, sugerimos que constituye un modelo óptimo para el estudio de los mecanismos celulares y sinápticos responsables del REM.

Palavras-Chave: REM, in vitro, NPO

Apoio Financeiro: PEDECIBA, PDT S/C/IF/54/090, USPHS grant MH59284

IS THERE DISSOCIATION OF MOTOR ACTIVITY RHYTHM IN MARMOSETS (*Callithrix jacchus*) UNDER T 21?

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Objective: To evaluate the circadian motor activity rhythm (CAR) of the diurnal primate *Callithrix jacchus* under a symmetric light-dark (LD) cycle with a period of 21 h.

Methods: Motor activity of 6 adult females, in individual cages, was continuously registered. The animals were isolated in a room with controlled temperature and humidity, and sound attenuated. The light intensity during light phase was $146,5 \pm 53,3$ lx whereas dark phase consisted in total darkness. They were maintained under a symmetric LD cycle with a period of 24h for 14 days (T24); then they were exposed to a LD cycle with period of 21 h during 30 consecutive days (T21).

Results: During T24 was seen only one component in CAR which had the same period of the external LD cycle. The Percentage of Variance (VSP) of this component was $45,0 \pm 8,7\%$ and the potency was $528,2 \pm 158,6$. The active phase in relation to total distribution of activity was $98,7 \pm 1,1\%$ during the light phase. During T21 was seen the presence of two simultaneous components in the CAR for all animals. One had the same period of LD cycle of 21 h, named synchronized component (SC) and the other had a period of $23,82 \pm 0,1$ h, named endogenous component (EC). The VSP of SC was $8,5 \pm 1,2\%$ whereas the VSP of EC was $17,0 \pm 5,9$. The potency of SC was $217,4 \pm 69,5$ whereas the potency of EC was $636,2 \pm 281,8$. Together these results indicate that EC was stronger than SC. Despite that, most of activity happened at the light phase ($69,9 \pm 3,0\%$) in relation to total distribution of activity.

Conclusion: The circadian motor activity rhythm of marmosets dissociate in two components under a symmetric LD cycle with a period of 21 h, being one synchronized to the external LD cycle and the other in free running.

Núcleo de Primatologia da UFRN – Registro no IBAMA no. 1/24/92/0039-0.

Palavras-Chave: Dissociation; circadian activity rhythm, circadian rhythms; entrainment; marmosets.

Apoio Financeiro: CAPES, CNPq e FAPERN.

EFFECT OF SUNLIGHT EXPOSURE IN THE MORNING ON THE SLEEP-WAKE CYCLE AND DAYTIME SLEEPINESS OF ADOLESCENTS IN THE RETURN TO CLASSES AFTER MID-YEAR VACATION

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Objectives: To evaluate the effect of sunlight exposure in the morning on the sleep-wake cycle (SWC) and daytime sleepiness of adolescents in the return to the classes after mid-year vacation. **Methods:** 83 high school volunteer students aged 15 ± 0.5 years old were enrolled. They were organized in two groups: the sunlight intervention (n = 38), that attended the first class (7:15-8:00 h) at shadow in an outdoor environment exposed to sunlight (11.500 ± 4.800 lux), and the control (n = 45), that stayed in usual classroom (256 ± 96 lux). The study had two stages: 1^a) Students characterization about chronotype, sleep knowledge and habits; 2^a) SWC and somnolence evaluation for two consecutive weeks after mid-year vacation. The intervention was developed on the second week (baseline: first week). The SWC was assessed by sleep logs and the daytime sleepiness by the Karolinska Sleepiness Scale (KSS), on 5 times: wake-up time, 8am, 11am, 2pm and bedtime. Subgroups used actimeter during the two weeks and underwent a psychomotor vigilance test (PVT) on weekdays at 8am and 11am. **Results:** At the second week, the bedtime of intervention group was earlier than baseline (Baseline: 23h48min, Intervention: 23h25min; Wilcoxon, $p = 0.0001$) and sleep duration increased in 16 minutes (Wilcoxon, $p = 0.0005$) on weekdays. The sleepiness levels, measured by the KSS showed no difference between the two weeks for both groups ($F_{5,1325} = 0.61$; $p = 0.68$), but the PVT detected a decrease in reaction time at 11am in the intervention group (Wilcoxon, $p = 0.0008$). Naps frequency diminished on weekdays from 46% to 31% on the intervention group ($X_2 = 10.31$; $p = 0.001$). Bedtime irregularity did not change (Wilcoxon, $p > 0.05$), but wake-up time irregularity decreased for the control (Wilcoxon, $p = 0.001$) and intervention group (Wilcoxon, $p = 0.0005$). **Conclusion:** the sunlight morning exposure can be used by school as a procedure to help students to adapt their sleep-wake cycle on the return from vacation to school.

Palavras-Chave: adolescent, sleep, sunlight

Apoio Financeiro: UFRN, CAPES, FINEP e Ctinfra

LUCID DREAM: SLEEP ELECTROENCEPHALOGRAPHIC FEATURES AND BEHAVIORAL INDUCTION METHODS

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Introduction: Lucid dream (LD) is a kind of dream in which the dreamer has awareness of being in a dream as it unfolds. LD is identified by pre-arranged ocular movements (PCOM) performed during REM sleep (REMS) and recorded by polysomnography (Sleep, 12:374, 1989; Sleep Med., 7:462, 2006). Because LD is not easily achieved, some techniques as suggestion, sleep deprivation and rebound (which decreases REMS latency and increases REMS duration) and stimulus incorporation (which penetrate into the dream and act as a cue for the dreamer to know that he/she is dreaming) increase the chance to have a LD. **Objective:** Characterize the LD at the electroencephalographic level and quantitatively compare the efficacy of these techniques to induce LD. **Material and methods:** Subjects were assigned to the following groups according to the technique used to induce LD: Suggestion (n=3), Sleep deprivation for one night and sleep rebound in the next day (n=3), Red light stimulus during REMS (n=3). A control group with paired age and sex (n=9) consisted of subjects not exposed to any technique to induce LD. Sleep recording was performed with 22 cortical, 2 ocular and 2 muscle channels, and also audio / video recording. **Results:** One sleep deprived subject reported two LD during the sleep rebound period; however, an analysis of the electroencephalogram showed that the first LD report was actually a hypnopompic hallucination, because PCOM were performed immediately after a brief awakening; the second putative LD was validated because it occurred well within a REMS episode. One subject performed PCOM after receiving red light pulses during REMS; this subject reported incorporation of the stimulus into the dream, which helped him to become lucid during the dream. In the same subject, red light pulses applied during slow-wave sleep (control) failed to produce any psychological or behavioral changes. One subject that received suggestion performed PCOM during REMS. Controls unexposed to any LD induction technique did not report LDs. **Conclusion:** a) PCOM registration during REMS was an efficient tool to identify LD; b) all techniques utilized were able to induce LD with a 33% success rate.

Palavras-Chave: REM sleep, dream, lucid dream, EEG

Apoio Financeiro: AASDAP, PEW Lat. Am. Fellow. Biomed. Prog., CNPq, FINEP, MCT

CARACTERIZAÇÃO ACÚSTICA DO RONCO NOS DIFERENTES ÍNDICES DE APNÉIA E HIPOPNÉIA OBSTRUTIVA DO SONO

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Introdução: Os métodos utilizados para detecção do ronco durante o registro de polissonografia são grosseiros e com baixa especificidade, dificultando assim, validá-los como métodos complementares no diagnóstico de apnéia/hipopnéia obstrutiva do sono (AHOS) (1). **Objetivo:** Caracterizar o ronco através de medidas acústicas e correlacioná-lo com a gravidade dos eventos respiratórios da polissonografia em pacientes com AHOS.

Métodos: Participaram da pesquisa 13 indivíduos, sexo masculino, entre 33 e 64 anos com idade média de 47 ± 11 , índice de massa corpórea de $26,19 \text{ Kg/m}^2 \pm 4,94$ e a média da circunferência cervical foi de $44 \text{ cm} \pm 2,7 \text{ DP}$ com índice de apnéia e hipopnéia (IAH) maior 5 eventos por hora previamente selecionados. O estudo foi realizado a partir do exame de polissonografia completo de noite inteira (Sonolab-Meditron 16 canais) associado a análise espectrográfica do ronco (GRAM 5.1.7), de onde foram retirados recortes de roncos com apnéia/hipopnéia, nos quais verificamos as principais características temporais da onda sonora (2). **Resultados:** Pacientes com Índice de apnéia/hipopnéia de leve a moderado mostraram roncos periódicos regulares, com presença de padrão harmônico, sem variabilidade de frequência, maior concentração de energia nas frequências grave e média e produzidos na inspiração (86%). Pacientes com Índices de apnéia/hipopnéia grave, os roncos foram aperiódicos e em salva. Entre o término de uma salva de roncos e o início da outra os intervalos foram irregulares. Após apnéia/hipopnéia, os pacientes apresentaram ronco brusco, seguido por uma respiração ruidosa. Os roncos foram inspiratórios, não apresentaram padrão harmônico com variabilidade de frequência. Em alguns indivíduos (57%) houve maior concentração de energia entre as frequências média e aguda. **Conclusão:** Há relação entre a acústica e a gravidade da apnéia/hipopnéia obstrutiva do sono detectado na polissonografia.

1- Chest 115: 762, 1999 2 - Am. Rev Respir Dis 147: 635, 1993

Palavras-Chave: apnéia, acústica, ronco, polissonografia

MOXIDECTIN'S INTERFERENCE ON SEXUAL BEHAVIOR OF MALE RATS: IMPAIRS MOTOR PERFORMANCE?

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Objective: The effects of an antiparasitic drug used in Veterinary Clinics, Moxidectin (MOX), on sexual and motor behavior were studied because in mammals its mechanism of action involves GABA, a neurotransmitter with an important role on these behaviors.

Methods: In these experiments were utilized adult male Wistar rats distributed in 3 experimental groups (EG) which received (SC) 0.2; 2.0, or 20 mg/kg of MOX. **1)** Motor coordination was evaluated through a wooden beam; at the end of the training period the scores of each rat were counted (base line) and, after that, the rats of each group received MOX and were analysed 24h and 72h later. **2)** Sexual Behavior - the rats were distributed in 3 EG and 1 control group (CG) (vehicle-0.1 ml/kg), 24h before behavior's observation; and 01 EG - 0.2 mg/kg, observed after 72 h. **3)** Penile Erection (PE= sexual performance)- 3 EG and 1 CG being treated like above; after 72h the rats received apomorphine (80 mg/kg) just before the observation for 1 hour.

Results:1)Motor Coordination— the Dunn test showed a significant increase in medians of the scores after 24h (2.0 and 20 mg/kg = 12.5; 10.0 respectively) and 72h (0.2; 2.0; 20mg/kg = 9.5; 15.0; 13.0) of the drug administration when compared to that obtained 24h before the injections (5.0; 6.5; 5.0 respectively); **2) Sexual Behavior**- were observed increased latencies to first mount (2.8 ± 0.6) and intromissions (3.0 ± 0.6) as well as in the inter-intromission interval (1.0 ± 0.1) after 72h of 0.2 mg/kg MOX-treated animals in relation to CG (1.1 ± 0.2 / 1.4 ± 0.2 / 0.6 ± 0.1 ; respectively), and the total mounts in 40 min was reduced, both at 24h (52.7 ± 6.3) and 72h (51.9 ± 4.2) post-treatments (CG = 74.8 ± 6.9); **3) PE** - EG animals showed a decreased PE frequency (1.2 ± 0.7 ; 1.6 ± 1.4 ; 2.0 ± 1.1) in relation to CG (3.3 ± 0.9); $p < 0.05$.

Conclusion: After 24h and 72h different doses of MOX tested here were able to impair motor coordination of male rats, but only 0.2 mg/kg dose harmed sexual behavior. The three doses of MOX also weakened sexual performance after 72h. These results suggest that MOX impairs sexual behavior of male rats by interference in other than motor control.

Palavras-Chave: moxidectin; motor coordination; wooden beam; sexual behavior, penile erection; rats.

Apoio Financeiro: CNPq e CAPES

LA ACTIVIDAD ELECTRICA CORTICO-AMIGDALINA ES SENSIBLE A LA ESTIMULACION VAGINOCERVICAL ARTIFICIAL. INFLUENCIA DEL CICLO ESTRAL.

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LA ACTIVIDAD ELECTRICA CORTICO-AMIGDALINA ES SENSIBLE A LA ESTIMULACION VAGINOCERVICAL ARTIFICIAL. INFLUENCIA DEL CICLO ESTRAL. Guevara, M.A., Reynoso-Orozco, O.,* Martínez-Pelayo, M., Hernández-González, M. Instituto de Neurociencias, Universidad de Guadalajara. Guadalajara, Jalisco, México.

La estimulación vagino-cervical (EVC) provocada durante la cópula, así como mediante estimulación artificial, induce cambios neuroendocrinos, potencia el despliegue de conductas reproductivas y afecta la actividad electroencefalográfica (EEG), unitaria y del marcaje con c-Fos en áreas hipotálamicas y mesencefálicas de ratas hembra. La amígdala juega un papel importante en la secreción de gonadotrofinas, conducta sexual y secreción de prolactina. **Objetivo:** Determinar si el ciclo estral afecta la funcionalidad de la corteza prefrontal (CPF) y amígdala (Amy) durante la EVC artificial de ratas hembra. **Método:** Ratas hembra Wistar de 250 gr, se implantaron bilateralmente en la CPF y Amy. Se registró el EEG de ambas estructuras durante el Proestro-estro (P-E) y Diestro (D) durante dos condiciones, el estado basal de vigilia-quieto y la EVC artificial de ratas. Para la EVC se introdujo el émbolo de una jeringa de 1ml en el orificio vaginal y se aplicó una fuerza de 300 gr. **Resultados:** Tanto en la CPF como en la amígdala, la PR de la banda de 4-7 Hz aumentó durante el P-E en estado basal respecto al D con y sin EVC, en tanto que las frecuencias rápidas (8-12 y 13-21 Hz) mostraron una mayor PR durante el D de ratas en vigilia quieto y la EVC de ratas en P-E y D. La correlación de la banda de 8-12 Hz entre las cortezas fue mayor durante la EVC de ratas en diestro respecto a aquella de las ratas en P-E y la correlación de la banda de 13-21 Hz entre la CPF y Amy derecha fue mayor solo durante la EVC de ambas fases estales. **Conclusiones:** Tanto la CPF como la amígdala presentaron una activación general asociada a la EVC que no fue afectada por el ciclo estral. El aumento de la correlación cortical durante la EVC de ratas en diestro y la mayor activación y acoplamiento funcional entre la CPF y la Amy durante la EVC de ratas en P-E y D podrían relacionarse con el procesamiento sensorial y/o procesos motivacionales implicados.

Palavras-Chave: estimulación vaginocervical, ciclo estral, EEG

E.07.003

Efecto de la estimulación vaginocervical in-cópula sobre la actividad eléctrica amigdalina de ratas

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Efecto de la estimulación vaginocervical in-cópula sobre la actividad eléctrica amigdalina de ratas
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La estimulación vaginocervical (EVC) que recibe la hembra durante la interacción copulatoria constituye una de las fuentes más importantes de estimulación sensorial que inducen cambios endocrinos, neurales y conductuales a corto y largo plazo. La amígdala medial (AmM) presenta una mayor activación neural durante, y hasta por varios minutos y días después de la estimulación copulatoria, participando directamente sobre la cuantificación temporal y el procesamiento de las señales aferentes recibidas durante la conducta sexual. **Objetivo:** Evaluar si la actividad electroencefalográfica (EEG) de la AmM de la rata muestra cambios en respuesta a diferentes cantidades de EVC copulatoria. **Método:** Catorce ratas hembra Wistar, vírgenes, de 250-300 gr, con un ciclo estral regular de 4 días, fueron implantadas bilateralmente en la AmM y se registró el EEG después de recibir 5 Intromisiones (I) o 25 I respectivamente. El EEG post-EVC se dividió en : 15 min iniciales; 20-40 min intermedios y 45-60 min finales. **Resultados:** El grupo de 5 I manifestó durante toda la interacción copulatoria conductas proceptivas, mientras que el grupo de 25 I presentó conductas de rechazo desde la I 10. El grupo de 5 I presentó una mayor PR de las tres bandas de frecuencia sólo durante los periodos intermedios y finales post-EVC, en tanto que el de 25 I, presentó estos incrementos sólo durante el periodo final (45-60 min). Conclusiones: La mayor PR de las tres bandas de frecuencia desde los 20 min post-EVC podrían asociarse al estado apetitivo, en tanto que en el grupo de 25 I podría reflejar el tiempo mínimo requerido para que en las ratas que recibieron EVC excesiva asociada con evidentes conductas de rechazo hacia el macho, comience a presentarse un estado relajado y de menor sobreexcitación conductual. Estos resultados muestran que la funcionalidad EEG amigdalina de las ratas en P-E es sensible a la cantidad de estimulación vaginocervical copulatoria.

Palabras-Chave: EEG, cópula, rats, estimulación vaginocervical

E.07.004

Secreção de prolactina e expressão de FOS em neurônios GnRH do prosencéfalo de ratas adrenalectomizadas e intactas em proestro sob estímulo olfatório de feromônios do macho.

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Objetivo: Avaliar a concentração plasmática de prolactina (PRL) e a expressão de FOS em neurônios produtores de hormônio liberador de gonadotrofinas (GnRH), em ratas adrenalectomizadas (*ADX*) e *sham* expostas à urina do rato na noite do proestro. **Métodos:** Ratas *ADX* e *sham* foram expostas por 1h durante noite de proestro à urina de ratos ou à solução NaCl 0,9% (controles). A seguir foram anestesiadas para coleta de sangue (para dosagem de PRL plasmática por radioimunoensaio) e perfusão para retirada do cérebro. Imunofluorescências para GnRH e FOS foram realizadas em seções do cérebro contendo bulbo olfatório principal (MOB), bulbo olfatório acessório (AOB), septo lateral (LS) e septo medial (MS). Para análise estatística, foi utilizada ANOVA de duas vias, seguida do teste Bonferroni. A significância aceita foi $P < 0.05$. **Resultados:** O estímulo olfatório pela exposição à urina do macho induziu aumento de: secreção de PRL no grupo *sham* ($336,5 \pm 34,21$ ng/mL) mas não no *ADX*; expressão de FOS no AOB ($8,93 \pm 1,06$; $2,75 \pm 0,69$ respectivamente), no SL ($4,39 \pm 0,87$; $3,16 \pm 0,96$ respectivamente) e no MS ($4,75 \pm 0,55$; $5,52 \pm 0,57$ respectivamente) dos grupos *sham* e *ADX*; número de neurônios com expressão de GnRH no AOB dos grupos *sham* ($18,92 \pm 1,54$) e *ADX* ($17,88 \pm 1,55$). Por outro lado, houve redução no número de células com expressão de GnRH ($4,3 \pm 1,5$; $5,5 \pm 1,7$ respectivamente) no MS dos grupos *ADX*, porém o estímulo olfatório parece reverter parcialmente esse efeito. A co-expressão FOS e GnRH ocorreu após estímulo olfatório apenas no SL, sem diferença significativa entre os grupos *sham* e *ADX*. **Conclusões:** Feromônios da urina do macho induzem aumento da secreção de PRL, de atividade neuronal em regiões prosencefálicas e do número de neurônios com expressão de GnRH em fêmeas, na noite do proestro. Os aumentos da secreção de PRL e da expressão de GnRH no MS em resposta ao estímulo olfatório são dependentes do eixo hipotálamo-hipófise-adrenal.

Palavras-Chave: ratas, proestro, feromônios, GnRH, FOS, prolactina, prosencéfalo

Apoio Financeiro: CNPq, FAPESP

E.07.005

EFFERENT CONNECTIONS OF THE ANTEROVENTRAL DIVISION OF THE MEDIAL AMYGDALOID NUCLEUS IN THE RAT

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Objectives:

The medial amygdaloid nucleus (Me) is crucially involved in reproductive and agonistic behaviors. The projections from its different parts have been described in detail, excepted those from the tiny anteroventral part (MeAV), which reportedly provides massive inputs to the ventromedial hypothalamic nucleus. Although the posteroventral part of Me (MePV) also heavily innervates this nucleus, Fos observations suggest that MeAV and MePV are functionally different. In an investigation on the amygdala connectivity, an injection of *Phaseolus vulgaris* leucoagglutinin (PHA-L), virtually confined to MeAV, was incidentally obtained. The analysis of this case will be here reported.

Methods:

6 adult female Wistar albino rats received iontophoretic injections of PHA-L in Me. After 10-14 days, they were perfused, sectioned coronally at 40 µm and processed for PHA-L immunohistochemistry by using the avidin-biotin-peroxidase technique. An adjacent series of sections was stained with thionin. The anterograde labeling, analysed under bright- and darkfield illumination, was mapped by the aid of AutoCad drawing program.

Results:

MeAV projects more heavily to itself than to other Me parts. Its efferents run in the stria terminalis and, to a lesser degree, in the ansa peduncularis to robustly innervate the strial extension, interfascicular and transverse nuclei of the posterior bed nuclei of the stria terminalis, the retrochiasmatic area, ventromedial hypothalamic nucleus and nuclei gemini, the central (capsular and medial parts), lateral (ventral part), posterior basomedial, and posterolateral cortical nuclei of the amygdala and the amygdalostratial transition area. Remarkably, MeAV terminates less densely than other Me components in structures pertaining to the main and accessory olfactory systems.

Conclusions:

MeAV shares connectional attributes with the anterodorsal and posteroventral parts of Me. Overall, the present data reinforce previous views indicating that these three Me parts are interrelated and differ markedly from the posterodorsal part of Me. They also undercore distinctive MeAV features, in particular a less intimate association with the main and accessory olfactory systems. **Palavras-Chave:** amygdala, hypothalamus, vomeronasal system, sexual behavior, PHA-L

Apoio Financeiro: FAPESP 02/07014-2; 08/50445-0

E.07.006

Role of the medial preoptic area in the regulation of maternal behavior across the postpartum period in rats

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A marked transition in the expression of maternal behavior occurs as the postpartum period progresses. Although there is considerable evidence that the medial preoptic area (mPOA) is a key neural substrate underlying the onset and early expression of maternal behavior, the specific role of the mPOA in the expression of maternal behavior later in the postpartum period remains unknown. The present study examined the effects of reversible inactivation of mPOA in maternal behavior across the postpartum period. Early (days 7-8) and late (days 13-14) postpartum female rats with bilateral guide cannulae aimed at the mPOA region were tested for maternal behavior after receiving site-specific bilateral infusions (1.0 µl per side) of 2% bupivacaine HCl or vehicle. Retest occurred the following day after microinfusion of the opposite treatment. Results so far show that transient inactivation of mPOA greatly impaired the expression of maternal behavior in early postpartum rats, whereas infusion of vehicle did not. In contrast, infusion of bupivacaine into mPOA facilitated the expression of maternal behavior in late postpartum females. No changes were observed after inactivation of dorsal adjacent sites in the maternal behavior of early or late postpartum females. The behavioral effects obtained in early postpartum after reversible mPOA inactivation confirm those earlier reported after permanent lesions and indicates the positive influence of mPOA on early maternal behavior. Later in the postpartum period, however, mPOA seems to exert an inhibitory influence, regulating the decline of maternal responsiveness characteristic of late postpartum. We are currently exploring the role of other components of the maternal circuit (BLA, NA and mPFC) in the regulation of maternal behavior across postpartum. **Palavras-Chave:** Bupivacaine HCl; maternal behavior; medial preoptic area; neuronal inactivation; postpartum period

Apoio Financeiro: NIH DA014025 to J.I. Morrell and NARSAD Young Investigator Award to M. Pereira

E.07.007

Food Restriction or Sleep Deprivation: Which Factor Affect Most Sexual Behavior in Male Rats?

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The purpose of this study was to determined the consequences of two factors: the food restriction (FR) and paradoxical sleep deprivation (PSD) in the different parameters (mount, intromission and ejaculation) of the sexual behavior in male rats. Diet restriction began at weaning with 6g/day and food was increased by 1 g per week until reaching 15g/day by adulthood. At adulthood, rats submitted to FR and those fed ad libitum were distributed into PSD for 96 hours or maintained as home-cage groups. The results indicated that both FR and ad libitum sleep deprived groups showed a significant decreased in the performance and motivation to initiate the sexual behavior reflected by the increased of mount and intromission latencies. When FR was associated to sleep deprivation, the rats showed an increased of the number of mounts prior the first ejaculation compared to control group. The testosterone concentrations were decreased after sleep deprivation regardless food availability while progesterone was significantly higher only in the FR-PSD group. Our data suggest that the PSD decreased the sexual behavior and the hormonal concentrations plays an important effect in this behavior and might be related to the motivation response.

Palavras-Chave: sleep deprivation, sexual behavior, food restriction, testosterone, progesterone, male, rat.

Apoio Financeiro: Associação Fundo de Incentivo à Psicofarmacologia (AFIP) and FAPESP (no 06/58274-5 to T.A.A, CEPID no. 98/14303-3 to S.T.) M. Andersen and S. Tufik are recipients of CNPq.

Does Sleep Deprivation Influence Female Motivation in Sexual Behavior?

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The present study investigated whether sleep deprivation could affect sexual receptivity (male acceptance) and proceptivity (male solicitation) behaviors in females. Adult female Wistar rats were distributed into three subgroups according to phase of the estrous cycle (proestrus, estrus and diestrus) and were subjected to paradoxical sleep deprivation (PSD) for 96h or maintained as control (CTRL) in their home-cages. After this period, the estrous phase was determined, and the females were placed with a sexually-experienced male. The receptivity (lordosis) and the proceptivity (genital sniff, ear vibration, hop and dart) were evaluated. In order to expand the investigation into the role of hormones in sexual behavior, additional groups artificially induced to be receptive by the administration of a combination of estradiol and progesterone were included. PSD caused a significant increase in proceptivity and receptivity behaviors in females exclusively in the proestrus phase in relation to respective proestrus-CTRL. At diestrus and estrus, PSD increased the rejection responses. As for hormone profile, PSD produced reduction of progesterone in proestrus in relation to respective estrus phase control group. The PSD-proestrus females that displayed the highest sexual motivation had greater concentrations of corticosterone than the PSD-diestrus with absence of sexual behaviors. Heightened sexual motivation in a sleep deprived paradigm is confined within the proestrus phase only. This can be indication that PSD might produce a distinct response of the hormonal profile according to phase of the estrous cycle.

Palavras-Chave: sleep deprivation, sexual behavior, receptivity, proceptivity, female rats, progesterone, corticosterone.

Apoio Financeiro: Associação Fundo de Incentivo à Psicofarmacologia (AFIP) and Fundação de Amparo à Pesquisa do Estado de São Paulo (CEPID #98/14303-3 to ST; #06/58274-5 to T.A. and #07/56620-6 to A.S.). Monica L. Andersen is the recipiente of a fellowship from CNPq.

E.07.009

Disfunção erétil como fator associado à depressão pós-parto paterna

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Introdução: A depressão pós-parto atinge também os homens, os sintomas mais frequentes são: presença do humor depressivo ou anedonia (diminuição ou perda do interesse nas atividades anteriormente agradáveis), mudança significativa de peso ou do apetite, insônia ou sono excessivo, fadiga, agitação ou retardo psicomotor, sentimentos de desvalia ou culpa, perda de concentração e idéias de morte ou suicídio. Alguns autores relatam que a diminuição da libido e disfunção sexual é comum a esse quadro. A disfunção erétil (DE) pode ter causa fisiológica ou psicológica e é uma das situações mais comuns e angustiantes que aflige a população masculina. É um problema em crescimento, não só nos países desenvolvidos, como também naqueles em desenvolvimento.

Objetivo: Identificar a associação entre disfunção erétil e depressão pós-parto em homens.

Método: Estudo transversal aninhado a uma coorte, que avaliou os homens no período de 30 a 90 dias após o nascimento do bebê. Os instrumentos de avaliação utilizados foram o International Index of Erectile Function (IIEF-5) para detectar a disfunção erétil e a Edinburgh Postnatal Depression Scale (EPDS) com ponto de corte superior a 12 pontos na escala, para indicativo de depressão.

Resultados: Foram analisados os dados de 524 pais com idade média de 36,8 (dp± 22,18); 50% com classificação socioeconômica C de acordo com ABEP; 86,1% viviam com a mãe do bebê; 39,9% dos pais apresentam sinais de disfunção erétil e 23,5% apresentaram sintomas depressivos. Houve associação estatisticamente significativa entre a depressão paterna e disfunção erétil (RO 10,93; 6,71-17,81); 78,9% dos pais deprimidos apresentaram disfunção erétil.

Conclusão: A disfunção erétil é especialmente prevalente nos homens com depressão pós-parto. Como se trata de um estudo transversal, a direção desta associação, entretanto, é incerta. Para esclarecer este ponto, estudos longitudinais são necessários.

Palavras-Chave: Depressão, Pós-Parto, Disfunção erétil

Apoio Financeiro: Não

E.07.010

Previous maternal experience affects the preference for pups or males without changing the maternal and sexual behaviors of postpartum estrous rats.

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Female rats display maternal and sexual behavior during the postpartum estrus, a unique period of their reproductive cycle in which pups and males acquire attractive properties at the same time.

Objective: To assess the preferences for pups or males as well as the maternal and sexual behaviors of primiparous and multiparous postpartum estrous females.

Methods: Postpartum estrus primiparous (PRIM) and multiparous (MULT) dams, and late proestrous cycling females (CYCL) were tested for preference in a star-maze with three choice chambers: pups, sexually active male or no-stimulus, during 20 min. The maternal behavior of postpartum dams was evaluated in a 30 min test. Sexual receptivity of the three groups was registered in a circular arena (50 cm diameter/15 min) with a sexually active male, after the preference task.

Results: Most of the postpartum estrous dams preferred the pups to the male (% of time in male and pup chamber: PRIM 11.1 (7.8) vs 29.5 (1.8), $T=3.0$, $p<0.05$ and MULT 7.5 (3.8) vs 40.8 (5.3), $T=2.0$, $p<0.05$), although MULT females spent more time in the pups' chamber ($U=13.0$, $p=0.05$) and made more effort to gain access to them than PRIM dams (33.0 (7.0) vs 14.0 (3.0), $U=10.0$, $p<0.05$). CYCL females in late proestrus strongly preferred the male (% of time in male and pup chamber: 40.5 (7.3) vs 9.5 (2.8), $T=0.0$, $p<0.01$). All groups exhibited high and similar lordosis quotients (PRIM: 1.0 (0.1), MULT: 1.0 (0.0) and CYCL: 1.0 (0.1)). No differences were found between the maternal behavior of PRIM and MULT females (i.e. corporal licking of PRIM: 17.0 (1.5) and MULT: 18.0 (4.8), $U=29.0$, $p=NS$).

Conclusions: During the postpartum estrous a previous reproductive experience increased maternal motivation in a challenging situation, without affecting the expression of the home cage maternal behavior. All postpartum estrous females showed low interest for the sexually active male, however their sexual receptiveness was as high as that observed in cycling females.

Palavras-Chave: postpartum estrus; maternal motivation; sexual motivation

Apoio Financeiro: FCE (MEC), PDT (MEC), PEDECIBA, ANII

E.07.011

Avaliação no labirinto em cruz elevado de ratos wistar, na idade pré pubere, cujas mães foram selecionadas quanto a intensidade (high e low) dos cuidados a prole na fase lactacional

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Objetivo: Avaliar o comportamento no labirinto em cruz elevada de ratos wistar (machos e fêmeas), entre 35-37 dias de idade, os quais as mães foram selecionadas pela metodologia High-Low-5, durante o período lactacional.

Métodos e resultados: Foram utilizados animais pré-púberes (machos e fêmeas) com idade entre 35-37 dias, divididos em dois grupos: grupo High (H com NM=10 e NF=11) e grupo Low (L com NM=10 e NF=11), animais selecionados pela metodologia High-Low-5 (5 critérios são considerados para seleção: mãe fica em cima dos filhotes no ninho; lambe os filhotes; fica fora do ninho; análise no Labirinto e do Comportamento Agressivo Maternal). Nesta seleção, 100 mães são avaliadas, sendo que as 20 que mais cuidam dos filhotes são as High e as 20 que menos cuidam são as Low. Cada animal foi filmado no labirinto por 5 min e depois os comportamentos foram analisados em um software específico. Os resultados foram analisados através de uma ANOVA seguidos de teste de Newman-Keuls, quando necessário, o nível de significância aceito foi de $p < 0,05$. As fêmeas H ($22,2 \pm 6$) mostraram um tempo total de permanência no braço aberto menor do que as fêmeas L ($62,8 \pm 24$). Os as medidas de latência, frequência e duração dos demais comportamentos não mostraram diferenças estatisticamente diferentes tanto em machos quanto em fêmeas.

Conclusão: Animais que tiveram suas mães selecionadas durante a lactação em mais (H) ou menos (L) cuidadoras mostraram quando pré puberes, que as filhas de mães High apresentam um aumento da ansiedade verificado pela diminuição do tempo de permanência no braço aberto quando comparado as fêmeas filhas de mãe Low, porém esse comportamento não é observado nos machos. Esses resultados encontrados nos animais pré púberes são contrários aos encontrados em experimentos realizados em nosso laboratório em animais na idade adulta.

Palavras-Chave: cuidados maternos, labirinto, pré pubere

Apoio Financeiro: UNIOESTE

E.08.001

BEHAVIOR COMPARED STUDY OF WISTAR, WILD (*Rattus norvegicus* sp) AND HYBRID RATS IN THE RESIDENT-INTRUDER MODEL.

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Objectives: Most kind of mammals display aggressive behavior to defend your domain against intruder. These aggressive behavior could be an furious attack (fight) or just treath signals of attack. The present work aimed to investigate the territorial aggressive behavior expressed by differents rats lineages in the resident-intruder test. **Methods:** It was used 15 rats of each lineages, Wistar, Wild (*Rattus norvegicus* sp.) and Hibrid (Wild versus Wistar). One rat was placed in a special box to domain the territory to one week period. After that, it was place one intruder rat (same or different lineage) in that box. The fight and flight behaviors were measured with videocameras inside and outside the box, and some key behaviors was registered (e.g. boxe, vocalization, lateral treath, bites). The data was submitted to ANOVA analysis an T test comparisons (p 0,05). **Results:** The Wild and Hibrid residents rats showed aggressive behaviors against intruders, except when a Wild rat were an intruder. The same response wasn't observed in the Wistar rats. **Conclusion:** The present data suggest the influence of genetic factors over all strains of rats investigated. Rats from the Wild strain were the more aggressives, whereas those of the hibred and Wistar strains were the least ones. **Palavras-Chave:** Aggression, Wild rat, behavioral model, genetic, strains

Apoio Financeiro: CNPq

E.08.002

Dorsal premammillary nucleus lesion leads to conspecific defense impairment.

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Neural system analyses indicate that the processing of predator- associated cues and the expression of anti-predatory fear responses depend on a specific hypothalamic circuit , where small cytotoxic lesions in the site most responsive to predatory threats (i.e., the dorsal premammillary nucleus) produce a dramatic reduction of the defensive responses to the predator. In the present investigation we questioned whether this concept of the hypothalamus as a key element in the processing and expression of fear responses could be generalized to another threatening situation where a rival conspecific represents the source of danger. The aim of this work was to evaluate the PMd role in the conspecific defense using the Resident x Intruder paradigm. Intruder rats were divided in three groups: PMd- NMDA lesioned, outside the PMd- NMDA lesioned, and intact intruders, which were exposed to a long evans resident rat for five minutes after the first attack. The animals were videotaped for further behavioral analyses. Ninety minutes after the agonistic behavior the intruders were perfused and their brains processed for lesion confirmation. PMd-lesioned rats showed a dramatic drop on flight and freezing ($21.57s \pm 47.27$; mean $\pm$ SD), in comparison to outside PMd – lesioned ($238.06s \pm 47.77$) and the intact group ($229.78s \pm 55.12$); while boxing, upright and on-the-back postures, were preserved in the PMd- lesioned ($72.51s \pm 44.76$), when compared to outside PMd – lesioned ($32.54s \pm 33.46$) and the intact group ($48.76s \pm 35.41$). There were no significant behavioral differences between PMd- lesioned and intact intruders. Thus, the results indicate a clear modification in intra specific defense made by the NMDA PMd lesion once it abolishes the defensive responses that are not compelled by the resident, such as flight and freezing, and maintains undamaged those behaviors required when they are agonistically interacting such as boxing, upright and on-the-back postures. Therefore, PMd-lesioned intruders are able to defend themselves, but they seem to lack the fear response to the rival conspecific, even when they are repeatedly attacked.

Palavras-Chave: Conspecific defense, Dorsal premammillary nucleus, Hypothalamus

Apoio Financeiro: Fapesp # 05/54511-0 and 05/59286-4.

E.08.003

MODULATION OF TONIC IMMOBILITY IN GUINEA PIG'S ANTERIOR CINGULATE CORTEX BY HOMOCYSTEIC ACID, A GLUTAMATE AGONIST.

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Introduction

Tonic immobility (TI) is a defensive behavior characterized by a temporary state of profound motor inhibition, elicited by the prey as the last form to survive against predator attack. The aim of this study was to investigate the effect of microinjection of the excitatory amino acid (EAA) agonist DL-homocysteic acid (DLH) and the NMDA receptors antagonist (MK-801) into the anterior cingulate cortex (ACC) over the duration of TI in guinea pig.

Methods

Adult male guinea pigs (n=37) were submitted to the physical restraint by the experimenter for TI induction. The TI episodes were recorded by a timer and repeated for 5 times to obtain the control mean. Following 24 hours, a guide cannula was implanted into the ACC. After 6 and 7 days, a new control (sham) and experimental TI session were performed, respectively. In the experimental TI sessions, the animals were microinjected: Group 1- DLH (30 nmol/ 0.2 µl); group 2- MK-801 (3.6 nmol/0.2µl) + DLH (30 nmol/ 0.2 µl); group 3 e 4- MK-801 (3.6 nmol/0.2µl) and saline (0.2µl), respectively.

Results

Microinjection of DLH into ACC promoted a decrease in the TI duration ($F_{(2, 20)} = 4,1$; $p=0.03$; ANOVA repeated measures) when compared to sham and control episodes (Duncan's post-test). This effect was blocked by previous MK-801 microinjection. The microinjection of MK-801 alone, or of saline, did not have effects over the TI duration when compared to sham and control episodes ($p > 0.05$; ANOVA repeated measures).

Conclusion

These results support the hypothesis that the EAA in the ACC are capable of modulating TI duration. The mechanisms seem to contribute to reduce TI episodes probably by means of the involvement of the NMDA receptors.

Palavras-Chave: Tonic immobility; anterior cingulate cortex; excitatory amino acid; DL-homocysteic acid; guinea pig

Apoio Financeiro: CAPES and FAEPA

E.08.004

Blockade of NMDA receptors and nitric oxide synthesis in the dorsal premammillary nucleus attenuates behavioral consequences of predator exposure

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The dorsal premammillary nucleus (PMd) has been hypothesized to play a key role in the expression of defensive behaviors. While electrical stimulation of the PMd induces defensive responses, chemical lesions of this region have been reported to reduce the defensive reactions of rats confronted with a cat. Moreover, Fos expression in this region is strongly increased after predator exposure. The neurotransmitters involved in these responses are not completely understood. Nitric oxide (NO) is an atypical neurotransmitter that is closely associated with glutamate-mediated neurotransmission in the central nervous system. Ionotropic glutamate receptors and NOS containing neurons are localized in the PMd. Moreover, rats exposed to live cat showed activation of NO producing neurons in the PMd. Objectives: To test the hypothesis that amino-7-phosphonoheptanoic acid (AP7), a glutamate NMDA-receptor antagonist, and N-propyl-L-arginine (N-propyl), a neuronal nitric oxide synthase inhibitor, injected into the PMd would attenuate the defensive reactions induced by exposure to a live predator. Methods: Male Wistar rats (n=4-7) with cannulae aimed at the PMd received injections of AP7 (2 nmol/0.2 microL), N-propyl (100 nmol/0.2 microL) or saline and were exposed to a toy (control) or live cat for 10 min in a Plexiglas box. A metal grid wall located in the middle of the box prevented direct contact between the animals. The results were analyzed by one-way Analysis of Variance (ANOVA) followed by the Duncan test. Results: Animals exposed to the cat showed a reduction in time spent near the cat (live cat, 8.19 ± 4.25 s, toy cat 373.3 ± 37.4 s, $p < 0.05$) and immobility (live cat, 341.00 ± 43.23 s, toy cat, 4.00 ± 1.76 s $p < 0.05$). AP7 and N-propyl pretreatment attenuated these effects induced by exposition to the predator. Conclusions: These results suggest that antagonism of glutamatergic and nitrgergic neurotransmission in the PMd can attenuate behavioral responses to threatening stimuli.

Palavras-Chave: defensive behavior, dorsal premammillary nucleus, glutamate and nitric oxide

Apoio Financeiro: FAPESP, FAEPA

E.08.005

Antagonism of TRPV1 receptors in the medial prefrontal cortex induces anxiolytic-like effects.

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Introduction: The so-called capsaicin vanilloid receptor, VR1, is a member of the TRP family of ion channels (TRPV1) and is widely found on small- and medium-sized primary afferent neurons and in diverse areas of the central nervous system involved in modulation of anxiety behaviors, including the medial prefrontal cortex (mPFC). The mPFC is activated by threatening or stressful stimuli, and lesions or pharmacological inactivation produce anxiolytic-like effects. Therefore, this structure has been closely associated with defensive responses. However, the role of local TRPV1 receptors in these responses is largely unknown. The aim of the present study was to investigate the effects of antagonism of TRPV1 receptors in the mPFC of rats submitted to a model of anxiety, the Vogel conflict test (VCT). **Methods:** Water-deprived male Wistar rats (n=4-5) with cannulae aimed at the mPFC (bilateral) received local microinjections of CPZ (10-60 nmol/0.2µL, a TRPV1 receptor antagonist) or vehicle (DMSO) and were submitted to the VCT. **Results:** CPZ significantly increased the number of punished licks indicating an anxiolytic-like effect (Vehicle: 96.0± 30.10; CPZ 10nmol: 455.50± 36.02; CPZ 60nmol: 265.8± 54.77; F_{2,11}= 16.88 p=0.0004). **Conclusions:** The present study suggests that the mPFC is a possible neuroanatomical site for anxiolytic-like effects mediated by TRPV1 antagonists. In addition, these results indicate that vanilloids may play a role in anxiety disorders. **Palavras-Chave:** Anxiety; TRPV1; Vanilloids; Prefrontal Cortex;

Apoio Financeiro: FAPESP, CAPES, CNPq.

Efectos del estrés postnatal en ratas sobre el comportamiento defensivo, el aprendizaje y la memoria espacial.

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Eventos estresantes durante la vida temprana inducen alteraciones de tipo fisiológico y comportamental. De hecho, la relación madre-hijo y el cuidado de los padres con el recién nacido, cumplen un papel importante en el desarrollo fisiológico y emocional de los individuos. Así, la utilización de modelos animales, con sus respectivas limitaciones, nos permiten una aproximación experimental para dilucidar las posibles consecuencias y los mecanismos involucrados en las alteraciones tanto en el comportamiento como en el desarrollo de los individuos. Por eso, una de nuestras líneas de investigación tiene como objetivo estudiar los efectos del estrés postnatal por separación maternal temprana (ESMT). El objetivo de este trabajo es utilizar un modelo animal para estudiar si el ESMT, afecta en el adulto su comportamiento defensivo y procesos de aprendizaje y memoria espacial. El modelo utilizado consistió en la separación de las crías de su madre durante 6 horas diarias a partir del día postnatal 1 hasta el 21, con ciclo de luz invertido (12h luz/12 oscuridad). A partir del día 22 las ratas se separaron de acuerdo al sexo y continuaron su desarrollo normal con comida y agua *ad libitum*. Cuando los sujetos alcanzaron un peso promedio de 230 g fueron evaluados en el laberinto en cruz elevado para evidenciar su comportamiento defensivo. Las medidas de interés fueron entrada y tiempo de permanencia del sujeto en los brazos abiertos del laberinto, frecuencia y tiempo de *deeping* y distancia recorrida durante los 5 minutos que duró la sesión. Otro grupo experimental sometido a las mismas condiciones, se utilizó para evaluar en el adulto joven la memoria espacial utilizando el laberinto circular de Barnes (LCB). Todos los animales recibieron una sesión de habituación al laberinto, una de entrenamiento (aprendizaje de una tarea) y una de evaluación. El protocolo de entrenamiento constaba de 8 ensayos de adquisición. Veinticuatro horas después se evaluó el aprendizaje utilizando una prueba con caja meta (PCC) y una prueba sin caja (PSC). Las medidas de interés fueron latencia de llegada al agujero meta (LLAM) y el número de errores de agujero (EA). Todas las sesiones fueron filmadas y analizadas *a posteriori*. Los resultados encontrados con respecto al comportamiento defensivo muestran que este protocolo de separación temprana disminuye las conductas relacionadas con ansiedad en las hembras pero no en los machos. Por otra parte, con respecto a los resultados obtenidos con el LCB muestran que tanto en los grupos control como experimental, la LLAM durante la adquisición es menor en machos vs. Hembras. Esta misma medida es menor en las hembras experimentales vs. Hembras control. Esta tendencia se mantiene al medir la LLAM durante las PCC y PSC. Estos resultados sugieren que en nuestro modelo el ESMT disminuye las conductas relacionadas con ansiedad en las hembras pero no en los machos y tiende a favorecer el proceso de adquisición y la recuperación de la memoria.

Palavras-Chave: Estres postnatal, comportamiento defensivo, memoria espacial.

Apoio Financeiro: Grant UN.DIB 2010100905. y Grant UN. FACMED 20301009691

Efectos del estrés postnatal en ratas sobre el comportamiento defensivo, el aprendizaje y la memoria espacial

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Eventos estresantes durante la vida temprana inducen alteraciones de tipo fisiológico y comportamental. La relación madre-hijo y el cuidado de los padres con el recién nacido, son fundamentales en el desarrollo fisiológico y emocional de los individuos. Utilizar modelos animales no permite una aproximación experimental para dilucidar posibles consecuencias y mecanismos involucrados en alteraciones tanto del comportamiento como del desarrollo de los individuos. El objetivo de este trabajo es utilizar un modelo animal para estudiar si el estrés postnatal por separación maternal temprana (ESMT), afecta en el adulto su comportamiento defensivo y procesos de aprendizaje y memoria espacial. Las crías fueron separadas de su madre durante 6 horas diarias del día postnatal 1 al 21. El día 22 las ratas se separaron de acuerdo al sexo, con comida y agua *ad libitum*. Cuando los sujetos alcanzaron un peso de 230 g fueron evaluados en el laberinto en cruz elevado para evidenciar su comportamiento defensivo. Las medidas de interés fueron entrada y tiempo de permanencia en los brazos abiertos, frecuencia y tiempo de *deeping* durante los 5 minutos que duró la sesión. Otro grupo experimental sometido a las mismas condiciones, se utilizó para evaluar memoria espacial utilizando el laberinto circular de Barnes (LCB). Los animales fueron habituados, entrenados y evaluados. El protocolo de entrenamiento constaba de 8 ensayos de adquisición. Veinticuatro horas después se evaluó el aprendizaje utilizando una prueba con caja meta (PCC) y una prueba sin caja (PSC). Las medidas de interés fueron latencia de llegada al agujero meta (LLAM) y el número de errores de agujero (EA). Con respecto al LCB los análisis muestran que tanto en los grupos control como experimental, la LLAM durante la adquisición es menor en machos vs. Hembras. Esta misma medida es menor en las hembras experimentales vs. Hembras control. Esta tendencia se mantiene al medir la LLAM durante las PCC y PSC. Estos resultados sugieren que en nuestro modelo el ESMT disminuye las conductas relacionadas con ansiedad en las hembras pero no en los machos y tiende a favorecer el proceso de adquisición y la recuperación de la memoria.

Palavras-Chave: estrés postnatal, comportamiento defensivo, memoria espacial.

Apoio Financeiro: Grant UN.DIB 2010100905. y Grant UN. FACMED 20301009691

AUSÊNCIA DE EFEITOS DA ADMINISTRAÇÃO DE MIDAZOLAM INTRA-AMÍDALA NA ANTINOCICEPÇÃO INDUZIDA PELO MEDO EM CAMUNDONGOS REEXPOSTOS AO LABIRINTO EM CRUZ ELEVADO

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Objetivos: O confinamento de camundongos no braço aberto (BA) do labirinto em cruz elevado (LCE) produz antinocicepção (Psychopharmacol.150:300, 2000) insensível a reexposição ao aparelho com ou sem administração de drogas (FeSBE 01.06.122, 2003; FeSBE 06.037, 2007). Este estudo avaliou os efeitos de duas administrações intra-amídala (intra-AMD) de midazolam (MDZ) na antinocicepção induzida pela reexposição de camundongos ao braço aberto (BA) do LCE.

Métodos e Resultados: Camundongos suíços machos (25-35g), foram submetidos à cirurgia estereotáxica e após um período de 4 dias expostos as seguintes condições experimentais: *1ª Exposição:* Administração intra-amídala de midazolam ou salina e confinamento no BA ou braço fechado (BF) do LCE por 5 minutos. *2ª Exposição:* Após vinte e quatro horas os animais passaram pelo mesmo procedimento de injeção, exceto que ao final da injeção de MDZ os camundongos receberam injeção de ácido acético 0,6% (i.p.), 0,1ml/10g de peso. Os animais foram alojados em gaiolas individuais até o início das contorções e posteriormente submetidos à reexposição ao BA ou BF do LCE para o registro do número de contorções abdominais (resposta nociceptiva). Somente os animais com análise histológica positiva foram utilizados neste estudo. A ANOVA de duas vias (tratamento x local do confinamento) mostrou diferença significativa em relação ao local do confinamento ($F_{(1,58)}=20,39$ $P < 0,05$), e ausência de efeito do tratamento ($F_{(1,58)}=1,85$, $P > 0,05$) e da interação entre tratamento e local do confinamento ($F_{(1,58)}=0,34$, $P > 0,05$). Comparações posteriores (teste de Duncan) revelaram que os animais confinados no BA apresentaram menor número de contorções abdominais quando comparados ao BF, independentemente do tratamento recebido, [salina (BA: $3,38 \pm 0,58$; BF: $7,21 \pm 1,04$, $P < 0,05$); MDZ 30nmol (BA: $4,13 \pm 0,94$; BF: $09,12 \pm 1,20$, $P < 0,05$)].

Conclusão: Os resultados mostram que o tratamento prévio intra-AMD com MDZ, não alterou a resposta antinociceptiva nos animais reexpostos ao BA, sugerindo que este tipo de inibição da dor parece independer da ativação de receptores do complexo GABA-Benzodiazepínico localizados na amídala.

Palavras-Chave: Antinocicepção , Benzodiazepínicos, Labirinto em Cruz Elevado

Apoio Financeiro: UFSCar, FAPESP

SEROTONERGIC 2A/2C RECEPTORS OF THE LATERAL SEPTUM AND BEHAVIORAL DEFENSIVE RESPONSES IN RATS

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INTRODUCTION AND PURPOSE: The lateral septum has been implicated in the modulation of defensive responses and thus in anxiety. One of the main neurotransmitters which have been related to the physiopathology of anxiety is serotonin (5-HT). It has been shown that 5-HT_{2A/2C} receptors are well distributed in the lateral septum (LS). Our study investigates the role of LS 5-HT_{2A/2C} receptors in animals exposed to the elevated T-maze (ETM) animal model of anxiety, which allows the measurement of two behavioral defensive responses in the same rat: one-way escape and inhibitory avoidance. In clinical terms, these responses have been related to panic (PD) and generalized anxiety disorder (GAD), respectively. **METHOD:** Male Wistar rats (300 g) were administered intra-LS with the 5-HT_{2A/2C} agonist DOI (8 and 16 nmols, 2 µl) and ten minutes later were submitted to the ETM. Avoidance was measured by placing the rat at the end of the enclosed arm and recording the time to withdraw from this arm in 3 consecutive trials (Baseline, avoidance 1 and 2). Soon afterwards, escape was measured by recording the time taken by the animals to withdraw from the open arm again in 3 trials (escape 1, 2 and 3). Immediately after, animals were tested in the open-field for locomotor activity assessment. Data was analysed by ANOVA followed by the Duncan test. **RESULTS AND DISCUSSION:** Results showed that DOI significantly increased ($p < 0.05$) inhibitory avoidance latencies in the two doses administered, an anxiogenic effect (Avoidance 1: SAL: 44.5 ± 31.9 , 8 nmols: 258.4 ± 41.6 , 16 nmols: 176.9 ± 50.8). On the other hand, the dose of 8 nmols significantly increased ($p < 0.05$) one-way escape, a panicolytic-like effect (Escape 1: SAL: 7.2 ± 1.6 , 8 nmols: 15.4 ± 1.4). Locomotor activity in the open field was not altered by treatment. These results suggest that 5-HT_{2A/2C} of the lateral septum regulate different defensive behavioral responses, and point to the importance of these receptors activation to the physiopathology of PD and GAD. **Palavras-Chave:** lateral septum, 5-HT_{2A/2C} receptors, elevated T-maze, defense, anxiety

Apoio Financeiro: CNPq and FAPESP (Brazil)

Córtex Pré-Frontal, Receptores 5-HT_{1B}, Provocação Social e Comportamento Agressivo em Fêmeas Pós-Parto

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A serotonina (5-hidroxitriptamina, 5-HT) é um dos neurotransmissores que tem sido implicado no controle neural da expressão do comportamento agressivo. O córtex pré-frontal (CPF), mais especificamente a região orbitofrontal (VO), possui receptores 5-HT_{1B} e é uma área relevante para o controle da agressão e do comportamento impulsivo. O objetivo desse trabalho foi de avaliar o efeito do agonista dos receptores 5-HT_{1B}, CP-93,129, microinjetado no CPF VO, sobre o comportamento agressivo de ratas submetidas à provocação social. Foram utilizadas ratas fêmeas Wistar, com 5 meses de idade, microinjetadas no 9º dia pós-parto com CP-93,129 nas doses de 0,56 (n=12) e 1,0 µg/0,2 µl (n=9), sendo que o grupo controle consistiu de solução salina (n=8). Para comprovar a especificidade do receptor, o antagonista dos receptores 5-HT_{1B}, SB-224,289, foi microinjetado na mesma região, sendo que, tivemos 3 grupos experimentais: 1) fêmeas microinjetadas com salina e depois de 15 min receberam novamente salina (controle, n=12); 2) fêmeas microinjetadas com SB-224,289 na dose 5,0 µg/0,2 µl e depois de 15 min receberam salina (n=13); e 3) ratas microinjetadas com SB-224,289 na dose 5,0 µg/0,2 µl e depois de 15 min receberam CP-93,129 na dose de 1,0 µg/0,2 µl (n=12). Os resultados mostraram que o CP-93,129 na dose de 1,0 µg/0,2 µl diminuiu significativamente as frequências dos comportamentos agressivos de ataque lateral, postura agressiva e dominar (2,8±1,1; 0,9±0,6 e 0,1±0,1) comparadas ao grupo controle (12,0±2,5; 6,1±1,8 e 4,6±1,2). O agonista 5-HT_{1B} teve efeito somente sobre o elemento ofensivo do comportamento agressivo das fêmeas e não alterou os elementos motores. A reversão dos efeitos antiagressivos foi constatada com a microinjeção do SB-224,289, comprovando desta forma a participação específica desses receptores. Nossos resultados demonstram que a região VO do PFC, juntamente com os receptores 5-HT_{1B} estão envolvidos na modulação do comportamento agressivo em ratas lactantes provocadas socialmente.

Palavras-Chave: Córtex Pré-Frontal, agressividade, receptores 5-HT_{1B}.

Apoio Financeiro: UNISINOS, TUFTS UNIVERSITY e CAPES.

EFEITO ANSIOLÍTICO DA SERTRALINA E AUSÊNCIA DE EFEITO DA IMIPRAMINA EM CAMUNDONGOS AVALIADOS NO MODELO DE TRANSTORNO DE ESTRESSE PÓS-TRAUMÁTICO (TEPT).

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Objetivo: Investigar o papel da sertralina (SER), inibidor seletivo da recaptação de serotonina (ISRS), e da imipramina (IMI), antidepressivo tricíclico (ADT) no modelo de TEPT.

Método e Resultados: Camundongos machos, suíço-albino (25-31g, n=168), foram confinados no lado escuro (LE) da caixa claro escuro (CE) por 2 min. A porta guilhotina foi aberta e após a entrada do animal no lado claro (LC) da caixa, uma lâmpada de 25W foi acesa, e o mesmo retornou imediatamente ao LE da caixa. A porta foi fechada e os grupos receberam choque nas patas (CCHO-0,5 mA) ou não (SCHO), por 10 seg. No 7º, 14º e 21º dia, os animais foram reexpostos ao LC da caixa por 2 min., sem choque. No 34º dia, 30 min. após tratamento com SER (0, 5,0 e 10 mg/kg, i.p.) e IMI (0, 5,0, 10 e 20 mg/kg, i.p.) os animais foram submetidos ao teste CE, para registro da latência de fuga (LF) e do tempo gasto no LC da caixa. A ANOVA de duas vias mostrou diferença significativa para as medidas de ansiedade, conforme apresentado na tabela abaixo.

Tratamento	Teste CE			
	SCHO		CCHO	
	LF	TC	LF	TC
SER mg/kg				
0	37,3±23,9	111,3±18,4	116,6±35,7#	250,7±13,2#
5	45,7±24,7	104,5±21,5	33,4 ± 7,5*	231,7±20,3#
10	23,6±11,7	89,2 ±20,2	120,7±32,9#	261,8±11,5#
IMI mg/kg				
0	42,7±24,7	151,7±20,9	207,9±28,5#	265,2±15,9#
5	15,7 ±4,8	149,9±18,0	165,2±32,5#	274,2±13,0#
10	10,4 ±3,4	161,6±15,4	230,4±30,6#	280,0±11,2#
20	46,7±23,7	147,1±22,3	180,4±35,3#	282,7 ±6,5#

* $P < 0,05$ comparado a salina (0) CCHO. # $P < 0,05$ comparado ao respectivo grupo SCHO.

Conclusão: O estímulo aversivo (choque nas patas), associado às lembranças no LC da caixa, produziu aumento da ansiedade no teste CE, conforme demonstrado anteriormente em ratos fêmeas e camundongos machos (Psychoneuroendocrinol. 30:316, 2005; FesBE 06.032, 2007). A imipramina não alterou a ansiedade avaliada no modelo de TEPT, o que sugere que os ADT não estão envolvidos na modulação desta psicopatologia. A sertralina (5,0 mg/Kg) produziu efeito ansiolítico no teste CE, sugerindo o envolvimento dos ISRS na modulação do TEPT em camundongos.

Palavras-Chave: Transtorno de estresse pós-traumático, imipramina, sertralina, teste claro-escuro.

Apoio Financeiro: UFSCar e CAPES

PÂNICO E ESTRATÉGIAS DEFENSIVAS: ESQUIVA ATIVA EM RATOS SENSÍVEIS À CORRIDA SELVAGEM

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A corrida selvagem (CS) é um comportamento exibido por alguns ratos em resposta à estimulação acústica de alta intensidade e pode ser interpretada como uma fuga de pânico pertencente ao repertório defensivo dos animais. Ratos sensíveis à CS podem ser utilizados como modelos animais no estudo do transtorno de pânico em humanos. Comportamentos de pânico são vistos como estratégias de defesa ativa por se manifestarem através de reações do tipo luta-ou-fuga, mas a defesa ativa pode ser acessada de forma direta através do teste de esquivas numa *Shuttle-box*. Portanto, o objetivo do trabalho foi determinar se animais com sensibilidade à CS apresentam o perfil de defesa ativa quando testados na *Shuttle-box*. Os sujeitos da pesquisa foram ratos Wistar machos submetidos ao teste de sensibilidade à CS, pela exposição ao estímulo acústico de 112 dB, por 60 s, com concomitante registro comportamental. Separados em animais sensíveis (n=11) e resistentes (n=11), os ratos foram testados na *Shuttle-box*. Foi registrado o número de transições entre um compartimento e outro, e o tempo total de cada sessão. Esses dados foram avaliados diariamente por um período de cinco dias e de forma individual para cada rato, após fases de habituação e treino. Analisando os animais, sem considerar a sensibilidade à CS, observou-se que o teste de esquivas na *Shuttle-box* diferenciou indivíduos de alto e baixo desempenho. Quanto ao número de transições entre os compartimentos, a média ($\pm$ E.P.M.) dos animais resistentes foi de $6,73 \pm 1,32$ e dos sensíveis, de $8,36 \pm 1,22$; não havendo diferença estatisticamente significativa entre os grupos ($p=0,22$). A duração do teste para os animais resistentes foi de $338,53 \pm 18,55$ s e para os sensíveis de $311,00 \pm 18,91$ s; sem diferença estatisticamente significativa ($p=0,31$). De acordo com os resultados, não é possível associar a sensibilidade à CS ao perfil de defesa ativa determinado no teste convencional da *Shuttle-box*. Isso conduz à idéia de que um animal propenso a manifestar reações de pânico não necessariamente se defende de forma pró-ativa em outras circunstâncias.

Palavras-Chave: Pânico, Corrida Selvagem, Esquiva ativa, Shuttle-box

Apoio Financeiro: FAPESP

AVALIAÇÃO DO CURSO TEMPORAL DA ANTINOCICEPÇÃO INDUZIDA PELA EXPOSIÇÃO AO LABIRINTO EM CRUZ ELEVADO ABERTO

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Objetivo: Camundongos expostos ao labirinto em cruz elevado aberto (LCEa: 4 braços abertos) apresentam antinocicepção de elevada magnitude (Mendes-Gomes & Nunes-de-Souza, Brain Res., 1047: 97, 2005). Porém, é desconhecida a duração dessa reação de defesa quando o animal permanece e quando é retirado da situação aversiva. Assim, este estudo objetivou uma avaliação temporal desse tipo de antinocicepção.

Métodos e Resultados: Camundongos Suíços machos (n=8-9/grupo) receberam injeção de 50 µl de formalina 2,5% no dorso da pata direita traseira e foram expostos a uma caixa de vidro (CV: 30 X 20 X 25 cm; situação controle), onde foi registrado por 5 min (Fase 1 do teste) o tempo gasto lambendo a pata. Após 25 min da injeção, 5 grupos foram formados, de acordo com o local de exposição: Grupo 1: exposto à CV (vidro-vidro) dos 25 aos 55 min; Grupos 2 e 3: expostos ao LCE fechado (LCEf: 4 braços fechados) e LCEa, respectivamente, por 10 min (25-35 min) e retornados à CV (fechado-vidro e aberto-vidro) até o final do teste (55 min); Grupos 4 e 5 (fechado-fechado e aberto-aberto): expostos ao LCEf e LCEa, respectivamente, até o final do teste. O tempo de lambidas da pata da Fase 2 do teste foi registrado dos 25 aos 55 min após a injeção de formalina. Os resultados são demonstrados em intervalos de 5 min.

Exposição	Tempo de lambidas (s)			
	0'-5'	25'-35'	35'-45'	45'-55'
vidro-vidro (controle)	23±3	130±19	130±26	71±17 ^{bc}
fechado-vidro	15±3	142±26	90±26 ^b	75±11 ^b
aberto-vidro	21±4	12±5 ^a	152±26 ^b	117±17 ^b
fechado-fechado	14±2	116±18	165±31	132±16
aberto-aberto	18±4	12±5 ^a	23±6 ^a	42±10 ^b

^{a,b,c} $p < 0,05$ vs vidro-vidro e intervalos 25-35 e 35-45, respectivamente.

Conclusão: Os resultados demonstraram que (1) o grupo controle apresenta uma queda da resposta de dor no último intervalo, corroborando dados da literatura que mostram que o teste de formalina dura aproximadamente 60 min; (2) a resposta antinociceptiva verificada no LCEa se mantém aproximadamente por 20 min de exposição, diminuindo nos últimos 10 min do teste; (3) quando retornam à situação controle (CV), o grupo aberto-vidro imediatamente expressa a resposta de dor semelhante à dos animais não expostos ao ambiente aversivo (vidro-vidro).

Palavras-Chave: teste de formalina, antinocicepção, labirinto em cruz elevado, medo/ansiedade, reação de defesa, camundongos

Apoio Financeiro: FAPESP, CNPq, PADCFar/UNESP-Araraquara.

Avaliação da imunorreatividade à proteína *FOS* em áreas do tronco encefálico envolvido na resposta defensiva de imobilidade tônica em cobaias.

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O comportamento de imobilidade tônica (IT) é uma resposta defensiva inata, exibida pela presa quando o contato físico com o predador é prolongado e a situação inescapável. O objetivo deste trabalho foi avaliar a expressão da proteína *FOS*, em áreas do tronco encefálico como o núcleo de Edinger-Westphal (EW), núcleo dorsal da rafe (NDR), núcleo cuneiforme (CUN) e no colículo superior (CS) e inferior (CI); após a emissão da resposta de IT.

Cobaias machos (± 450 g, N= 12), foram divididas em 2 grupos. Os animais do grupo controle (N= 6) não foram induzidos ao comportamento de IT. E o grupo de IT, os animais foram induzidos ao comportamento de IT (N= 6) feito por meio de inversão postural e contenção física por pelo menos 60 min. Depois de 2 horas do começo dos experimentos, os animais foram anestesiados, perfundidos com salina tamponada seguida por paraformaldeído a 4%. Os encéfalos foram removidos, crioprotetidos e processados pela técnica da imunocitoquímica para a proteína *FOS*.

Os resultados foram expressos em média da densidade para $0,2\text{mm}^2 \pm \text{EPM}$. A análise estatística demonstrou que a resposta de IT promove acentuada marcação de células imunorreativas a proteína *FOS* (IR-*FOS*) no EW ($161,49 \pm 18,63$), NDR ($124,36 \pm 14,37$), CUN ($57,27 \pm 13,19$) e no CS ($19,90 \pm 3,81$) quando comparado com seus respectivos controles (EW $1,38 \pm 7,21$; NDR $50,75 \pm 12,74$; CUN $9,10 \pm 2,11$ e CS $4,05 \pm 1,30$). No entanto, no CI não ocorreu diferença significativa no grupo IT ($4,73 \pm 1,52$) quando comparado com o grupo controle ($1,84 \pm 0,96$).

Nossos resultados demonstram que a resposta de IT em cobaias é capaz de promover a ativação da proteína *FOS*, um marcador de atividade neuronal, no EW, NDR, CUN e no CS podendo estar envolvidos e participar na emissão e modulação deste comportamento, desde que foi evidenciado aumento da IR-*FOS* nestas estruturas após a emissão da resposta de IT.

Palavras-Chave: Imobilidade tônica, tronco encefálico, *FOS*.

Apoio Financeiro: Fapesp e Capes.

Avaliação Comportamental e Farmacológica da Relação entre Ansiedade e Pânico em Modelos Animais

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Neste trabalho investigamos a relação entre ansiedade e ataques do pânico em modelos animais. O condicionamento contextual de medo parece ser um excelente modelo de ansiedade enquanto a que a resposta de fuga induzida pela microinjeção de NMDA (N-metil-D-aspartato) na MCPD (matéria cinzenta periaquedutal dorsal) é um modelo de pânico. Neste estudo investigou-se se a relação entre a ansiedade através do condicionamento contextual de medo (primeiro experimento) ou do pentilenotetrazol (PTZ) sobre a resposta de fuga induzida pela microinjeção de NMDA na MCPD. No primeiro experimento, ratos foram submetidos à cirurgia para o implante de cânula, divididos em quatro grupos e treinados na caixa de condicionamento ao contexto. Depois do condicionamento, os ratos eram deixados na gaiola habitat. Passado um período, era realizado a microinjeção de NMDA ou salina e suas respostas de fuga registradas. No segundo experimento, ratos foram submetidos à cirurgia para o implante da cânula, divididos em dois grupos. Um grupo recebeu salina (0,9%), intraperitonalmente (i.p.), o segundo recebeu PTZ (15.0 mg/kg i.p.) antes do experimento ter início. Em seguida os ratos foram microinjetados com NMDA na MCPD, e suas resposta de fuga registradas. Nossos dados indicaram que o condicionamento contextual de medo do primeiro experimento e o PTZ do segundo experimento inibiram a fuga evocada pela estimulação química da MCPD (todas as comparações foram estatisticamente significativas, $p < 0,05$). Os resultados sugerem que a circuitaria neural envolvida na ansiedade poderia inibir a atividade da circuitaria envolvida nas respostas de fuga associada a reações de pânico. **Palavras-Chave:** Ansiedade; Medo ao contexto; Comportamento Defensivo; NMDA; Ataque de Pânico; MCPD; PTZ

Apoio Financeiro: CNPq Capes

EXPRESSÃO DA PROTEÍNA FOS EM ÁREAS AMIGDALÓIDES APÓS EMISSÃO DE DIFERENTES ESTRATÉGIAS COMPORTAMENTAIS EM COBAIAS.

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Considerando que a amígdala está envolvida na emissão de respostas comportamentais, este trabalho comparou a expressão da proteína FOS, nesta estrutura, após diferentes comportamentos defensivos, a resposta de imobilidade tônica (IT, emitida após confronto inescapável com o predador) e o congelamento condicionado e incondicionado por exposição ao predador vivo (*Boa constrictor amarali*).

Cobaias machos (± 450 g), foram divididos em 4 grupos. No grupo 1 (N=9), foi induzida a resposta de IT por contenção física e inversão postural (15min). No grupo 2 (N=5), os animais foram expostos ao predador em uma arena (15min), emitindo congelamento incondicionado. Para o congelamento condicionado, as cobaias (grupo 3, N=5) foram expostas ao predador (15min), e 24hs depois colocados no mesmo ambiente sem a serpente (15min). No controle parte dos animais permaneceram nas mesmas condições do grupo IT (N=4) e parte na arena por 15min, sem a presença do predador (N=4). Após 2hs do início, os animais foram anestesiados, perfundidos com salina seguida por paraformaldeído a 4%. Os cérfalos foram removidos, crioprotégidos e processados pela técnica imunocitoquímica para a proteína FOS.

Os dados mostraram que todos os comportamentos estudados promoveram aumento da densidade ($0,1\text{mm}^2/\text{tecido}$) de céls imunorreativas à FOS (IR-FOS) nas diferentes regiões da amígdala. Entretanto, há diferença de intensidade nos diferentes núcleos dependendo da estratégia comportamental adotada. Assim, após emissão de IT ocorreu maior densidade de céls IR-FOS na região posteroventral do núcleo medial (MEA), enquanto que o congelamento incondicionado e condicionado recruta maior número de céls IR-FOS na região posterodorsal do MEA (207 ± 9 e 166 ± 18 , respectivamente). Nos núcleos central, basolateral (BLA) e lateral (LA) maior densidade de céls IR-FOS foi encontrada após o congelamento incondicionado (97 ± 6 ; 72 ± 11 ; 53 ± 5 , respectivamente). Ainda, a IT não aumentou a IR-FOS na região anterior do BLA e do LA.

Nossos dados sugerem que a amígdala está envolvida na elaboração de diferentes respostas defensivas, tal como IT e congelamento, mas o comprometimento de cada núcleo depende da estratégia comportamental escolhida.

Palavras-Chave: Imobilidade Tônica, FOS, Amígdala

Apoio Financeiro: FAPESP

Pentylenetetrazol reduces the number of footshocks required to conditioning an olfactory neutral stimulus

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Objectives. The olfactory fear conditioning (OFC) is generally obtained after the association of 5 mild footshocks (US) with a neutral odor (CS). Pentylenetetrazol (PTZ) is a drug capable to induce anxiogenic-like effect in rodents. Here we test the hypothesis that the PTZ-induced aversive state could strength the US-CS pairing.

Methods. Male Wistar rats were conditioned to an olfactory neutral stimulus in a conditioning box (CB) after being systemically treated with saline or PTZ (15mg/kg). Thirty-min later each subject was exposed to an olfactory stimulus (coffee-odor) paired with 0, 1, 3 or 5 footshocks (0,4mA, 2s duration, 30s inter-trial). The occurrence of US-CS pairing was assessed 24h later by measuring the freezing time (FT) to the same CB and odor. The effectiveness of this pairing was also assessed by testing the CS ability to induce an aversive learning to a different context (odor box, OB). Three 10-min sessions (24, 48 and 72h later) were performed starting 24h after the pairing with the familiarization to the OB. During test session (48h), a cloth containing the CS was placed in the OB. In the context session (72h) a neutral odor cloth was used. The percentage of time approaching the odor source (%AT) was the defensive response index.

Results. ANOVA results showed an increase ($p<0.05$) in the FT in saline/5 shocks ($137\pm 20s$), as well as in PTZ/3 ($141\pm 27s$) or in PTZ/5 ($161\pm 24s$) shocks, when compared to saline/no shock ($17\pm 5s$) or saline/3 shocks ($67\pm 10s$) groups. There was also a reduction ($p<0.05$) in the %AT during the OB test session in subjects from the group PTZ/3 shocks (7 ± 2) when compared to subjects from saline/3 shocks (17 ± 2) or PTZ/no shock (14 ± 2). The same profile of results was found in the OB context session.

Conclusion. There was a reduction in the number of US-CS pairing in subjects pretreated with PTZ. The aversive nature of the PTZ/US-CS pairing was confirmed by showing an increased defensiveness of rats towards both the odor source and the context where it was previously presented. The efficiency of a CS-US in the olfactory fear conditioning depends on the internal mood state, in this case, elicited by the PTZ.

Palavras-Chave: Olfactory fear conditioning; anxiety

Apoio Financeiro: CNPq, CAPES, FAPESP, FAPESC, UFSC

MIDAZOLAM BLOQUEIA O EFEITO ANSIOGÊNICO PRODUZIDO PELO ESTÍMULO AVERSIVO EM CAMUNDONGOS FÊMEAS.

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Objetivo: Estudos prévios mostraram que a administração intraperitoneal interfere nos resultados da reversão do efeito ansiogênico promovido pelo estímulo aversivo no “Step-down” (SD) em camundongos fêmeas (FeSBE, 04.013, 2006). Este estudo avaliou se a via de administração por gavagem poderia interferir no efeito ansiogênico produzido pelo estímulo aversivo e avaliado no labirinto em cruz elevado (LCE) e se o tratamento com midazolam poderia reverter este efeito.

Métodos e Resultados: Camundongos fêmeas, Suíço-albino (peso 37-42g e n= 12), distribuídos aleatoriamente em dois grupos: sem estimulação elétrica (SCh) e com estimulação elétrica (CCh - 0,3mA), foram submetidos à caixa de SD por cinco minutos. Vinte e quatro horas depois os grupos SCh e CCh receberam administração via gavagem de salina ou midazolam (MDZ, 2,0 mg/Kg, v.o.) e após 30 minutos os animais foram submetidos ao SD sem estimulação elétrica. Em seguida, foram submetidos ao LCE para o registro dos índices de ansiedade [porcentagem do número de entradas (%EBA) e de tempo gasto nos braços abertos (%TBA)] e o número de entradas nos braços fechados (EBF), como atividade locomotora. O teste *t* para amostras independentes mostrou que o estímulo aversivo produziu no grupo salina CCh, diminuição da %EBA [$t_{(10)} = 3,63, P < 0,05$] e da %TBA [$t_{(10)} = 2,32, P < 0,05$] e aumento de EBF [$t_{(10)} = 4,77, P < 0,05$], quando comparado ao grupo salina SCh. O tratamento com MDZ no grupo CCh, produziu aumento da %EBA [$t_{(11)} = 4,15, P < 0,05$] e da %TBA [$t_{(11)} = 4,99, P < 0,05$] e diminuição de EBF [$t_{(11)} = 3,65, P < 0,05$], quando comparado ao grupo salina CCh.

Conclusão: A administração por gavagem restaurou o efeito ansiogênico produzido pelo estímulo aversivo (choque) e avaliado no LCE. Também alterou a atividade locomotora no grupo salina CCh. O MDZ produziu diminuição da ansiedade no grupo CCh, ou seja, efeito ansiolítico confirmado pelo aumento da %EBA e da %TBA. O MDZ também alterou a atividade locomotora no grupo CCh. Os resultados sugerem que os receptores GABA-benzodiazepínicos estão envolvidos na modulação da ansiedade induzida pelo choque e avaliada no LCE em camundongos fêmeas.

Palavras-Chave: Camundongos Fêmeas, Midazolam, Gavagem

Apoio Financeiro: PIBIC/ CNPq

E.08.019

Anandamide administration into dorsolateral periaqueductal gray attenuates flight reactions induced by SIN-1

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Activation of cannabinoid CB1 receptors by administration of the endocannabinoid anandamide (AEA) into the dorsolateral periaqueductal gray (dlPAG) induces anxiolytic-like effects in rats submitted to the elevated plus-maze (Neuropharmacology 3:958, 2007), while facilitation of nitrenergic transmission in this region leads to flight reactions (Neurosci Biobehav Rev 7-8:679, 2001; Neurosci Biobehav Rev 8:1313, 2005). The nitric oxide synthase enzyme in its neuronal form (nNOS) and cannabinoid CB1 receptors are present in the dlPAG (J Neurosci 2:563, 1991; Brain Res 1:77, 1993). Although the cannabinoid and nitrenergic systems have been suggested to interact in the central nervous system regions (Eur J Neurosci 3:561, 2001; Clin Exp Pharmacol Physiol 3:223, 2007) it is still unknown if this interaction occurs in dlPAG. **Objective:** To verify if local pretreatment with AEA would attenuate flight reactions induced by SIN-1 (a nitric oxide donor) administration into dlPAG. **Methods:** Male Wistar rats (n=37) with cannulae aimed at the dlPAG received a first microinjection of vehicle or AEA (5 pmol/ 200 nL) followed, 5 minutes later, by a second microinjection of vehicle or SIN-1 (150 nmol/ 200 nL). The rats were immediately placed in a circular arena after the last microinjection for evaluation of flight reactions and total distance travelled during 10 minutes. **Results:** SIN-1 induced flight reactions and increased total distance travelled. These effects were significantly attenuated by pre-treatment with AEA (Vehicle: 12.8 ± 2.0 , SIN-1: 36.8 ± 9.9 , AEA + SIN-1: 20.2 ± 6.1 , $F_{3,16}=3$, $P=0.062$, Duncan, $P<0.05$). **Conclusion:** These results suggest that administration of endogenous cannabinoids into the dlPAG can modulate flight reaction induced by nitric oxide donors.

Palavras-Chave: Endocannabinoids, nitric oxide, defensive reaction, dorsolateral periaqueductal gray

Apoio Financeiro: FAPESP, CNPq and CAPES

ARE THE PERIAQUEDUCTAL GRAY MATTER 5-HT_{2C} RECEPTORS INVOLVED IN THE ONE-TRIAL TOLERANCE PHENOMENON IN MICE?

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Elevated plus-maze (EPM) experienced rodents no longer respond to systemic treatment with anxiolytic drugs on second trial (Trial 2) in this animal model of anxiety. A learned avoidance response hypothesis has been proposed to explain this 'one-trial-tolerance' (OTT) phenomenon in maze-experienced rats and mice. Previous results have demonstrated that the dorsal periaqueductal gray matter (dPAG) inactivation reinstates the anxiolytic-like effect of midazolam on trial 2, and activation of 5-HT_{2C} receptors located within the dPAG modulates fear/anxiety states.

Objectives: The present study investigated the role of the 5-HT_{2C} receptors located in the midbrain PAG on the OTT phenomenon in mice.

Methods: Mice (n=9-14/group) received intra-dPAG infusions of saline or MK212 (21.2 nmol/0.1 microliter), a preferential 5HT_{2C} receptor agonist, 5 min prior Trial 1 and Trial 2 in the EPM.

Results: Two-way ANOVA (drug x trial) showed a decrease in the open arm exploration on Trial 2 [percent of open entries (%OE: (F_{1,42}=12.6, p<0.05); percent of open time (%OT: F_{1,42}=16.9, p<0.05)]. Neither drug nor drug x trial effect was revealed on these behavioral parameters (all F ≤ 2.02, p>0.05). A trial effect was also observed in closed arms entries (F_{1,42}=10.7, p<0.05). Post hoc comparison confirmed a general decrease in closed arm entries, the parameter considered to be a valid index of locomotor activity in the EPM.

Conclusions: These results corroborate previous studies showing that maze-experienced mice explore less the open arms during Trial 2. The failure of MK212 to change anxiety indices on both Trial 1 and Trial 2 suggests that the 5-HT_{2C} receptors located within the dPAG do not play a role on the anxiety modulation in maze-naïve and maze-experienced mice. Further studies with higher doses of MK212 are encouraged to confirm or reject such conclusions.

Palavras-Chave: 5HT_{2C} receptor, periaqueductal gray, one-trial tolerance, elevated plus-maze, mice

Apoio Financeiro: FAPESP, CNPq, PADCFar-UNESP

Effects of chronic treatment with imipramine on BDNF levels in the dorsal periaqueductal gray matter and hippocampus

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Introduction: Antidepressant drugs have been successfully used for the treatment of mood and anxiety pathologies such as depression and panic disorder. It has been suggested that these compounds act on depression by increasing the synthesis and signaling of neurotrophic proteins, mainly BDNF (brain-derived neurotrophic factor), in key depression-associated areas such as the hippocampus and frontal cortex [1]. In the hippocampus, BDNF are shown to increase neurogenesis and this finding has been correlated to the antidepressive effect of imipramine- and fluoxetine-like drugs [1]. However, it is presently unknown if interference with BDNF signaling may also be of relevance for the antipanic effect caused by antidepressants.

Objective: In the current study we compared the effect of chronic administration of imipramine on BDNF levels in the rat dorsal periaqueductal gray matter and hippocampus. The dorsal periaqueductal gray in the midbrain has been considered a critical structure in the mediation of panic attacks [2].

Methods: Male Wistar rats were treated (i.p.) with imipramine (10 mg/kg) or saline for 21 days. Three hours after the last injection, the animals were decapitated and brains were removed on ice. BDNF levels were determined by ELISA and values obtained were expressed as a ratio of the total of protein present in each area.

Results: Animals treated with imipramine caused a significant increase in BDNF levels both in the dorsal periaqueductal gray [Mean $\pm$ SEM of pg BDNF/ μ g total protein – saline=0.7 $\pm$ 0.04; imipramine=0.9 $\pm$ 0.03] and hippocampus [saline=1.4 $\pm$ 0.07; imipramine=2.1 $\pm$ 0.2].

Conclusions: Chronic treatment with imipramine enhances BDNF levels not only in brain areas associated with depression such as the hippocampus, but also in a panic-related structure, the dorsal periaqueductal gray. This latter effect may be of importance for the drug well-know panicolytic activity.

Reference:

1 - Biol Psychiatry 59:1116, 2006.

2 - Neuropharmacology 52:1188, 2007.

Palavras-Chave: Antidepressant, BDNF, Periaqueductal gray

Apoio Financeiro: Fapesp and CNPq, Brazil.

Anxiolytic-like effect of capsazepine administered in the rat ventral hippocampus

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Objectives. The ventral hippocampus (VH) has been implicated in the regulation of defensive behaviors associated with anxiety in rodents. The transient receptor potential vanilloid type 1 (TRPV1) channel is widely expressed in various rodent brain regions, including the HV. Since the role of TRPV1 channels in the central nervous system still remains to be completely understood, the present study evaluated whether the blockade of TRPV1 channels located in the VH would attenuate anxiety-related behaviors.

Methods: Male Wistar rats were bilaterally-implanted with guide cannulas aimed at the VH. One-week later, each subject was infused into the VH with vehicle or the TRPV1 antagonist capsazepine (0.2-6.0 nmol in 0.4 µl), and 10 min later exposed to the elevated-plus maze (EPM) or the cat-odor apparatus (COA). The EPM consists of two open-arms perpendicular with two enclosed-arms, set up 50 cm above the floor. Behavioral measures scored for 5 min in the EPM were the percentage of open-arm time (%OAT), the percentage of open-arm entries (%OAE), stretched-attend postures (SAPs) and enclosed-arm entries (EAE). The COA comprises two compartments, one open and another enclosed, connected by one square hole. A cloth that had been rubbed against a cat back fur/skin was used as an odor source and placed in the open-compartment. During the 10 min session in the COA were scored the percentage of approach time (%AT) and the percentage of hide time (%HT).

Results: One-way analysis of variance followed by Duncan test ($p < 0.05$) showed an increase in %OAT (44 ± 5) and %OAE (58 ± 3) in the group administered with capsazepine (2.0 nmol) when compared to controls (14 ± 3 and 32 ± 5 , respectively). No differences were observed for SAPs and EAE. In the COA, there was an increase in the %AT (128 ± 17 s) and a reduction in the %HT (348 ± 48 s) in the group administered with capsazepine (6.0 nmol) when compared to controls (56 ± 12 s and 476 ± 22 s, respectively).

Conclusions: Capsazepine-treated rats showed less inhibitory avoidance behavior than controls, suggesting a role for VH TRPV1 channels in regulating anxiety-related defensive behavior.

Palavras-Chave: Anxiety; Hippocampus; TRPV1 channel; Capsazepine

Apoio Financeiro: CNPq and FAPESC, Brazil.

AVALIAÇÃO DO PERFIL DE SECREÇÃO DE CORTICOSTERONA EM CAMUNDONGOS SUBMETIDOS AO TESTE DE EXPOSIÇÃO AO RATO (RET).

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O RET é um modelo de interação presa-predador que permite avaliar comportamentos defensivos de camundongos (ex., esquiva, avaliação de risco, congelamento, etc.) diante do rato. Constituído por uma caixa com dois compartimentos similares divididos por uma tela de arame, o RET apresenta um dos compartimentos conectado via um túnel a uma caixa menor que serve de abrigo ao camundongo durante o teste. Estudos têm demonstrado que a exposição ao predador consiste em um modelo de estresse etologicamente relevante e capaz de produzir robusta ativação do eixo hipotálamo-pituitária-adrenal (HPA). Os objetivos do presente estudo foram: (i) investigar se o estresse da exposição ao predador no RET altera a secreção de corticosterona em camundongos e (ii) determinar a magnitude e a duração desta secreção. Camundongos Swiss machos foram colocados no RET com um rato Long Evans (predador) por 10 min. Após o teste os animais foram mantidos isolados em caixas viveiros por 5, 20 e 50 min, sendo em seguida decapitados e os níveis de corticosterona plasmática (CP) medidos. Grupos controles foram mantidos isolados por 5, 20 e 50 min em suas caixas viveiros e submetidos ao mesmo procedimento para a coleta de sangue. Foi observado um aumento significativo nos níveis de CP após 5 min ($14,12 \pm 2,14$ mg/dL) de exposição quando comparados aos níveis após 20 min ($5,92 \pm 0,54$ mg/dL) e 50 min ($5,21 \pm 1,06$ mg/dL). Adicionalmente, a CP após 5 min de exposição ao rato foi significativamente maior em relação ao seu controle (5 min: $5,65 \pm 1,21$ mg/dL; 20 min: $2,97 \pm 0,45$ mg/dL; 50 min: $3,94 \pm 0,99$ mg/dL). Os resultados mostraram que a exposição de camundongos ao seu predador no RET influencia o perfil de secreção de corticosterona, sobretudo após 5 min da exposição, quando foi observado um declínio na CP, provavelmente refletindo os efeitos do *feedback* negativo na adaptação dessa resposta.

Palavras-Chave: Teste de Exposição ao Rato, Corticosterona, camundongos

Apoio Financeiro: PADC/FCFar-UNESP

AVALIAÇÃO DO PAPEL MODULATÓRIO DE RECEPTORES NK1 NO MODELO DE ANSIEDADE EXPERIMENTAL DO ODOR DE GATO.

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Objetivo: O presente estudo teve por objetivo investigar a participação dos receptores NK1 no modelo experimental de odor de gato. **Métodos e Resultados:** Este modelo consiste em uma caixa retangular de madeira, dividida em dois compartimentos, um aberto (40 x 26 x 36 cm) e um fechado (20 x 26 x 36 cm). Na parede frontal da porção fechada da caixa há uma pequena abertura (6 x 6 cm) com espaço suficiente para o animal transitar entre os dois lados do modelo. Na parede oposta do compartimento fechado, um pano, previamente impregnado com odor de gato, é utilizado como estímulo aversivo. Um pano idêntico, porém sem o odor serve como controle. Quando os ratos são expostos ao modelo, mede-se o tempo de *head-out*, tempo de aproximação e tempo escondido, assim como medidas relacionadas à locomoção do animal. Os ratos previamente canulados foram tratados pela via intracerebroventricular com WIN, um antagonista seletivo de receptores NK1, nas doses de 1, 10, e 100 pmol, e, 5 min após, foram avaliados comportamentalmente no teste de odor de gato. Nossos resultados mostraram que o WIN, nas doses de 1 e 10 pmol, induziu um aumento no tempo de aproximação da fonte de odor (PBS=10,41±3,50; WIN 1=18,20±3,24; WIN 10=21,4±3,32; WIN 100=11,41±2,97; F(3,21)=2,4397; p = 0,092, ANOVA de 1 via) e uma diminuição no tempo escondido (PBS=65,55±8,49; WIN 1=51,18±8,25; WIN 10=35,54±8,35; WIN 100=50,72±13,50; F(3,21)=1,4560. p= 0,2551; ANOVA de 1 via). **Conclusões:** Nossos resultados parciais mostram que o bloqueio dos receptores NK1 promove um conjunto de alterações comportamentais em ratos (aumento do tempo de aproximação e diminuição do tempo escondido) sugestivo de um efeito do tipo-ansiolítico, reforçando o papel modulador dos receptores NK1 na ansiedade experimental. **Palavras-Chave:** Neurocininas, Ansiedade Experimental e Odor de Gato

Apoio Financeiro: CAPES

Effects of amygdaloid lesions on contextual fear conditioning in two new lines of rats selectively bred for high and low conditioned freezing

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Rats selectively bred for high or low emotionality represents an important tool to investigate the role of genetic variables in the occurrence of different anxiety disorders. In the present study, albino rats were selectively bred for differences in defensive freezing behavior to contextual cues previously associated with footshock, an animal model of general anxiety disorder. Results indicated that these two new lines of rats, named Carioca High Freezing (CHF) and Carioca Low Freezing (CLF), presented a reliable difference in conditioned freezing after 3 selected generations. Males from both lines consistently presented more freezing than females. A reliable difference between CHF and CLF male lines was detected in the third (S3) generation ($t(74)=4.47$; $p<0.001$). This same pattern was observed among females. A reliable difference between CHF and CLF lines within females was detected in S3 ($t(80)=5.31$, $p<0.001$) but not in S1 ($t(74)=0.55$; $p>0.5$) or S2 ($t(67)=0.92$; $p>0.3$). Overall, males rats froze 59.9% (± 2.03) while the females froze 50.9% (± 1.90). A student t-test indicated a significant difference between these two groups ($t(446)=3.24$; $p<0.01$). These results represent the first attempt to successfully select rats with reliable differences in conditioned freezing and extend previous report, which also found a bidirectional short-term selection of this conditioned response among mice (Ponder et al.: Genes, Brain and Behavior, Nov;6(8):736-49, 2007). A second study employed male rats from the fourth generation to investigate the participation of the amygdala during contextual fear conditioning in CHF and CLF lines. Results indicated that post-training amygdaloid electrolytic lesions led to similar disruption in conditioned freezing behavior in both lines of animals. The analyses revealed that amygdaloid lesion led to a reduction of 60.6% in the CHF and 63.0% in the CLF lines. These results are discussed in terms of possible neural circuitry involved in defensive freezing behavior.

Palavras-Chave: Anxiety; Selected Lines; Amygdala

Apoio Financeiro: CNPQ

ANXIETY AND ANTINOCICEPTION INDUCED BY MICE EXPOSURE TO DIFFERENT TYPES OF ELEVATED PLUS-MAZE: EFFECTS OF BILATERAL LESIONS OF DORSAL PERIAQUEDUCTAL GRAY.

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The midbrain periaqueductal gray is one of the structures involved in the modulation of the defensive reaction and of the descendent inhibitory pain system. We have demonstrated that bilateral lesion of the dorsal column of this structure (dPAG) attenuated anxiety in the standard elevated plus-maze (sEPM) in mice (Mendes-Gomes & Nunes-de-Souza, IBNS: abstract 143, 2007). In addition, mice exposure to an open EPM (oEPM: an EPM with 4 open arms) induces a marked antinociception (Mendes-Gomes & Nunes-de-Souza, Brain Res., 1047: 97, 2005). This study investigated the effects of bilateral lesion of dPAG on anxiety (assessed in the sEPM) and oEPM-induced antinociception.

Swiss male mice (n=9-18/group) had the dPAG bilaterally lesioned with NMDA (N-methyl-D-aspartic acid, 2 µg/0.2 µl) injection. Six days later, animals received 50 µl of formalin 2.5% into the dorsal right hind paw and 25 minutes later they were individually exposed to the sEPM (2 open arms and 2 closed arms), oEPM, or enclosed EPM (eEPM: four closed arms; control situation) to record the time spent on licking the formalin injected paw during a 10-min period. For sEPM exposed animals, the frequency of closed arms entries (CE), percent open arms entries (%OE) and percent open arms time (%OT) were also recorded for 5 minutes.

dPAG	Time spent on licking (s)			sEPM		
	eEPM	sEPM	oEPM	CE	%OE	%OT
Sham	150±24	100±20 ^a	8±4 ^{ab}	10±1	32±5	20±4
Lesioned	138±12	93±17 ^a	13±3 ^{ab}	14±2 ^c	26±4	22±6

a,b,c $p < 0.05$ vs eEPM, sEPM and sham group, respectively.

Present results showed that bilateral dPAG lesion did not alter anxiety indices in mice that had received formalin injection. Considering our previous results in that bilateral lesion of dPAG reduces anxiety in animals under normal test conditions (i.e., in absence of nociception), we suggest that pain stimulus has somehow impaired the anxiolytic effect of the dPAG bilateral lesion. Furthermore, oEPM-induced antinociception was not prevented by dPAG lesion, suggesting that this type of environmentally induced pain inhibition does not recruit the dorsal columns of this midbrain structure. It remains undetermined the role of ventrolateral portion of the PAG on oEPM-induced antinociception. **Palavras-**

Chave: fear/anxiety, antinociception, formalin test, dorsal periaqueductal gray, elevated plus-maze, mice

Apoio Financeiro: FAPESP; CNPq; PADCFar-UNESP

ADMINISTRAÇÃO DE ONDANSETRON NA AMÍDALA DE CAMUNDONGOS PRODUZ EFEITO ANSIOLÍTICO

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Objetivo: Avaliar os efeitos do ondansetron (OND), antagonista dos receptores 5-HT₃, administrado intra-amídala (AMI) em camundongos submetidos ao labirinto em cruz elevado (LCE).

Métodos e Resultados: Camundongos machos, Suíço-albino, foram submetidos a cirurgia estereotáxica e após 4 dias de recuperação receberam injeções intra-AMI de OND (0, 0,01, 0,1, 0,3, 1,0 µg/0,1µl). Após 7 minutos da injeção, os animais foram expostos individualmente ao LCE para o registro dos índices convencionais de ansiedade [porcentagem de entradas (%EBA) e de tempo gasto nos braços abertos (%TBA), e entradas nos braços fechados (EBF), como atividade locomotora] e os índices etológicos [frequência de levantar (TLEV), mergulhar (TMERG), esticar (TEST) e imobilidade (TI) e porcentagem de mergulhar (%MERGP) e de esticar protegido (%ESTP)]. A ANOVA seguida do teste de Duncan mostrou que o tratamento com OND alterou significativamente os índices de ansiedade e etológicos conforme tabela abaixo.

Ondansetron (ug/0,1ul)					
Comportamentos	0 (n=12)	0,01 (n=11)	0,1 (n=8)	0,3 (n=9)	1,0 (n=11)
EBF	9,08 ± 1,1	9,45 ± 0,8	7,6 ± 0,8	10,7 ± 1,4	7,6 ± 1,1
%EBA	31,4 ± 5,8	38,1 ± 6,3	55,2 ± 6,9*	47,7 ± 6,8	45,8 ± 6,1
% TBA	12,9 ± 3,5	22,4 ± 4,9	32,3 ± 6,2*	31,7 ± 7,1*	32,6 ± 5,2*
TMERG	25,6 ± 4,6	16,9 ± 4,8	23,2 ± 5,2	38,0 ± 4,7	31,1 ± 5,1
%MERGP	65,8 ± 6,2	48,8 ± 7,5	34,7 ± 7,3	40,7 ± 11,3	48,1 ± 8,3
TEST	44,9 ± 4,6	33,4 ± 3,1	31,0 ± 4,4	29,1 ± 6,3	40,5 ± 5,5
%ESTP	81,1 ± 4,4	39,6 ± 6,0*	57,4 ± 9,2*	63,1 ± 9,2	56,1 ± 7,8*
TLEV	20,3 ± 3,3	12,1 ± 3,8	8,9 ± 2,7*	24,8 ± 4,3	12,0 ± 2,9
TI	15,8 ± 6,7	18,2 ± 12,2	25,0 ± 16,4	10,7 ± 7,4	22,2 ± 13,4

*P < 0,05 comparado ao veículo (0).

Conclusão: O OND produziu diminuição dos índices de ansiedade, enquanto que a dose mais baixa não produziu efeito. Nenhuma das doses testadas afetou a atividade locomotora. Os índices etológicos, foram seletivamente diminuídos pelo OND, corroborando com a redução da ansiedade. O bloqueio dos receptores 5-HT₃ da AMI produz efeito ansiolítico, sugerindo que a serotonina pode apresentar papel ansiogênico nestes receptores.

Palavras-Chave: Ansiedade, Serotonina, Receptores 5-HT₃, Labirinto em Cruz Elevado, Amídala, Camundongos.

Apoio Financeiro: UFSCar, FAPESP e CAPES

E.08.028

The prelimbic prefrontal cortex may be involved in the attenuation of contextual fear induced by cannabidiol

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Previous results showed that systemic administration of cannabidiol (CBD), a non-psychotomimetic constituent of *Cannabis sativa*, reduces the behavioral and cardiovascular changes induced in rats by re-exposition to a chamber previously paired with footshocks. The brain structures related to these effects, however, remain poorly understood. In the present study c-Fos immunohistochemistry was used to try to identify these brain sites. Twenty-four after being exposed to an experimental box where they had been submitted to a series of 6 foot shocks (1s, 0.5 mA) male Wistar rats received i.p. injections of either vehicle (tween 1% plus propilenoglicol 10% in saline) or CBD (10 mg/Kg). Thirty min later the animals were re-exposed to the same experimental box for 10 min where freezing behavior was recorded. Two h later the rats were sacrificed and the brains removed and processed for c-Fos immunohistochemistry. Conditioned animals show increased freezing and higher c-Fos expression in several brain structures such as the prefrontal cortex, central amygdaloid nucleus, hypothalamus and periaqueductal gray. Pretreatment with CBD attenuated c-Fos expression in prelimbic (PL) and infralimbic prefrontal cortex. In an additional experiment CBD (15, 30 or 60 nmol) was microinjected into the PL 10 min before re-exposition to the aversively conditioned box. CBD (30 nmol) was able to significantly decrease freezing time in the box. The results suggest, therefore, that the prelimbic prefrontal cortex may be a brain site for the attenuation of contextual fear observed after systemic administration of CBD. **Palavras-Chave:** cannabidiol, fear conditioning, prefrontal cortex

Apoio Financeiro: FAPESP and CNPq.

EFEITO DA NEUROTRANSMISSÃO OPIOIDÉRGICA DO NÚCLEO DORSAL DA RAFE SOBRE O COMPORTAMENTO DEFENSIVO DE IMOBILIDADE TÔNICA EM COBAIAS.

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Objetivo: Alguns trabalhos demonstraram a participação dos receptores opioidérgicos do núcleo dorsal da rafe (NDR) na elaboração de algumas respostas comportamentais defensivas. Estudos de nosso laboratório comprovaram o envolvimento do sistema opioidérgico na modulação da resposta defensiva de imobilidade tônica (IT) em diferentes estruturas encefálicas. Assim, o objetivo do presente trabalho foi investigar a participação do sistema opioidérgico do NDR sobre a resposta de IT em cobaias (*Cavia porcellus*).

Metodologia: Cobaias machos (420 - 480 g) foram submetidos a 5 episódios de IT (controle). A indução da IT consiste de inversão e contenção postural, sendo a duração dos episódios registrada em segundos (s) até o instante em que o animal recupera sua postura habitual. Implantou-se cânula-guia no NDR e após 5 a 7 dias, todos os animais foram novamente submetidos a sessões de IT para controle pós-cirúrgico (sham). No dia seguinte, após a microinjeção das drogas, registrou-se a duração dos episódios de IT. Todas as microinjeções foram realizadas em um volume de 0,2 µl. A morfina microinjetada nas duas doses (1,1 e 2,2 nmol) no NDR aumentou significativamente a duração dos episódios de IT ($p < 0.05$, ANOVA), cujos valores médios foram: controle ($158,64 \pm 40,11$ s), sham ($217,99 \pm 55,69$ s) e morfina ($506,42 \pm 95,48$ s) para a primeira dose ($n = 10$); e controle ($295,82 \pm 114,15$ s), sham ($295,81 \pm 81,61$ s) e morfina ($784,31 \pm 107,48$ s) para a segunda dose ($n = 7$). O efeito de aumento da IT promovido pela morfina na dose de 1,1 nmol foi bloqueado pela microinjeção prévia de naloxone (2,7 nmol; $n = 8$), cujas durações médias foram: controle ($147,78 \pm 38,25$ s), sham ($202,58 \pm 61,45$ s), morfina ($510,70 \pm 111,81$ s) e naloxone+morfina ($228,71 \pm 87,52$ s). Entretanto, as microinjeções de salina ($n = 10$) e naloxone ($n = 11$) no mesmo sítio não alteraram a duração da IT ($p > 0,05$; ANOVA).

Conclusão: A microinjeção de morfina no NDR promoveu o aumento na duração dos episódios de IT, indicando que a ativação de receptores opioidérgicos no NDR tem ação facilitatória para a resposta comportamental defensiva de IT.

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Beta-adrenergic receptors in the dorsal premammillary nucleus of rats mediate defensive responses exhibited during an olfactory fear conditioning paradigm

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Objectives: In rodents, the olfactory system is critical to detect threatening stimulus. Recent studies revealed an increased Fos protein activity in the dorsal premammillary nucleus (PMd) of rats during the olfactory fear conditioning (OFC) paradigm, obtained after the association of 5 mild footshocks (US) with a neutral odor (CS). In addition, propranolol, a beta-adrenergic antagonist, systemically applied, reduced the defensive behavior (DB) exhibited during the confrontation to the olfactory CS. For this reason, cued OFC was conducted to verify the role of adrenergic receptors in the PMd during the exposure to the olfactory CS in a novel environment.

Methods: Wistar rats received cannula implants aimed at the PMd. Seven days after the surgery, all subjects received 5 pairings of US (footshock, 0,4mA, 2s duration, 40s inter-trial)-CS (250 µl, 5%amyl acetate) in a conditioning box (CB). Furthermore, 24 h after the rats were placed in a different context (odor box, OB) for 10 min familiarization. During the test session (48h), ten min after receiving (0.3µl) PBS or Atenolol (ATE; 40nmol, a beta1-antagonist) the rats were confronted to the cloth containing the CS placed in the OB. During the context session (72h), the rats were confronted to OB with a neutral odor cloth. The percentage amount of time the rats spent near the odor source (%AT), and the time spent hiding (%HT) were taken as an index of DB.

Results: ATE produced a significant ($p<0.05$) increase in the %AT (27 ± 3) during the test session when compared to subjects from the PBS (15 ± 6) or ATE-out (11 ± 1) groups. The same profile of results was obtained during the context session [ATE (30 ± 5); PBS (5 ± 2); ATE-out (9 ± 1)]. ATE reduced ($p<0.05$) the %HT (41 ± 6) when compared to the ATE-out (84 ± 2) and PBS (76 ± 7) groups. The same profile of results was obtained during the context session [ATE (38 ± 6); ATE-out (79 ± 2); PBS (87 ± 3)].

Conclusion: ATE in the PMd reduced the DB elicited by the olfactory CS and also by the context. These results suggest a role of the beta-adrenergic receptors in the PMd in the expression of DB to a learned OFC in addition to its well known effects in reducing the DB to predatory odor. **Palavras-**

Chave: Defensive behavior, olfactory fear conditioning, dorsal premammillary nucleus, adrenergic receptors

Apoio Financeiro: CNPq, CAPES, FAPESP, FAPESC, UFSC

**ANTINOCICEPÇÃO INDUZIDA PELO MEDO EM CAMUNDONGOS FÊMEAS:
CORRELAÇÃO COM O LOCAL DAS CONTORÇÕES E A FASE DO CICLO ESTRAL**

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Objetivos: Evidências enfatizam que situações de medo podem inibir a resposta de dor (Behav. Brain. Sci, 3:291, 1980; Psychopharmacol, 150(3):300, 2000). Além disso, a flutuação de hormônios ovarianos (estrógeno e progesterona) durante as fases do ciclo estral pode interferir em mudanças comportamentais em ratos fêmeas (Psychoneuroendocrinol, 609, 1996). Este estudo avaliou a antinocicepção induzida pela presença do predador em camundongos fêmeas e a correlação com o local das contorções e a fase do ciclo estral.

Métodos e Resultados: Camundongos suíços fêmeas (25-35g) foram submetidos à habituação por 3 dias consecutivos (10 min.) no aparato com exposição ao estímulo neutro (rato de brinquedo-RB). No 4º dia receberam injeção de ácido acético (0,6%, 10 ml/Kg, i.p.). Permaneceram em gaiolas individuais até o início das contorções (máximo 5 min.) e então cada animal foi exposto à presença do rato de verdade (RV), para o registro do número de contorções abdominais (resposta nociceptiva) e coleta da secreção vaginal para avaliação do ciclo estral. A ANOVA de duas vias (estímulo x local de contorções) mostrou diferença significativa em relação ao local de contorções ($F_{(1,40)} = 20,26, P < 0,05$), e interação entre estímulo x local de contorções ($F_{(1,40)} = 15,13, P < 0,05$) e ausência de efeito do estímulo ($F_{(1,40)} = 2,18, P > 0,05$). O teste de *post hoc* mostrou que os animais expostos à presença do RV apresentaram diminuição no número de contorções abdominais quando permaneciam no local desprotegido da caixa ($p < 0,05$), quando comparados à presença do RB e ao grupo (RV) que contorcia no local protegido do aparato ($p < 0,05$). Para o ciclo estral os resultados foram: RB (64% metaestro, 18% proestro, 9% diestro e 9% estro) e RV (73% diestro, 18% proestro e 9% metaestro).

Conclusão: A exposição de camundongos fêmeas ao estímulo aversivo, rato de verdade, produz antinocicepção, principalmente quando os animais contorcem no lado desprotegido do aparato. No diestro os animais estão mais sensíveis ao estímulo aversivo e com mais ansiedade/medo, o que ocorreu no grupo RV.

Palavras-Chave: Antinocicepção; camundongos fêmeas; ciclo estral

Apoio Financeiro: UFSCar/Fapesp

Comportamentos agressivos na adolescência e a presença de Transtornos Mentais Comuns – Um estudo de base populacional

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Introdução: A agressão física é uma forma comum de violência interpessoal entre os adolescentes de diferentes culturas. Em geral, tais atitudes estão ligadas ao caráter exploratório desta etapa do desenvolvimento, porém podem levar à consolidação destes comportamentos com significativas consequências negativas nos níveis individual, familiar e social. Os fatores de risco para comportamentos violentos podem ser encontrados no indivíduo, no meio em que ele vive ou na capacidade individual de responder às demandas do ambiente.

Objetivo: Avaliar a prevalência dos comportamentos agressivos e a associação destes comportamentos com transtornos mentais comuns (TMC) em adolescentes de 15 a 18 anos, em uma cidade de porte médio no Sul do Brasil.

Métodos: Estudo transversal de base populacional com uma amostra de 960 adolescentes entre 15 e 18 anos, residentes na cidade de Pelotas/RS. Sortearam-se, aleatoriamente, 90 setores censitários a partir de 448 da cidade de Pelotas e foram visitados 86 domicílios de cada setor. Como comportamento agressivo considerou-se separadamente duas variáveis dicotômicas: portar alguma arma ou participar de briga com agressão no último ano. A ocorrência de TMC, nos últimos 30 dias, foi avaliada através do Self-Report Questionary (SRQ-20). Na análise estatística utilizou-se a Regressão de Poisson com um modelo hierárquico para cada um dos desfechos e controle para efeito de delineamento.

Resultados: A prevalência dos comportamentos estudados na presente amostra foi de 22,8% para participação em brigas com agressão e 9,6% para porte de armas. Após a análise ajustada, concluiu-se que a presença de transtornos mentais comuns acrescenta 63% de risco (RR:1,63 – IC:1,18 a 2,56) para portar armas nos últimos 12 meses ($p=0,003$) e aumenta em 40% as chances (RR:1,40 – IC:1,12 a 1,74) destes jovens envolverem-se em brigas ($p=0,003$), em relação aos adolescentes que não apresentavam os problemas psicológicos estudados.

Conclusões: Problemas psicológicos do indivíduo se configuraram como fator de risco para comportamentos violentos; os jovens que apresentaram transtornos mentais comuns estavam mais propensos a portar armas e brigar.

Palavras-Chave: Adolescente, agressão, briga, comportamento agressivo, epidemiologia, porte de armas.

DOS ESPECIES DE PECES ELÉCTRICOS CON ESTRATEGIAS SOCIALES DIFERENTES COMO MODELO DE AGRESIÓN EN VERTEBRADOS.

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Dos especies de peces eléctricos de descarga débil con estrategias sociales diferentes son usados como modelos neuroetológicos para el estudio de la conducta agonística-agresiva: *Brachyhypopomus pinnicaudatus* (gregario, reproductor estacional, con dimorfismo sexual) y *Gymnotus omari* (solitario, reproductor estacional, sin dimorfismo sexual). Los comportamientos agonísticos-agresivos se encuentran ampliamente conservados en peces teleósteos, por lo que las diferencias interespecíficas pueden explicarse por modulación de estos circuitos ancestrales.

Objetivos: Caracterizar la conducta agresiva en diadas de *B. pinnicaudatus* y *G. omari* para realizar un estudio comparativo entre ambas especies. Analizar los despliegues eléctricos y locomotores y evaluar el efecto de la administración de AVT (neuropéptido hipotalámico modulador del comportamiento social en otros vertebrados) sobre estos despliegues.

Métodos: Se caracterizaron los despliegues locomotores (unidades dinámicas y posturales) y las señales eléctricas sociales (SES, chirps e interrupciones) de diadas en libre movimiento de ambas especies (por protocolo residente-intruso y de compuerta) a partir del registro simultáneo de imagen y actividad eléctrica. Se diseñaron índices para la comparación interespecífica. Se evaluó el efecto de AVT (IP, 10 µg/gr).

Resultados y discusión: La conducta agonística de ambas especies incluye despliegues locomotores y eléctricos. Como resultado de cada combate, se establecen marcadas relaciones de dominancia y subordinación. Las unidades más características fueron las mordidas, aproximaciones del dominante y alejamientos del subordinado. Los despliegues eléctricos incluyeron chirps e interrupciones preferentemente emitidos por el subordinado. Particularmente, los índices aproximación-alejamiento del dominante y mordidas del subordinado-dominante, y el mayor número de SES observadas en *G. omari*, la señalan como una especie más agresiva. El AVT afectó los despliegues locomotores y eléctricos de la conducta agresiva de ambas especies y se observaron acciones diferenciales que podrían estar en la base de los mecanismos que subyacen a las diferentes estrategias sociales.

Palavras-Chave: Agresión, neuromodulación, Vasotocina, pez eléctrico.

Apoio Financeiro: Fondo Clemente Estable

EFEITO DA MICROINJEÇÃO DE SOMATOSTATINA NA AMÍGDALA MEDIAL PÓSTERO-DORSAL SOBRE O COMPORTAMENTO AGRESSIVO DE RATOS.

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Objetivos: Analisar o papel da somatostatina na amígdala medial póstero-dorsal (MePD) no comportamento agressivo de ratos machos.

Métodos e Resultados: Foram utilizados animais do biotério da UFCSPA, com cerca de 3 meses de idade, pesando aproximadamente 300g. Os machos eram separados por 7 dias em caixas individuais. No outro dia, (dia 1) sofreram contensão manual por 1 min e esta era repetida no 3º dia, sendo que após 1 min da contensão foi realizado o *screening* do comportamento agressivo. Este consistiu em colocar um intruso na caixa do residente e observar o comportamento agressivo do residente por 10 min (sem nenhuma injeção de droga). No 4º dia foi realizada cirurgia estereotóxica para implante unilateral (lado direito) da cânula na MePD no encéfalo. No 7º dia, os animais foram divididos em 2 grupos: grupo 1 (n=4) recebeu microinjeção de salina (0,3µL); grupo 2 (n=4) recebeu somatostatina (1µM/0,3µL). No 8º dia, a droga foi microinjetada na MePD e, 10 min após, foi realizado o teste comportamental que teve duração de 15 min. Os comportamentos analisados foram: investigação social, lutar, morder, postura agressiva, boxear e caminhar. No 9º dia foi feito o pós-teste (sem nenhuma injeção de droga) por 10 min. Após o pós-teste, os animais foram eutanasiados para análise histológica e os registros foram analisados com auxílio do software Noldus (Holanda). As médias das frequências dos comportamentos ($\pm$ E.P.M.) foram analisadas através de um teste t ($p < 0,05$). Nossos resultados são preliminares e mostraram que não houve diferença significativa na frequência dos comportamentos de lutar, morder, boxear em ambos os grupos durante o teste comportamental sem injeção de droga. Além disso, após a microinjeção de somatostatina na MePD, observamos que não houve diferença significativa na frequência dos comportamentos de lutar ($2,10 \pm 2,0$; $2,00 \pm 1,5$), boxear ($0,0 \pm 0,0$; $0,0 \pm 0,0$), investigação social ($22,0 \pm 15,0$; $25,0 \pm 4,0$; respectivamente, droga e salina) quando comparado ao grupo salina.

Conclusão: nossos resultados embora preliminares demonstraram que a microinjeção de somatostatina na MePD não altera o comportamento agressivo de ratos machos.

Palavras-Chave: Comportamento Agressivo; Somatostatina; MePD

Apoio Financeiro: Apoio Financeiro: CAPES e PIC/UFCSPA

**VARIABILIDADE COMPORTAMENTAL OBSERVADA NO LABIRINTO EM “T” ELEVADO:
UMA PROPOSTA DE INTERPRETAÇÃO**

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O Labirinto em “T” Elevado (LTE) é um modelo animal que distingue, no mesmo sujeito, dois níveis de estados motivacionais relacionados ao comportamento defensivo: pânico via fuga e ansiedade via esquivas inibitórias. Ratos testados desta forma tendem a aumentar o tempo de permanência no braço fechado e reduzi-lo no braço aberto ao longo das repetições de exposição. Entretanto, observa-se que alguns indivíduos contrariam essa tendência, fato que estaria relacionado a outras estratégias de defesa. Com base nessa hipótese, este estudo investigou se há correlação entre a variabilidade dos comportamentos manifestados nos testes de LTE e em outros testes de comportamento defensivo. Para tanto, 24 ratos Wistar machos adultos (365-505g) foram inicialmente habituados por 30 minutos (restritos ao braço aberto) e submetidos a cinco exposições consecutivas, com intervalos de 30 segundos, a cada tipo de braço (fechado com paredes transparentes e aberto, nesta ordem) no LTE. Após três semanas, os ratos foram submetidos à Caixa de Esquiva Passiva (CEP). Os mesmos animais foram expostos ao Campo Aberto (CA) depois de cinco dias e posteriormente testados na CEP. Segundo a matriz de correlação, com determinação do coeficiente de Spearman (r) estabelecido como significativo se $p < 0,05$, houve correlação positiva entre: as repetições da esquivas no LTE ($r = 0,63$ a $0,97$); as repetições de fuga no LTE ($r = 0,69$ a $0,99$); as esquivas no LTE e o tempo que os ratos desciam da plataforma no último teste na CEP apenas com uma das patas ($r = 0,60$ a $0,91$); a descida com uma das patas no primeiro teste na CEP e as três primeiras fugas no LTE ($r = 0,72$ a $0,79$); a quarta e quinta fugas com o tempo de permanência no centro do CA ($r = 0,55$ a $0,56$). Não houve correlação com demais parâmetros do CA, nem das fugas com as esquivas no LTE. Conclui-se que a variabilidade do mesmo indivíduo é coerente para as esquivas, assim como para as fugas, mas não entre esses parâmetros. Elevadas latências no LTE podem estar associadas com a avaliação de risco. A repetição de exposição ao braço aberto acarreta o aumento da latência de fugas, que pode estar relacionado com a redução de ansiedade.

Palavras-Chave: Labirinto em "T" Elevado; Esquiva Passiva; Campo Aberto; avaliação de risco; pânico; ansiedade

Apoio Financeiro: FAPESP

INFLUENCE OF FOOD RESTRICTION ON THE GROWTH RATE AND DOMINANCE HIERARCHY IN PIAU FISH *Leporinus piau*

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Objectives: The animal size is described as the main factor for the dominance hierarchy and, in many studies, the individual growth rate is straightly proportional to the hierarchical rank in the shoal. The objectives of this study were investigating the agonistic interactions in juvenile piau fish *Leporinus piau*, and check the effect of food restriction on the growth rate and hierarchical organization.

Methods: The first experiment was carried out with pairs of fish and the agonistic behaviors were identified and characterized. The most aggressive fish was characterized as the dominant. After that, the animals were grouped in shoals with five subjects with no food restriction (control group: ratio of food/fish of 3% w/w, n=10) or with restricted food supplement (1%, n=10). All occurrence samples of agonistic behaviors were videotaped during 21min, in three consecutive stages (1 and 2 – baseline; 3 – experimental), in which measurements of body weight were taken to evaluate the growth rate.

Results: In 20 pairs, 75 % of the biggest piaus gave out more agonistic behaviors than the smaller ones. Among the shoals, after changing the food supply, animals submitted to food restriction had significantly lower growth rates when compared to the control group in the baseline ($W = -55.0$; $P = 0.002$) and in the experimental phase ($T = 155.0$; $P \leq 0.001$). Quantifying of aggressive acts, the food restriction group presented bigger values than the control in the stage 3 ($T = 71.0$; $P = 0.011$). In 90 % of the control shoals, the dominant, in spite of not being the biggest it was always bigger than subordinates. However, in the food restriction group, the relation between size and aggressiveness just occurred in 40 % of the shoals.

Conclusions: Piaus submitted to food restriction grew less and they are more aggressive to conspecifics than the control ones. It suggests that hunger can increase aggression and to make the least stable hierarchical relations. In addition, the animal size is important for the dominance, but it is not the exclusive factor.

Palavras-Chave: *Leporinus piau*, agonistic behavior, food restriction, growth rate, dominance hierarchy

Apoio Financeiro: PIBIC/UFSJ, PIBIC/FAPEMIG and UFSJ

UMA ABORDAGEM MULTIDISCIPLINAR DA OBESIDADE: Imagem Corporal X Traços Depressivos

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TEMA: Em um contexto sócio cultural onde a estética corporal é supervalorizada, pessoas obesas tornam-se, em geral, presas fáceis da discrepância existente entre o peso da realidade física (RF) e a leveza da imagem corporal idealizada (ICI). O presente trabalho, de caráter neuropsicológico cognitivo, visa avaliar a incidência de tal discrepância em mulheres que optaram pelo procedimento cirúrgico para reduzir a obesidade e a influência da obesidade no estado emocional das mesmas.

OBJETIVOS: Avaliar a incidência de discrepância entre a RF e a ICI idealizada em mulheres obesas decididas a se submeterem à cirurgia Bariátrica (CB). Avaliar a incidência de traços depressivos (TD) na personalidade de mulheres obesas, decididas a se submeterem à CB, que apresentem discrepância entre a RF e a ICI.

MÉTODOS: Trata-se de uma pesquisa descritiva exploratória, que se configura como pesquisa bibliográfica e de campo. A amostra constituiu-se por pacientes obesos do Hospital São José do Avaí, em Itaperuna, RJ, em fase pré-operatória da CB. A seleção de sujeitos adultos, do sexo feminino, com idade entre 20 e 60 anos, seguiu o critério de amostra não probabilística do tipo “acidental”. Utilizaram-se, como instrumentos de pesquisa, para medir a incidência de TD, o Inventário de Depressão de Beck (BDI), e para medir o grau de discrepância entre o RF e o ICI, a Escala de Figuras de Stunkard (FRS).

HIPÓTESES: Sujeitos que optaram pela CB não apresentam um nível de discrepância significativa entre a RF e a ICI. Sujeitos que optaram pela CB apresentam discrepância significativa entre a RF e a IC. Sujeitos que apresentam discrepância significativa entre a RF e a ICI também apresentam uma incidência significativa de TD em sua personalidade. Sujeitos que não apresentam discrepância significativa entre a RF e a ICI não apresentam uma incidência significativa de TD em sua personalidade.

CONCLUSÃO: A presente pesquisa encontra-se em processo de análise dos dados. Os primeiros resultados sugerem que há uma prevalência de algum nível de depressão em 81,25% da amostra. No entanto, os níveis mais altos de depressão ocorrem nos níveis mais baixos de obesidade. Quanto a imagem corporal dos sujeitos da amostra, os resultados insinuam que há maior discrepância entre a RF e a ICI nos níveis mais baixos de obesidade, mostrando a correlação existente com a depressão.

Palavras-Chave: OBESIDADE; IMAGEM CORPORAL; TRAÇOS DEPRESSIVOS.

LA VASOTOCINA MODULA LOS DESPLIEGUES ELÉCTRICOS Y LOCOMOTORES DEL COMPORTAMIENTO SOCIAL EN PECES ELÉCTRICOS

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La participación de los neuropéptidos como moduladores del comportamiento social se ha demostrado en varias especies de vertebrados, desde peces hasta mamíferos (vasopresina y oxitocina en mamíferos, y vasotocina, AVT e isotocina en no mamíferos).

Objetivos: explorar el rol del AVT en la modulación de los comportamientos reproductivo y agresivo en *Brachyhypopomus pinnicaudatus*, a través del análisis de despliegues locomotores y eléctricos.

Métodos: *B. pinnicaudatus* es una especie gregaria, de hábitos nocturnos, con reproducción estacional y que presenta conducta agresiva entre machos sólo durante el período reproductivo. Se registraron simultáneamente las descargas eléctricas y despliegues locomotores de diadas macho-hembra y macho-macho dentro y fuera del período reproductivo. Se analizaron dos tipos de despliegues eléctricos: el incremento nocturno de la frecuencia basal de la descarga del órgano eléctrico (INFB-DOE) y señales eléctricas sociales (SES). El incremento nocturno es mayor durante el período reproductivo (Physiol Behav. 525,90, 2007). Las SES son fácilmente identificables y propias de cada tipo de conducta. Los despliegues locomotores se analizaron utilizando las unidades conductuales posturales y dinámicas.

Resultados y Conclusiones: La administración diurna de AVT a machos aislados o en diadas produce un incremento persistente de la frecuencia basal de la DOE que remeda el INFB-DOE. En diadas macho-hembra, el efecto del AVT sobre la frecuencia basal de la DOE presenta variaciones estacionales similares a las que exhibe naturalmente el INFB-DOE. En diadas macho-macho en período reproductivo, el efecto es similar a lo observado en diadas macho-hembra. El efecto del AVT sobre el INFB-DOE no parece depender del tipo de interacción social. Estos resultados sugieren que el AVT podría ser el mediador del INFB-DOE descrito en esta especie; lo que se evaluó mediante la acción de bloqueantes de AVT sobre el INFB-DOE natural en peces aislados. El AVT modificó número y tipo de SES en diadas macho-macho, y no en diadas macho-hembra, por lo que este efecto sí depende del contexto social. Asimismo, el AVT incrementó la actividad locomotora durante el día.

Palavras-Chave: social behavior vasotocin electric fish

Apoio Financeiro: DICYT PDT 043

E.09.003

Development of affiliative and hedonic cognitive stimuli for functional neuroimaging studies

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Objectives: Human motivations have been framed within a hedonic-aversive continuum (e.g. negative vs positive valence). Human emotional experience transcends this framework, however. Affiliative emotional experience (attachment) may occur both in the context of positive (joy, awe, love) and negative emotion (guilt, compassion, pity), and can thus be treated provisionally as an independent variable. Recent functional magnetic resonance imaging (fMRI) studies suggest that certain brain regions, such as the subgenual area, is involved in social attachment. The present study aims to create an emotional stimulus battery to evoke psychological and brain responses to attachment experience while controlling for emotional valence.

Methods: The stimulus battery comprises written statements describing an agent in diverse social scenarios. Stimuli were created by combining the variables: valence (positive or negative); agency (you, other); and attachment (yes, no), resulting in 8 categories. Using 10- or 15-point Likert scales, participants (n=30) scored the statements according to two main variables of interest: valence and affiliative sentiments. Analyses were performed using Shapiro-Wilks, Mann-Whitney and Kruskal-Wallis tests, and Spearman correlation.

Results: Based on calculated distances from category centroids, 20 stimuli per category (160 total) were selected from a initial pool of 30 stimuli per category (240 total). As intended, stimuli discriminated between valence ($p < 0.0001$) and affiliation ($p < 0.0001$) across categories. As expected, the correlation between these two variables was weak ($r = 0.29$), indicating that they can be treated as independent variables. Both valence and affiliation independently explained attachment feelings, and there was also an interaction effect between these two variables in explaining the level of attachment ($p < 0.0001$).

Conclusions: An emotional stimulus battery capable of evoking attachment or hedonic feelings as independent parameters was successfully developed. This stimulus set will be used in forthcoming fMRI studies to investigate the common and dissociable effects of hedonic and affiliative emotions on brain activation.

Palavras-Chave: moral, emotion, attachment, fMRI

Apoio Financeiro: LABS-DOr Hospital Network, PRONEX/CNPq

EFEITO DA MICROINJEÇÃO DE SOMATOSTATINA NA AMÍGDALA MEDIAL PÓSTERO-DORSAL SOBRE O COMPORTAMENTO DE ANSIEDADE/MEDO EM RATOS

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Objetivos: analisar o papel da somatostatina na amígdala medial póstero-dorsal (MePD) sobre o comportamento de ansiedade/medo no labirinto em cruz elevado (LEC) de ratos machos.

Métodos e Resultados: foram utilizados animais do biotério da UFCSPA, com cerca de 3 meses de idade e peso aproximado de 300g. Os ratos sofreram cirurgia estereotáxica para implante unilateral (lado direito) da cânula na MePD no encéfalo. Após 4 dias, os animais foram divididos em 3 grupos: grupo 1 (n=5) recebeu microinjeção de salina (0,3µL); grupo 2 (n=3) recebeu somatostatina (1µM/0,3µL); grupo 3 (n=2) recebeu substância P. Após 10 min da microinjeção, os animais foram testados no LEC e, filmados por 5 min. No LEC analisamos a frequência e duração nos braços aberto e fechado, percentual de frequência e tempo de permanência no braço aberto. Após o teste comportamental, os animais foram eutanasiados para análise histológica e confirmação do local da microinjeção. Os registros foram analisados com auxílio do software Noldus (Holanda). As médias das frequências e duração dos comportamentos ($\pm$ E.P.M.) foram analisadas através de uma ANOVA seguida de Newman-Keuls ($p < 0,05$). Nossos resultados são preliminares e mostram diminuição significativa na percentagem de frequência no braço aberto dos animais que receberam somatostatina ($0,01 \pm 0,01$) e substância P ($0,01 \pm 0,01$) na MePD quando comparado ao grupo salina ($0,33 \pm 0,02$). Além disso, observamos diminuição significativa no tempo de permanência no braço aberto dos animais que receberam somatostatina ($0,01 \pm 0,01$) e substância P ($0,01 \pm 0,01$) na MePD quando comparado ao grupo salina ($0,16 \pm 0,04$).

Conclusão: as conclusões são preliminares e, indicam, que a somatostatina e a substância P microinjetadas na MePD promovem efeito anisogênico em ratos machos testados no LCE.

Palavras-Chave: amígdala medial póstero-dorsal, somatostatina, substância P, ratos

Apoio Financeiro: CAPES e PIBIC/CNPq

E.09.005

Aspectos da transmissão e processamento de informação em canto de anuros.

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Objetivos: Por hipótese, a seleção natural leva a uma diminuição na geração de entropia nos processos. No intuito de testar essa hipótese no nível do processamento de sinais de comunicação, o presente estudo visou medir a regularidade de séries temporais de vocalização em 3 espécies de anuros.

Métodos: Gravações de canto de 3 espécies foram obtidas de indivíduos que estivessem interagindo numa população. Os padrões de vocalização de cada indivíduo foram isolados e os tempos entre as vocalizações foram obtidos. A regularidade foi abordada através de ferramentas não-lineares (análise espectral de potência, plot de Poincaré e entropia aproximada volumétrica). Para comparação, as ferramentas foram aplicadas em séries periódicas e em séries aleatórias, Ruído Branco (RB) e Passeio de Lévy (PL), geradas artificialmente.

Resultados: O valor médio de vApEn encontrado para os indivíduos analisados (2575,9) é próximo ao valor encontrado para PL (2833,5), sendo muito superior ao valor encontrado para RB (1143,6).

Conclusão: A análise intra-individual indica um comportamento semelhante ao PL, ou seja, intervalos de frequentes emissões sonoras acompanhados por longos períodos de silêncio. Esse tipo de padrão é observado no deslocamento na busca por alimento em várias espécies. No caso das emissões sonoras, esse é pelo que conhecemos o primeiro relato de tal padrão. Aqui, o fator determinante do PL ainda não nos é claro, podendo ser fruto desde imposições metabólicas da musculatura de canto até processos cognitivos mais elaborados.

Os resultados mostram que os intervalos de tempo de vocalização entre-indivíduos têm alta variabilidade, sendo um indicio de que os indivíduos estejam vocalizando de maneira independente uns dos outros. A modelagem via sistemas dinâmicos mostra que a ausência de acoplamento entre indivíduos é uma das maneiras de haver minimização de geração de entropia num sistema.

Os resultados aqui apresentados sugerem que este tipo de análise neste tipo de comunicação pode ser de grande valia para a inferência a respeito de processos evolutivos neurais subjacentes.

Palavras-Chave: Comunicação, processamento, entropia, informação, série temporal.

Apoio Financeiro: FAPESP

E.09.006

Avaliação no campo aberto de animais adultos que tiveram suas mães selecionadas quanto a intensidade (high e low) dos cuidados aos filhotes durante a lactação.

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Objetivo: Avaliar o comportamento no campo aberto de ratos wistar, aos 60 dias de idade, cujas mães foram selecionados pela metodologia High-Low-5, durante o período lactacional.

Métodos e resultados: Foram utilizados ratos wistar (machos e fêmeas) com 60 dias de idade, divididos em dois grupos: grupo High (H com NM=26 e NF=25) e grupo Low (L com NM=26 e NF=25). Os animais foram selecionados pela metodologia High-Low-5 (5 critérios são considerados para seleção: mãe fica em cima dos filhotes no ninho; lambe os filhotes; fica fora do ninho; análise no Labirinto e do Comportamento Agressivo Maternal). Nesta seleção, 100 mães são avaliadas, sendo que as 20 que mais cuidam dos filhotes são as High e as 20 que menos cuidam são as Low. Os animais foram filmados no campo aberto por 5min e posteriormente os comportamentos foram analisados em um software específico. Os resultados foram analisados por ANOVA, seguido de teste de Newman-Keuls, quando necessário, o nível de significância aceito foi de $p < 0,05$. As fêmeas H ($15,8 \pm 1,9$) tiveram uma frequência menor de andar do que L ($20,4 \pm 1,3$), porém em relação a parada ocorreu o contrário: H ($13,3 \pm 1,9$) e L ($4,8 \pm 1,1$). As fêmeas H ($7,2 \pm 1,5$) também foram menos vezes ao meio do campo que L ($16,5 \pm 2$). Em relação a duração dos comportamentos, as fêmeas H ficaram menos tempo andando ($137 \pm 13,1$) do que L ($175,3 \pm 5,7$). Nos machos H ($13,46 \pm 1,64$) a frequência de paradas foi maior do que L ($7,5 \pm 1,1$). Assim como o tempo que permaneceu parado, H ($124,8 \pm 12,6$) foi maior do que L ($86,50 \pm 13,57$), porém o tempo de andar no meio do campo aberto mostrou que os machos H ($116,2 \pm 15$) permaneceram mais tempo no meio do que L ($84,2 \pm 9,5$). Os demais comportamentos avaliados não mostraram diferenças significativas tanto em machos quanto em fêmeas.

Conclusão: Animais que tiveram suas mães selecionadas durante a lactação em mais (H) ou menos (L) cuidadoras mostraram de um modo geral que quando adultos, os filhotes provenientes de mães High diminuem os comportamentos de exploração principalmente em fêmeas, os machos apresentaram uma diminuição não tão clara quanto as fêmeas.

Palavras-Chave: comportamento maternal, labirinto

Apoio Financeiro: UNIOESTE

Comportamento no labirinto em cruz elevado de ratos wistar adultos que tiveram suas mães selecionadas quanto a intensidade dos cuidados aos filhotes durante a lactação

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Objetivo: Avaliar o comportamento no labirinto em cruz elevada de ratos wistar (machos e fêmeas) os quais as mães foram selecionadas pela metodologia High-Low-5, durante o período lactacional.

Métodos e resultados: Foram utilizados ratos wistar (machos e fêmeas) com 60 dias de idade, divididos em dois grupos: grupo High (H com NM=45 e NF=50) e grupo Low (L com NM=45 e NF=50). Os animais foram selecionados pela metodologia High-Low-5 (5 critérios são considerados para seleção: mãe fica em cima dos filhotes no ninho; lambe os filhotes; fica fora do ninho; análise no Labirinto e do Comportamento Agressivo Maternal). Nesta seleção, 100 mães são avaliadas, sendo que as 20 que mais cuidam dos filhotes são as High e as 20 que menos cuidam são as Low. Cada animal foi filmado no labirinto por 5 min e depois os comportamentos foram analisados em um software específico. Os resultados foram analisados através de uma ANOVA seguidos de teste de Newman-Keuls, quando necessário, o nível de significância aceito foi de $p < 0,05$. As fêmeas H ($15,0 \pm 3,5$) foram ao braço aberto mais vezes do que as L ($3,1 \pm 1,1$). Em relação à frequência no braço fechado, as fêmeas H ($17,8 \pm 3,4$) foram mais vezes do que as L ($5,6 \pm 0,5$). No tempo total de estar no braço fechado, as fêmeas H ($159,3 \pm 20,98$) ficaram mais tempo do que as L ($101,8 \pm 19,4$). Os demais comportamentos não foram estatisticamente diferentes tanto em machos quanto em fêmeas.

Conclusão: Animais que tiveram suas mães selecionadas durante a lactação em mais (H) ou menos (L) cuidadoras mostraram na idade adulta, que as filhas de mães High aumentam o comportamento de locomoção, visto pelo aumento geral da frequência de entradas nos braços porém esse comportamento não é observado nos machos. Portanto fêmeas provenientes de mães que cuidam mais seus filhotes mostram uma diminuição da ansiedade quando avaliadas no labirinto na idade adulta.

Palavras-Chave: cuidado maternal, labirinto, ansiedade

Apoio Financeiro: UNIOESTE

ORIENTAÇÃO ENCOBERTA DA ATENÇÃO VISUO-ESPACIAL NA DISTROFIA MUSCULAR DE DUCHENNE

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A Distrofia Muscular de Duchenne (DMD) é uma doença de herança genética recessiva que gera um quadro de fraqueza muscular progressiva muitas vezes associada à deficiência mental. A atenção, considerada como o mecanismo cerebral que permite o processamento de uma informação em detrimento de outras, poderia estar alterada na doença contribuindo pelo menos em parte para o comprometimento cognitivo global observado. **OBJETIVO:** Investigou-se o desempenho atencional de meninos portadores de DMD utilizando-se testes psicofísicos específicos. **MÉTODOS:** Testou-se 30 meninos com DMD (GD) e 30 meninos saudáveis (GC), com idade entre 10 e 16 anos, em uma tarefa de tempo de reação de escolha, que consistia em responder o mais rapidamente possível a um estímulo alvo visual lateralizado com mão do mesmo lado. Antes do aparecimento deste estímulo, orientava-se a atenção automática do participante por meio de um estímulo visual periférico não-informativo espacialmente ou então sua atenção voluntária, por meio de um estímulo visual central que informava o local de maior probabilidade de aparecimento do estímulo alvo. **RESULTADOS:** Os tempos de reação (TR) foram maiores para o GD do que para o GC tanto no teste de atenção automática ($p < 0,001$) quanto no teste de atenção voluntária ($p < 0,001$). Os TR no teste de atenção voluntária foram menores do que no teste de atenção automática no caso do GD ($p < 0,001$) mas não no caso do GC ($p = 0,20$). O efeito atencional (diferença entre o TR na condição oposta/inválida e o TR na condição mesma/válida) não diferiu entre os dois grupos no caso da atenção automática ($p = 0,846$), mas foi maior no GD do que no GC no caso da atenção voluntária ($p < 0,001$). Não foram observadas quaisquer assimetrias interlaterais. **DISCUSSÃO:** Os resultados sugerem que os meninos com DMD apresentam prejuízo na capacidade de orientar a atenção no tempo e também gerenciam de modo anômalo para a idade a atenção espacial voluntária. O grande efeito atencional apresentado por eles é compatível com um atraso na maturação do seu sistema nervoso.

Palavras-Chave: Distrofia Muscular de Duchenne, Atenção, Testes Psicofísicos

F.01.002

O que acontece com a informação não atendida em uma tarefa de busca visual?

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Diariamente uma grande quantidade de estímulos relevantes e irrelevantes é apresentada ao sistema visual humano, que de maneira geral, apresenta uma capacidade limitada de processamento. Muitos resultados experimentais apontam para o descarte eficiente da informação irrelevante, o que corrobora com o modelo de gargalo atencional proposto inicialmente por Broadbent (1958). Na presente pesquisa foi investigado um possível efeito no tempo de reação do participante em decorrência de um estímulo relevante apresentado em meio a estímulos irrelevantes para a tarefa. Para tanto foi delineado um experimento de busca visual no qual os participantes foram solicitados a localizar um alvo em meio a distratores (elementos relevantes com a mesma luminância do alvo) e uma textura irrelevante com uma luminância menor que os estímulos relevantes. Em 50% das provas um elemento com muitas características do alvo foi apresentado em meio à textura (elemento intruso). O tempo de reação médio (TR) de cada participante obtido na condição experimental foi submetido a uma análise da variância para medidas repetidas (ANOVA) (Alfa = 0,05). Os tempos de reação que excederam dois desvios padrão além da média do TR não foram incluídos na análise dos dados. A análise dos dados confirmou um aumento linear do TR em relação ao número de estímulos relevantes para a busca visual, com 5, 10 ou 15 estímulos ($F(2,48) = 424,58$; $p < 0,01$). De maneira geral, houve também um efeito significativo do fator presença ou ausência do intruso na textura ($F(1,24) = 45,44$; $p < 0,01$) e nas provas com contraste da luminância ($F(1,24) = 21,29$; $p < 0,01$). Estes resultados sugerem que o descarte da informação irrelevante é eficiente nas condições nas quais o contraste entre estímulos relevantes e textura é grande. Nas condições em que o contraste é pequeno há um efeito significativo associado ao descarte da textura possivelmente causado pela presença do elemento intruso. Tais resultados sugerem um sistema hierárquico de seleção da informação relevante e apontam para novos experimentos que venham a contribuir com uma melhor compreensão deste processo.

Palavras-Chave: Busca visual; Processamento de informação; Atenção visual; Tempo de reação

Apoio Financeiro: CNPq/UFU

A PERCEPÇÃO DA IMAGEM CORPORAL E SUA RELAÇÃO COM O ESTADO NUTRICIONAL E A PRÁTICA DE ATIVIDADE FÍSICA EM ADOLESCENTES

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O objetivo do presente estudo foi verificar as relações entre percepção da imagem corporal, o estado nutricional e a prática de atividade física em alunos do ensino médio. Avaliou-se 275 alunos de 10 escolas públicas e particulares ($16,2 \pm 1,0$ anos), sendo 139 meninos e 136 meninas. Aplicou-se o Questionário Internacional de Atividade Física (IPAQ) e a Escala de Figuras de Silhuetas (EFS) e aferiu-se peso e estatura para avaliação do estado nutricional através do cálculo do Índice de Massa Corporal (IMC). Os resultados foram submetidos a uma Análise de Variância (ANOVA), seguida do teste *Post-Hoc* de Newman-Keuls, além da Correlação de Pearson. Encontrou-se uma correlação significativa entre IMC e distorção ($r=+0,33$) e também entre IMC e insatisfação corporal ($r=-0,68$). A EFS mostrou que as meninas possuem mais distorção que os meninos ($p<0,01$), independente do estado nutricional e que o grupo de indivíduos subnutridos obteve os menores índices de distorção [$F_{(3,271)}=5,41$; $p<0,01$)] quando comparado aos grupos “sobrepeso” e “obesidade” ($p<0,05$). Com relação à insatisfação, a Escala também indica uma maior prevalência no sexo feminino ($p<0,01$), com diferença estatisticamente significativa entre todas as categorias de estado nutricional [$F_{(3,271)}=37,27$; $p<0,01$)] em ambos os sexos. Em se tratando de atividade física, observou-se que não houve diferença significativa entre nível de atividade e distorção da imagem corporal [$F_{(3,271)}=0,71$; $p>0,05$)] ou insatisfação [$F_{(3,271)}=1,06$; $p>0,05$]]. Assim, conclui-se que as meninas possuem maiores índices de distorção com a própria imagem que os meninos, bem como de insatisfação. Além disso, em ambos os sexos, há um aumento na distorção e na insatisfação com a própria imagem conforme o aumento do IMC. Entretanto, a percepção da imagem independe do nível de atividade física praticado tanto por meninos quanto por meninas.

Palavras-Chave: imagem corporal, estado nutricional, atividade física, adolescentes

Apoio Financeiro: FAPESP

F.01.004

É possível uma divisão da atenção visual automática em um hemisfério visual?

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Objetivos: Há controvérsia na literatura sobre a possibilidade de divisão espacial da atenção visual. Em três experimentos anteriores do nosso laboratório obtivemos evidências bastante sugestivas de que há uma divisão espacial da atenção visual automática em um hemisfério visual. Neste experimento tentamos replicar nossos resultados anteriores utilizando posições alinhadas de apresentação dos estímulos ao invés de posições de mesma excentricidade, mas não alinhadas.

Métodos e Resultados: Foram testadas 12 voluntárias que realizaram uma sessão de treino e uma sessão de prova, constituídas de 192 tentativas. Cada tentativa iniciava-se com o aparecimento de um ponto de fixação (PF) central na tela e de três anéis de cada lado deste, um superior e um inferior, a uma excentricidade de 9 graus, e um intermediário a uma excentricidade de 8,5 graus. Entre 1750 e 2250 ms depois aparecia um estímulo precedente (E1), representado por um escurecimento do anel superior, do anel intermediário, do anel inferior ou dos anéis superior e inferior. Cem ms depois aparecia um estímulo alvo (E2), representado por uma linha vertical ou um anel pequeno, ou no anel superior, ou no anel intermediário, ou no anel inferior, à direita ou à esquerda. O E2 podia aparecer na mesma posição (50% das tentativas) ou em posição oposta (50% das tentativas) ao E1. As voluntárias foram instruídas a manter o olhar no PF e responder com o dedo indicador esquerdo à linha vertical, e com o dedo indicador direito ao anel. O tempo de reação na condição mesma foi menor do que na condição oposta (média $\pm$ epm: 469 $\pm$ 10 e 498 $\pm$ 10 ms; $p < 0,001$). Este efeito da atenção foi menor para a condição E1 duplo do que para a condição E1 único (média $\pm$ epm: 18 $\pm$ 5 e 40 $\pm$ 6 ms, respectivamente; $p = 0,008$). A interação entre o tipo do E1 e a posição do E2 não foi significativa ($p = 0,219$), indicando que os efeitos atencionais não diferiram entre as condições do E2 superior, intermediário e inferior, quando o E1 era único (35 $\pm$ 9, 50 $\pm$ 8 e 36 $\pm$ 9 ms, respectivamente) e quando o E1 era duplo (31 $\pm$ 8, 12 $\pm$ 9 e 10 $\pm$ 11 ms, respectivamente).

Conclusões: Os presentes resultados em conjunto com aqueles obtidos nos experimentos anteriores do nosso laboratório sugerem que o foco atencional não se divide, mas pode assumir uma forma bastante longa e estreita.

Palavras-Chave: Atenção, psicofisiologia

Apoio Financeiro: CAPES

ASSIMETRIA INTERLATERAL DA ATENÇÃO VISUOESPACIAL AUTOMÁTICA EM UMA TAREFA DE DISCRIMINAÇÃO DE LOCAL.

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Objetivos

Investigar a possibilidade de haver uma assimetria interlateral no efeito produzido por um estímulo pré-ativador visual em uma tarefa de discriminação de local.

Métodos

Testamos 24 homens destros. Apresentamos a eles uma tela com um ponto de fixação e dois anéis periféricos, um de cada lado. Em seguida a borda de um ou de ambos os anéis piscava (E1). Trinta e quatro, 67 ou 100 ms depois, aparecia uma linha vertical (E2) na região superior ou inferior de qualquer um dos anéis. Dependendo da posição da linha vertical (superior ou inferior) o voluntário deveria apertar uma tecla à esquerda ou à direita o mais rápido possível assim que o E2 aparecesse. Foi medido então, o tempo de reação (TR) dos voluntários.

Resultados

- o Quando E2 aparecia do lado direito, o TR foi menor do que quando aparecia do lado esquerdo (474 ± 10 ms; 481 ± 11 ms respectivamente; $p = 0,04$).
- o O efeito atencional (TR na condição em que E1 e E2 apareciam em lados opostos menos o TR na condição em que apareciam do mesmo lado) foi maior quando E1 aparecia do lado direito do que quando E1 aparecia do lado esquerdo (37 ± 4 ms; 22 ± 4 ms respectivamente; $p = 0,01$).

Conclusão

Nossos resultados sugerem que há uma discriminação mais eficiente da localização dos estímulos do lado direito do espaço. Esta assimetria seria pelo menos em parte de natureza atencional.

Palavras-Chave: atenção, visão, assimetria, discriminação

Apoio Financeiro: FAPESP

F.01.006

Importância do conflito semântico em uma tarefa Stroop avaliada através da técnica de potenciais evocados.

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Nomear a cor de um estímulo é mais difícil quando este é composto por uma palavra incongruente à cor em que ele se apresenta, por exemplo, a palavra AZUL escrita com tinta vermelha - "efeito Stroop". Um ponto frequentemente debatido é em que nível de processamento (semântico ou motor) ocorre a interferência presente na tarefa Stroop. Neste trabalho, objetivamos avaliar a participação da atenção e da ativação semântica involuntária sobre este efeito através de manipulações temporais em uma tarefa denominada Stroop pareado. Os voluntários (n=40) julgavam se a cor de um primeiro estímulo (E1) era igual ao significado do segundo e, neste caso, apertavam uma tecla. O E1 era uma palavra relacionada à cor em amarelo, vermelho ou azul (estímulo Stroop), enquanto que o E2 era uma palavra relacionada à cor em branco. Os estímulos poderiam aparecer simultaneamente, ou separados por intervalos de 400 ou 1200ms. Como os sujeitos deveriam responder somente após o acender do E2, pressupõe-se que a apresentação do E1 induziria um conflito puramente semântico. O efeito Stroop foi claramente modulado pelo intervalo, sendo reduzido com o aumento do intervalo entre os estímulos. A análise dos potenciais evocados mostrou uma onda fronto-central associada ao acender do E1 e que é compatível com a N450, um componente previamente descrito como um indicador do processamento do conflito por mecanismos atencionais voluntários. A análise de variância revelou que a amplitude média de pico desta onda foi maior para a condição incongruente (e.g. a palavra AZUL em vermelho) em relação à congruente (e.g. a palavra AZUL em azul); $F(1,39) = 5.49$, $p < 0,05$; $\epsilon = 1,00$. Os resultados indicam que durante o intervalo ocorre o processamento do conflito semântico presente no estímulo Stroop por estruturas possivelmente envolvidas no sistema executivo atencional.

Palavras-Chave: Efeito Stroop, Atenção, Potenciais Evocados

Apoio Financeiro: CAPES, CAPES-GDU/MECD nº086/05, PRONEX-FAPERJ e PROPP-UFF.

F.01.007

Modulação de tamanhos visualmente percebidos por meio de exposições breves de informações de profundidade provenientes de gradientes de textura e horizonte

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Objetivo: Investigar se o tempo de exposição das informações de profundidade afeta a comparação de tamanho de objetos pictóricos.

Método: Duas barras verticais foram apresentadas simultaneamente na tela de um computador pelo método dos estímulos constantes, de modo que 120 observadores julgassem qual delas era a maior. A apresentação podia ser de 50ms, 100ms ou 200ms sobre um fundo sem textura, ou com uma linha do horizonte, ou com gradiente de perspectiva ou de compressão. Foram traçadas curvas psicométricas dos julgamentos de tamanhos e determinou-se o ponto de igualdade subjetiva (PIS) e, conseqüentemente, o erro relativo (E%).

Resultados: ANOVA aplicada aos erros relativos indicou diferença significativa entre os fundos de tela [$F(3,108)=4,668$, $p=0,004$] e um pós-teste de Bonferroni apontou que as diferenças ocorreram entre o gradiente de perspectiva e os fundos sem textura ($p=0,032$) e com linha do horizonte ($p=0,004$). Embora não tenha havido diferença significativa entre os tempos de exposição [$F(2,108)=1,199$, $p=0,305$], notou-se uma maior superestimação do tamanho nas apresentações de 100ms nas condições com indícios de profundidade: perspectiva (E%=6%, EPM=1,28) e compressão (E%=4%, EPM=0,88). Para a condição controle, à medida que se aumentava o tempo de exposição, os erros relativos diminuía (E%=3% e EPM=1,02 para o tempo de 50ms; E%=2% e EPM=0,78 para 100ms; e E%=1% e EPM=0,61 para 200ms). Na condição com a linha do horizonte, maiores tempos de exposição resultaram em aumento dos erros relativos (E%=1% e EPM=1,07; E%=1,5% e EPM=1,13; e E%=4% e EPM=1,18, respectivamente).

Conclusão: Os erros relativos maiores na condição de gradiente de perspectiva confirmam evidências de que este é uma informação de profundidade eficiente na modulação do tamanho percebido. Além disso, parece que essa eficiência é maximizada nas situações em que os estímulos são expostos em torno dos 100ms.

Palavras-Chave: Percepção de Tamanho; Gradiente de Textura; Tempo de Exposição

Apoio Financeiro: CNPq

F.01.008

Bandas de frecuencia espacial alta y baja afectan el reconocimiento de caras en campo visual lateralizado.

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Objetivo: Identificar la asimetría hemisférica en el reconocimiento de caras con predominio de bandas de frecuencia espacial alta (FEA) y bandas de frecuencia espacial baja (FEB).

Método: Cuarenta voluntarios debían memorizar 14 fotos de caras frontales sin filtraje de frecuencia espacial (SF); luego, debían reconocerlas entre 56 fotos de caras con FEB, FEA y SF, presentadas individual y dicópticamente por 300 ms en un monitor. A veinte voluntarios (10 hombres, 10 mujeres) se les presentaron fotos en el hemicampo visual derecho (priorizando el procesamiento de la imagen en el hemisferio cerebral izquierdo (HE)) y a los otros veinte (10 hombres, 10 mujeres) en el hemicampo visual izquierdo (priorizando el procesamiento en el hemisferio cerebral derecho (HD)). Las frecuencias de respuestas permitieron calcular las curvas *Receiver Operating Characteristic* y el parámetro Az de la Teoría de Detección de Señal.

Resultados: En las imágenes procesadas por el HD el desempeño de los hombres para caras SF, FEB y FEA fue de $Az=0.75$, $Az=0.70$ y $Az=0.61$, respectivamente; y la ejecución de las mujeres para caras SF, FEB y FEA fue de $Az=0.74$, $Az=0.74$ y $Az=0.65$, correspondientemente. En las imágenes procesadas por el HE, el desempeño de los hombres para caras SF, FEB y FEA fue de $Az=0.79$, $Az=0.75$ y $Az=0.66$, respectivamente; y la ejecución de las mujeres para las caras SF, FEB y FEA fue de $Az=0.87$, $Az=0.72$ y $Az=0.77$, correspondientemente.

Conclusiones: Las caras SF fueron más fácilmente reconocidas que las caras FEA o FEB. Independientemente de la condición de filtraje, el HE hizo un reconocimiento de caras más eficaz que el HD. El HD fue tan competente como el HE para procesar caras FEB, pero menos competente que el HE para procesar caras FEA. El HD de hombres y mujeres tuvo un desempeño similar. Con el HE, las mujeres reconocieron más las caras FEA que las FEB, en cambio los hombres, reconocieron más las caras FEB que las FEA.

Palavras-Chave: Reconocimiento de caras, Frecuencias Espaciales, Asimetría Hemisférica

Apoio Financeiro: CNPq

F.01.009

Endogenous orienting of visual attention by rats?

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Objectives: In typical covert visual attention tests a person focuses on a central point of a screen and pushes a button as soon as a target is detected in peripheral locations of the screen; when a preceding cue stimulus correctly informs the location of the pending target (valid cue), the reaction time (RT) is smaller than when the cue incorrectly informs the target location (invalid cue); this RT difference is known as validity effect. Subjects trained with 50% of valid and 50% of invalid trials exhibit the validity effect when the cue-target time interval (stimulus onset asynchrony, SOA) is smaller than about 300ms; this result is ascribed to exogenous influences of the cue on attention. Differently, subjects trained with 80% of valid and 20% of invalid trials also exhibit the validity effect when the SOA is greater than 300ms; this is ascribed to endogenous attention. The aim of this study was to investigate if rats exposed to analogous testing conditions exhibit similar results.

Methods and results: A computerized operant box was used; one of its walls had three adjacent holes. At the end of each hole there was a lamp that could be lit with either 50 (cue) or 200 (target) lux. In each trial, the animals inserted their noses into the central hole maintaining it there during the cue presentation in one of the lateral holes; then, at variables SOAs (200, 400, 800 or 1200ms) the target was presented either in the same (valid) or in the opposite (invalid) hole; reward was given when the rat removed its nose from the central hole (RT) and inserted it where the target was presented. One session included 500 trials. The mean ($\pm$ standard error) validity effects in each SOA were, respectively, 29 $\pm$ 4, 17 $\pm$ 2, 12 $\pm$ 1, 13 $\pm$ 2ms for Group 50-50 and 36 $\pm$ 2, 19 $\pm$ 2, 20 $\pm$ 2, 10 $\pm$ 4ms for Group 80-20. The ANOVA revealed a significant Group x SOA interaction effect ($F_{3,27}=3.36$, $p<0.033$); a post hoc test indicated a significant groups' difference when the SOA was 800ms.

Conclusions: The greater validity effect for Group 80-20 when the SOA was 800ms suggests that rats exhibit attentional effects that, in human beings, are ascribed to endogenous attention.

Palavras-Chave: Attention; Endogenous Orienting; Rats; Reaction Time

Apoio Financeiro: CAPES; FAPESP; CNPq

F.01.010

Influência da estimulação precedente visual sobre o tempo de reação a um estímulo alvo visual em tarefas de localização espacial e de identificação da forma.

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Objetivo: Examinamos, em que medida, a facilitação do tempo de reação produzida por um estímulo precedente visual dependeria do tipo de processamento do estímulo alvo visual requerido, se identificação de sua posição espacial ou identificação de sua forma.

Método: Testamos vinte e quatro adultos jovens de ambos os sexos. Metade dos participantes realizou uma tarefa de tempo de reação que exigia a identificação do lado de aparecimento do estímulo alvo visual para a escolha da mão de resposta (à esquerda ou à direita) e a outra metade dos participantes realizou uma tarefa de tempo de reação que exigia a identificação da forma (círculo ou elipse) para a escolha da mão de resposta. A resposta consistiu em pressionar uma tecla o mais rápido possível. O estímulo alvo visual era precedido pelo piscar de um retângulo com as dimensões da tela de um monitor de vídeo. A assincronia entre o início dos dois estímulos podia ser de 50, 100, 200, 400, 800 e de 1600 ms em blocos separados de tentativas.

Resultados: O estímulo precedente visual foi capaz de reduzir o tempo de reação em ambas as tarefas visuais. Na tarefa de localização espacial, este efeito facilitador ocorreu apenas para as assincronias de 100 ms (7 ± 1 ; $p=0,037$) e de 400 ms (11 ± 1 ; $p=0,010$). Na tarefa de identificação da forma, o efeito facilitador ocorreu para todas as assincronias ($p=0,018$) sem variação.

Conclusões: Um mesmo estímulo precedente visual produz efeitos facilitadores diferentes dependendo da demanda discriminativa da tarefa. Este efeito facilitador é ligeiramente maior na tarefa de discriminação da forma do que em uma tarefa de localização espacial.

Palavras-Chave: estímulo precedente, tempo de reação, tarefa de localização espacial, tarefa de identificação da forma

Apoio Financeiro: Capes

F.01.011

Seleção de estratégia atencional em uma tarefa de tempo de reação vai/ não-vai

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Introdução: Em um estudo prévio com tarefa vai/não-vai, observamos que um estímulo periférico não-informativo espacialmente, representado por um anel cinza (E1), causa um tempo de reação (TR) menor à linha vertical no interior do anel (LV) quando ocorre na mesma posição que o E1. Não observamos o mesmo efeito atencional para a cruz no interior do anel (CR). Por serem apresentados em blocos separados, os voluntários assumem estratégias atencionais diferentes.

Objetivos: Para cada estímulo, uma estratégia é adotada. No caso da LV, o E1 seria capaz de capturar mais eficientemente a atenção, o mesmo não acontecendo para a CR. No presente experimento, colocamos em conflito a adoção de uma destas estratégias, adicionando os 2 estímulos alvos positivos nos mesmos blocos de tentativas.

Métodos: Testamos 12 adultos jovens, destros e com visão normal ou corrigida. Os estímulos alvos positivos foram uma LV e uma CR e o estímulo alvo negativo um anel pequeno dentro do anel grande, apresentados no mesmo bloco de tentativas. Eles eram precedidos pelo aparecimento adicional do E1 na posição mesma ou na oposta. Os voluntários pressionaram uma tecla com o dedo indicador direito na mesa. O aparecimento da LV foi de 40% enquanto que o da CR foi de 10%.

Resultados: Os tempos de reação para a CR tanto na posição mesma como na posição oposta (403 ± 10 ms e 413 ± 14 ms) foram similares à LV (388 ± 12 ms e 410 ± 17 ms) ($p > 0,05$), não havendo efeito atencional significativo.

Conclusão: Não houve efeito atencional, mesmo em uma condição em que a estratégia atencional normalmente utilizada para a LV deveria prevalecer. Talvez, isto se explica pela estratégia adotada para a CR ser mais adaptativa aos voluntários, uma vez que o E1 apenas funciona como distraidor e dificultador da tarefa. O mais plausível é que o estímulo precedente capture a atenção automática, porém diminua o seu processamento ao longo da tarefa.

Palavras-Chave: atenção, estratégia atencional, efeito atencional, vai/ não-vai, tempo de reação

Apoio Financeiro: FAPESP

F.01.012

Comparação entre os efeitos comportamentais da atenção voluntária e da atenção automática.

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Objetivo: Comparamos os efeitos atencionais produzidos por um estímulo pré-ativador periférico e uma pista central em uma tarefa de discriminação de local e uma tarefa de discriminação de forma de um estímulo alvo visual.

Métodos: Testamos 24 adultos jovens. Em um primeiro experimento 12 participantes responderam ao local de aparecimento de um estímulo alvo visual no hemisfério esquerdo ou direito. Em metade das tentativas um estímulo precedente visual central indicava o lado, de maior probabilidade, de aparecimento do estímulo alvo. Na outra metade das tentativas o estímulo precedente visual era periférico e não informativo espacialmente. No segundo experimento os outros 12 voluntários responderam à forma do estímulo alvo visual. Os mesmos estímulos precedentes foram utilizados. Avaliamos o tempo de reação.

Resultados: No primeiro experimento encontramos um grande efeito atencional (83 ± 7 ms) do estímulo pré-ativador periférico, sendo o benefício de 44 ± 6 ms e o custo de 39 ± 5 ms. O efeito atencional produzido pela pista central foi menor (14 ± 6 ms; $p < 0,001$), sendo o benefício de apenas 12 ± 3 ms e não havendo praticamente custo. O tempo de reação com a pista central foi menor do que com o estímulo pré-ativador periférico (312 ± 9 ms e 327 ± 9 ms, respectivamente; $p < 0,001$). Houve menos erros nas tentativas em que a atenção era orientada pela pista central do que naquelas em que era orientada pelo estímulo pré-ativador periférico (total de erros: 31 e 170, respectivamente; $p < 0,001$). No segundo experimento encontramos efeitos atencionais semelhantes para o estímulo pré-ativador periférico e para a pista central (respectivamente 53 ± 13 ms e 34 ± 17 ms; $p = 0,457$).

Conclusão: Os efeitos da atenção automática e da atenção voluntária podem diferir ou não, dependendo da demanda sensorial da tarefa realizada. Este achado sugere certa independência entre os dois tipos de atenção, que poderia indicar mediações de mecanismos, pelo menos, parcialmente distintos.

Palavras-Chave: Atenção automática, atenção voluntária

Apoio Financeiro: FAPESP

F.01.013

ORIENTAÇÃO ENDÓGENA DA ATENÇÃO VISUAL EM RATOS?

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Objetivo

O objetivo do presente trabalho foi desenvolver uma tarefa comportamental para avaliar a orientação endógena da atenção visual em ratos, utilizando sons como sinalizadores.

Método

Foi utilizada uma caixa de condicionamento operante; uma de suas paredes possui um conjunto de três buracos adjacentes. No fundo de cada um dos buracos há um LED que pode ser aceso por curtos intervalos de tempo, e na sua entrada há um fotossensor que detecta quando o rato insere o focinho no buraco. Um alto-falante posicionado no teto da gaiola emite sons de frequências desejadas, usados como pistas; i.e., cada frequência indica um provável local de aparecimento do alvo; e.g., sons de 1 kHz indicam a provável ocorrência do alvo (acendimento do LED) no buraco lateral esquerdo e sons de 5 kHz indicam o provável acendimento do LED no buraco lateral direito. Cada um desses componentes está sob controle de um microcomputador.

Os animais foram treinados a (1) inserir o focinho no buraco central, (2) manter o focinho no buraco central durante a apresentação do som, (3) manter o focinho no buraco central até o aparecimento do alvo luminoso em um dos buracos laterais; os intervalos de tempo entre o início da apresentação da pista e do alvo foram de 50, 100, 200 ou 400ms, e (4) inserir o focinho no buraco lateral no qual o alvo foi apresentado. Durante o treino, 100% das tentativas foram válidas, i.e., o som sinalizou corretamente o provável local de aparecimento do alvo. Durante os testes, 80% das tentativas foram válidas e 20% das tentativas foram inválidas (i.e., o som sinalizou incorretamente o local de aparecimento do alvo).

Resultados

A análise de variância mostrou que o tempo de reação nas tentativas válidas foi significativamente menor que o tempo de reação nas tentativas inválidas [$F(1,3) = 10,7328$; $p=0,046563$], indicando que os animais orientaram a atenção para o lado sinalizado pela pista.

Conclusão

Ratos são capazes de orientar a atenção visuo-espacial baseando-se em sinalização por pistas auditivas. Como os sons foram apresentados a partir de um mesmo alto falante, pode-se excluir a eventual participação de atenção exógena nesse fenômeno.

Palavras-Chave: atenção visual, orientação endógena, pista auditiva e ratos

Apoio Financeiro: CNPq/PIBIC, FAPESP e CAPES

FATORES DE RISCO PARA DESENVOLVIMENTO DE TRANSTORNOS ALIMENTARES EM ADOLESCENTES FREQUENTADORES DE ACADEMIAS DE GINÁSTICA DE RIBEIRÃO PRETO.

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O objetivo deste estudo foi verificar a prevalência para o risco de desenvolvimento de transtornos alimentares em adolescentes frequentadores de academias de ginástica de Ribeirão Preto. Para tanto, foram estudados 100 sujeitos frequentadores de academias de ginástica, não atletas, de ambos os sexos, com idade entre 14 e 18 anos, sendo 52 meninos e 48 meninas. Foram aplicados: Questionário sobre a Imagem Corporal (BSQ), Escala de Dedicção ao Exercício Físico (EDE), Escala de Figuras de Silhuetas (EFS) e também foram coletados dados sobre peso corporal e altura para cálculo de Índice de Massa Corporal (IMC) e conseqüente diagnóstico de estado nutricional. Os resultados mostraram que, em relação ao IMC, 74% dos sujeitos (n=74) foram classificados como eutróficos. A atividade física em excesso é praticada por 70,8% (n=34) das adolescentes e por 46,1% (n=24) dos adolescentes. Segundo o BSQ, 71,1% dos adolescentes (n=37) não apresentaram qualquer tipo de distorção da imagem corporal, enquanto 28,8% (n=15) apresentaram algum grau de distorção. Com relação às adolescentes, 27% (n=13) não possuem algum tipo de distorção, mas 72,9% (n=35) apresentaram distorção da imagem corporal nos seus três graus. A distorção da imagem corporal foi avaliada pela EFS e as adolescentes, em média, se vêem com 4,62 kg a mais do seu IMC real. Já os adolescentes, se vêem com 2,57 kg a mais do que na verdade possuem (IMC real). As adolescentes também apresentaram insatisfação da imagem corporal, mostrando, na EFS, que gostariam de ter (IMC meta) 5,36 kg a menos do peso que pensam ter (IMC atual). Por outro lado, os adolescentes gostariam de ter 0,16 kg a mais do que pensam ter. Observa-se, portanto, que parece existir uma tendência, por parte das meninas em especial, de praticar exercícios físicos de forma excessiva. Como elas também apresentaram maiores índices de distorção e insatisfação da imagem corporal, em relação aos meninos, pode-se supor que o corpo ideal esteja sendo buscado através da prática excessiva de exercícios físicos na academia de ginástica. De acordo com a literatura, estes dois fatores se configuram como de risco para desenvolvimento de transtornos alimentares.

Palavras-Chave: Palavras - chave: transtornos alimentares, exercício físico, adolescentes, imagem corporal.

Apoio Financeiro: Universidade de Ribeirão Preto - UNAERP/ FAPESP

Perturbation of the attention as the first physiological step towards the memory impairment, dysfunctionality of the sensory recognition components and then the typical abnormal behaviors in the Alzheimer's process

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Objectives: To establish behaviors that show distraction, lack of concentration and very marked absorption into painful thoughts as the ways in which impairment of the attention is shown in Alzheimer cases, in the transition between the initial psychological phenomenon and the beginning of the pathological dialogue with the biological structures. **Methods:** We made an abduction process by way of articulate the know ledges and what comes along with it in order to an holistic understanding of the Alzheimer's phenomenon starting from its most universal and remarkable epiphenomena. **Results:** In the first place the distraction, the lack of concentration, and the absorption into (painful) thoughts is observed, that can be oscillating or persistent and resistant. The fixation of the person in the desire of dying, or even the transference of the present reality that rejects to an idyllic familial abstract past, naturally causes the progressive loss of the recognition with the nine channels that are a typical phenomenon of this type of dementia, due to the programmed biological mechanism of sensory blocking besides the fixation of the attention in that attacks or that is wished so strongly. In time, the other channels start adding impairments of sensory recognition in the channels that are still functioning, leading to the total bi-directional lack of communication. Nevertheless, at some sporadic moments, which could be caused by themselves or by another agent, it could occur an occasional reinstallation in the reality, or the recovery of the attention and of the sensory recognition elements, which indicates that until that moment it does not exist any neuronal damage, such as it is further demonstrated by few spontaneous remissions, or by means a programmed rehabilitations. **Conclusions:** Alzheimer is basically an attention problem, and not of memory. In the progressive process of dissolution of the sensory perception and of the processing of the stimuli by the brain (recognition), some impairment of many kinds appear in a course towards what could be similar to a state of coma, that is irreversible to such an extent that could create a complete lack of communication for the person and for the others in respect to the person.

Palavras-Chave: Alzheimer, attention, memory, sense recognition, distraction, absorption, behavior, death, translocation.

Apoio Financeiro: Universidad Autónoma de Entre Ríos, BBVA Banco Francés, Fundación Alzheimer

F.01.016

Influência dos processos sensoriais sobre o efeito facilitador do estímulo precedente

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Objetivos: Recentes pesquisas indicam a importância relativa do efeito facilitador de um estímulo precedente em otimizar o comportamento. Em estudo realizado anteriormente investigou-se em que medida esse efeito envolvia processos sensoriais e/ou motores do processamento sensório-motor. Diante do fato de que houve uma sugestão para etapas sensoriais e de que não houve evidências para a etapa motora, este estudo visou esclarecer a influência de processos sensoriais sobre o efeito facilitador proporcionado pelo estímulo precedente.

Métodos: Participaram 12 jovens. Experimento com apresentação de estímulos visuais em um monitor de vídeo e apresentação de estímulos auditivos, realizado em duas etapas. Na primeira etapa a tarefa tinha início com a apresentação de um anel central, com 0,60° de diâmetro, em torno de um ponto de fixação. As intensidades do anel variavam a cada tentativa de acordo com a resposta emitida, sendo apresentadas acima e abaixo do limiar sensório. Após solicitada a resposta, o voluntário apertava a tecla da direita para afirmar que havia sido apresentado estímulo visual e a tecla da esquerda para negar que havia sido apresentado. Ao término de todas as tentativas era definido o limiar sensório visual. Não havia nesta etapa a apresentação de estímulo auditivo. Na segunda etapa, o estímulo apresentava intensidade determinada na etapa anterior para cada voluntário. A emissão da resposta era da mesma forma que a emitida na primeira etapa. Estímulo alvo visual foi apresentado em 50% das tentativas e houve ausência do estímulo alvo visual em outros 50% das tentativas. Nas tentativas em que havia alvo visual, 25% destas havia estímulo precedente auditivo e em 25% não havia. A mesma proporção foi utilizada para as tentativas sem alvo visual.

Resultados: Realizou-se análise da detectabilidade e do critério, por meio da teoria de detecção dos sinais. A comparação da detectabilidade indicou diferença ($p = 0,01$) entre as condições sem som ($d' = 2,33 \pm 0,22$) e com som ($d' = 2,75 \pm 0,22$), com aumento da detectabilidade na condição com som. Também houve diferença na comparação do critério ($p < 0,01$) entre as condições sem som ($C = 0,47 \pm 0,08$) e com som ($C = 0,22 \pm 0,10$), com redução do critério.

Conclusão: Evidenciou-se a influência dos processos sensoriais sobre o efeito facilitador da responsividade de um estímulo precedente auditivo.

Palavras-Chave: percepção visual, estímulo precedente auditivo, processos sensoriais, teoria de detecção dos sinais

F.01.017

Percepção de tempo e sua modulação atencional em idosos e pacientes com demência de Alzheimer

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Objetivos: a percepção de tempo é um fator fundamental para a interação com o meio e sabe-se, por outro lado, que funções cognitivas têm papel importante nesta percepção, influenciando o modo como o tempo é analisado pelo cérebro, destacando-se entre estas funções a atenção. No envelhecimento e particularmente na demência de Alzheimer podemos verificar uma menor disponibilidade de recursos atencionais e uma percepção de tempo menos acurada. Neste estudo objetivamos avaliar a percepção de tempo e a influência da atenção usando tarefas de reprodução de tempo e de discriminação temporal.

Métodos: realizaram as tarefas 9 jovens ($23,44 \pm 0,97$ anos), 11 idosos ($69,5 \pm 1,1$ anos) e 4 pacientes com demência de Alzheimer ($80,0 \pm 3,7$ anos). Realizamos em cada tarefa 3 blocos: com um estímulo, 1 alvo com 3 distratores com conhecimento prévio do alvo e sem conhecimento prévio do alvo. As medidas de desempenho foram: diferença relativa (%) para reprodução temporal e detectabilidade (d') e critério (β) para discriminação de tempo, considerando a teoria de detecção de sinais.

Resultados: usando ANOVA $3 \times 3 \times 6$, encontramos para a diferença relativa como efeito principal a duração ($F(5, 105)=27,87$ e $p<0,0001$) e as interações: grupo e duração ($F(10, 105)=2,45$ e $p=0,0112$) e grupo, duração e tipo de experimento ($F(20, 210)=3,5720$ e $p<0,00001$). Para d' encontramos, usando ANOVA $3 \times 3 \times 2$, temos como efeito principal: $|\Delta t|$ ($F(2, 21)=8,70$ e $p=0,0018$) e grupo ($F(2, 21)=31,29$ e $p<0,00001$). Não encontramos diferenças significativas considerando β .

Conclusão: verifica-se uma diferença entre o grupo de jovens e os grupos de idosos e pacientes com demência de Alzheimer, que é mais pronunciada quanto maior a demanda atencional. O fato de encontramos diferenças em relação ao d' corrobora o fato de uma menor demanda atencional poder explicar as diferenças na percepção de tempo em pacientes com Alzheimer e oriundas do envelhecimento.

Palavras-Chave: percepção de tempo, atenção, envelhecimento, Alzheimer

Apoio Financeiro: CNPQ

F.01.018

VISUALLY PERCEIVED SIZE COMPARISONS REVEAL EXOGENOUS ATTENTIONAL ASYMMETRY BETWEEN THE RIGHT AND THE LEFT BRAIN HEMISPHERE

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Objectives: By analyzing the point of subjective equality (PIS) and the slope of psychometric curve, we investigated asymmetries between the right and the left brain hemisphere for comparing visually perceived sizes as attention is oriented automatically or voluntarily to the right or to the left visual field. **Methods:** Applying the psychophysical method of the constant stimuli, observers under binocular or under only the right eye or under only the left eye viewing were asked to indicate between two horizontal bars, the standard and the test, which one was the bigger. The bars were simultaneously presented for 50 ms, randomly one at the right and the other at the left of the fixation point (center of the computer screen) in four conditions: (cond.1) without cues, (cond.2) with cues presented simultaneously and flanked at the test bars (cond.3) with cues at the location of the test bar but after 50ms later from its presentation (exogenous attention), and (cond.4) with an arrow at the center of the screen indicating to observers if they had to pay attention to the right or left visual field (endogenous attention). **Results:** PIS from the left visual field trended to be smaller than those one from the right visual field and PIS are reduced by exogenous attention but not by the endogenous attention. Slopes of psychometric curve were steeper for cond.2 and cond.3 as the test bar was presented at the right visual field in the binocular viewing, but not on other viewing conditions. **Conclusions:** There should be a natural lateral brain asymmetry for exogenous attention but not for endogenous attention. Also, there is evidence that exogenous attention amplifies visually perceived size and increases the discriminative sensitivity of the left brain in binocular viewing for comparing perceived size. **Palavras-Chave:** visual attention, perceived size, brain asymmetry

Apoio Financeiro: FAPESP e CNPq

F.01.019

EFEITO ATENCIONAL AUTOMÁTICO NA AUSÊNCIA DE POSIÇÕES DEMARCADAS.

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Objetivo: Observa-se um efeito atencional automático em tarefas de tempo de reação quando as posições de aparecimento dos estímulos são demarcadas na tela do monitor de vídeo, mas não quando essas posições não estão demarcadas. Neste estudo, procuramos avaliar se o ruído sensorial (ou menor saliência do estímulo alvo), conseqüente da presença das demarcações, é o fator crítico para a ocorrência do efeito.

Métodos: Testamos 12 voluntários em uma tarefa de tempo de reação de escolha. Eles deveriam responder a um estímulo alvo, representado por uma linha vertical, que aparecia à esquerda ou à direita de um ponto de fixação central, com a mão do mesmo lado. Esse estímulo era precedido por um sinal que ocorria no mesmo local ou no local correspondente do lado oposto, 50, 100, 150, 200 ou 250 ms antes (AIE). Um padrão quadriculado formava o fundo da tela em que esses estímulos eram apresentados.

Resultados: Houve uma redução do tempo de reação com o aumento da AIE (média $\pm$ e.p.m.: 390 $\pm$ 11, 357 $\pm$ 9, 344 $\pm$ 10, 324 $\pm$ 8, 324 $\pm$ 9, para as AIE de 50 a 250 ms; $p < 0,001$). Observamos também um efeito atencional significativo (média $\pm$ e.p.m.: 9 $\pm$ 7, 14 $\pm$ 6, 16 $\pm$ 6, 21 $\pm$ 8, 21 $\pm$ 10 para as AIE de 50 a 250 ms respectivamente; $p < 0,05$).

Conclusões: O aparecimento de um consistente efeito atencional quando se introduziu o fundo de tela quadriculado sugere que a saliência relativamente pequena do estímulo alvo é um fator crítico para a mobilização da atenção automática.

Palavras-Chave: atenção automática, saliência

VIÉS ATENCIONAL PARA PISTAS ASSOCIADAS AO COMPORTAMENTO DE FUMAR

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Fumantes podem apresentar viés atencional (VA) para pistas ambientais associadas ao comportamento de fumar, contribuindo para a transição do uso ocasional à dependência da droga. O processo atencional é dividido em dois subsistemas: orientação inicial, avaliada em tempos de exposição (TE) curtos (≤ 200 ms) e manutenção da atenção, influenciada por variáveis motivacionais e avaliada em TE maiores (≥ 2000 ms).

Objetivo: investigar a presença de VA para pistas associadas ao fumar em fumantes (F) e não fumantes (NF). **Método:** participaram 38 universitários, de ambos os sexos, da UFRGS: 17 F (média 22 anos, $dp=4,28$) e 21 NF (média 21 anos, $dp=2,33$). O VA foi investigado através de uma tarefa computadorizada (*Visual-Probe Task*). Nesta tarefa, 24 imagens (12 relacionadas ao cigarro e 12 controle) eram apresentadas aos pares, em três TE (200, 500 e 2000 ms). Após, uma delas era substituída por uma seta à qual o participante deveria localizar e indicar a direção (para cima ou para baixo). Calculou-se o VA subtraindo os TR das vezes que a seta substituiu imagens relacionadas ao fumar dos TR das vezes que a seta substituiu imagens controle. O *Fagerström Test for Nicotine Dependence* (FTND) foi usado para avaliar a dependência de nicotina. **Resultados:** Apesar do grupo F apresentar VA nos TE 200 ($M=4,9$; $dp=35,3$) e 500 ms ($M=10,8$; $dp=19,3$), enquanto o grupo NF apresentou apenas VA baixo no TE 200 ($M=0,7$, $dp=25,8$), não houve diferença significativa entre os grupos ou interação entre grupo e TE. A maioria do grupo F (94%) apresentou baixo nível dependência de nicotina no FTND, e fumava há menos de 5 anos (64,7%). **Conclusão:** A Visual probe task é uma tarefa adequada para mensurar o VA em F. Os resultados tendem a confirmar a literatura, revelando que F apresentam maior VA para estímulos relacionados ao fumar do que NF, mas a variabilidade dos TR foi muito grande. É possível que fosse encontrada diferença significativa entre os grupos se F mais experientes tivessem sido incluídos na amostra, pois a magnitude do VA parece sofrer influência do nível de dependência e do tempo de uso. Assim, novos estudos devem incluir F novatos e experientes, com diferentes níveis de dependência de nicotina.

Palavras-Chave: Viés atencional; fumantes; visual probe task

Apoio Financeiro: CNPq e CAPES

F.01.021

Visual stimuli with different level of coherence proportionally modulate evoked related potentials.

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Objective: Visual perception involves the grouping of individual elements in objects diminishing the descriptive complexity of a visual scene. The physiological bases of this perceptual simplification are still poorly understood. Recent evidence shows that the activation of different visual areas depends on the coherence of the visual stimuli (1). Based on in this evidence, we hypothesized that the cortical activity measured by electroencephalography (EEG) would reflect a differential processing of stimuli with different levels of coherence.

Methods: Electroencephalographic recordings were obtained (n=13) during a passive discrimination task of geometric figures, which were shown in 5 levels of coherence, that varied from a complete figure to the lines that they compose it, separated in an arbitrary ways. Event related potentials were used as measure of the cerebral activity in response to the different degrees of coherence of the stimulus.

Results: We found that the amplitude of the evoked potentials diminishes proportionally with the coherence of the objects. For the P2 component (175-275 ms), an analysis of variance for two factors showed a significant effect by stimulus type ($F(4,375) = 5,33$, $p < 0.001$) and by electrode location ($F(2,375) = 64,07$, $p < 0.001$). The N1 component (115-175 ms) showed the same significant levels that P2.

Conclusions: Our results suggest that the level and locations of the cerebral activity, is strongly influenced by the level of coherence of the visual stimuli. This activation would reflect the functional relation among different areas of the visual pathway, presumably through a top-down modulation mechanism.

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Palavras-Chave: Visual Psychophysics, Visual Cortex, EEG, ERP

Apoio Financeiro: Iniciativa Científica Milenio P04-068F

PREMILINARY FINDINGS ON THE MULTIPLE-FACES PHENOMENON USING BOLD TECHINIC

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Objective: The Multiple-Faces-phenomenon was studied with fMRI, using BOLD contrast.

Method: A non-naïve healthy male subject (26 years old) was tested. A printed face (female) was presented binocularly, either at center, right or left sides. The six conditions were: three with face presentations, two with index finger motion and one with neither. These were consecutively repeated, yielding 12 conditions. Presentation was continuous for 35.25 seconds and focus sustained at a black dot. Whole-brain fMRI data were acquired on a Magnetom Sonata (Siemens) of 1.5 tesla equipped with a head volume coil. Six blocks of 36 axial slices (3mm) per condition were obtained (i.e. three with and three without any of the stimuli). Time of repetition (TR) was 3520 ms and trials lasted 3:35 min.

Result: A paired t-test showed activity at the left fusiform gyrus and both occipital areas. Repetition effect was higher for the centered face than for faces at periphery.

Conclusion: We concluded that the multiple-faces phenomenon may be related to the observed sustained activation of visual areas.

Reference: Perception, 29: 1393, 2000.

Palavras-Chave: Perceptive processes; Face perception; Object perception; Multiple Faces phenomenon; psychophysics.

Apoio Financeiro: CNPq/ Propesq

O FENÔMENO MUITAS FACES: ESTUDO COMPARANDO A PERCEPÇÃO DO FENÔMENO QUANDO UTILIZADOS ESTÍMULOS DE FACE E DE OBJETO.

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Objetivos: O Fenômeno Muitas Faces (1) consiste na percepção de ilusões quando imagens de faces são apresentadas na periferia do campo visual. Caracteriza-se pela observação de variações na imagem, como movimentos, mudanças de expressão facial, surgimento de outras características ou outras faces sobrepondo a face apresentada na foto-estímulo. O presente estudo visou comparar a frequência de mudanças percebidas quando apresentada a imagem de um objeto (cadeira) e de uma face.

Método: Participaram 60 voluntários (30 homens/ 30 mulheres), adultos (18 à 30 anos), com visão normal ou corrigida. Duas imagens foram utilizadas como estímulo: uma cadeira e uma face feminina, frontal. Ambas acromáticas e mediam 25 graus de ângulo visual de altura e continham três pontos de fixação: um no centro e dois nas laterais (15 graus de ângulo visual do ponto central). Cada imagem foi apresentada em ordem aleatória, em um monitor 21'', tela plana, durante 3 minutos e observada com o olho direito e esquerdo separadamente. Os sujeitos foram instruídos a fixar o olhar, em um dos pontos laterais e posicionar-se até o ponto central coincidir com o campo cego. Mantendo o olhar fixo no ponto lateral, foi solicitado que pressionassem a tecla 1 cada vez que percebessem movimento ou mudanças de expressão facial (categoria 1) ou a tecla 2 para o surgimento de outra característica ou faces (categoria 2).

Resultados: Os resultados, analisados com a ANOVA e pós-teste Newman-Keuls, apontam para maior incidência do Fenômeno quando usado o estímulo de face, tanto com olho direito ($X_{\text{face}}=16$; $X_{\text{objeto}}=9$; $p=0,00169$) quanto com o esquerdo ($X_{\text{face}}=15,5$; $X_{\text{objeto}}=11$; $p=0,00574$), principalmente com relação à categoria 2 ($X_{\text{face}}=19$; $X_{\text{objeto}}=7$; $p=0,000133$) com olho direito ($X_{\text{face}}=17,25$; $X_{\text{objeto}}=8,25$; $p=0,000414$) com olho esquerdo.

Conclusão: Concluiu-se que embora o Fenômeno ocorra com maior frequência, com estímulos de faces, ele não é restrito a faces, podendo ocorrer com outro objeto visual (cadeira). A maior frequência observada da face pode ser pertinente à maior especialização na identificação e reconhecimento de faces.

Referência: Perception; 29:1393, 2000.

Palavras-Chave: Palavras-Chave: Processos Perceptivos; Percepção de faces; Percepção de objetos; Fenômeno Muitas Faces; Psicofísica.

Apoio Financeiro: CNPq/ Propesq

F.01.024

DESENVOLVIMENTO DA ATENÇÃO VISUO-ESPACIAL EM CRIANÇAS

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Objetivo: Investigar a ontogenia de Tempos de Reação (TRs) a estímulos apresentados em uma ampla área do campo visual em escolares de 8, 10 e 12 anos.

Métodos: Participaram deste estudo 3 grupos de crianças: 8 anos(n=13), 10 anos(n=14) e 12 anos(n=10). Tarefa: apertar o botão de um joystick quando aparecesse um círculo branco (0,2° de ângulo visual), em uma de 158 posições, distribuídas na tela do computador. Movimentos oculares eram monitorados pelo experimentador. Os participantes foram instruídos a prestar atenção em uma moldura cinza, quadrada, com lados de 4°, localizada no centro da tela (ExpI), ou em duas molduras, centradas 10° à direita e à esquerda do ponto de fixação (ExpII).

Resultados: Os TRs médios(em ms)dentro e fora da moldura foram: 8 anos(375±6 e 408±7), 10 anos(346±8 e 370±9) e 12 anos(316±8 e 341±7), mostrando TRs mais rápidos com o aumento da idade(p< 0,0001), confirmando parcialmente os achados de Surnina & Lebedeva(2002) onde os participantes mais velhos apresentaram TRs menores e baixas taxas de erros, porém nossos dados não corroboram totalmente estes achados pois não houve diferença estatisticamente significativa nos TRs e nos erros em função do sexo dos voluntários.

Conclusões: No exp I os TRs foram menores no centro. No exp II(atenção dividida) não houve diferença dos TRs das regiões atendidas e da área foveada(participantes não desengajaram a atenção do ponto de fixação). Estudos futuros são necessários para verificar se voluntários acima de 12 anos dividem a atenção como os adultos, e verificar se existem diferenças no desempenho em função do sexo.

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Palavras-Chave: atenção, psicofísica,crianças

Apoio Financeiro: CNPQ

F.02.001

Evidências de validade para o Teste Pictórico de Memória: relação com a inteligência

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O objetivo do estudo foi verificar evidências de validade para o Teste Pictórico de Memória. Participaram 436 indivíduos, sendo 182 do sexo masculino e 254 do feminino, com idades variando de 10 a 60 anos, que cursavam desde a segunda série do Ensino Fundamental até cursos universitários. Foram aplicados o Teste Pictórico de Memória e o Teste de Raciocínio Inferencial (RIn) de forma coletiva. Os resultados revelaram, segundo o Coeficiente de Correlação de Pearson, correlações variando de 0,19 até 0,44 para os três agrupamentos que compõe o Teste Pictórico de Memória e o Teste de Raciocínio Inferencial, e correlações variando de 0,40 até 0,56, quando considerada a pontuação total do Teste de Memória e o RIn. Quanto aos grupos extremos, os indivíduos com alta e baixa pontuação no Teste de Raciocínio Inferencial apresentaram, ao se realizar uma prova t de student, diferenças significativas nas quatro medidas do Teste de Memória. Dessa forma, evidências de validade de critério concorrente e por grupos extremos foram verificados para o teste Pictórico de Memória. **Palavras-Chave:** memória; inteligência; avaliação psicológica; validade; testes.

F.02.002

Visual neurons that would play a critical role in the process involved in the long-term expression of an acquired memory

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It has been assumed that endogenous modulatory systems play a major role in memory storage. Our recent results obtained investigating retrieval and reconsolidation phases of memory point to a completely new and original interpretation of the neuromodulator's action on memory. Studying the role of the neuromodulator angiotensin during reconsolidation in *Chasmagnathus* we obtained evidence of memory traces, building up by weak-trainings, that are not expressed unless and enhancement treatment mediated by the neuromodulator is provided. Such unexpressed memories are however mRNA and protein synthesis dependent, indicating that they are in fact long-term consolidated. Present poster shows an approach to study the cellular basis of neuromodulation by optophysiological recording (calcium imaging) of visual neurons involved in this memory process. Results show that a strong-training, unlike a weak training ($p > 0.1$ for all areas) produce a significant global fall of the signal during training ($p < 0,0005$ for all evaluated areas). However, during short-term memory test a signal decrease in these neurons is disclosed only in the neuronal process located in lateral protocerebrum ($p < 0.05$) but not in lobula neuropil ($p > 0.1$ and $p > 0.1$). This decrease is stimulus-specific because a mechanic stimulus applied with the same frequency that the strong-training trigger a different dynamic. Altogether, results show that these neurons also would play a critical role in the process involved in the long-term expression of an acquired memory, but not in information's storage *per se*. The finding of memory traces determining memory's expression, that are different of traces where the information would be stored, would insert a new viewpoint into the general proposition that behavioral memory is guided from multiple and discrete memory trace elements .

Palavras-Chave: learning, memory, calcium-imaging

Apoio Financeiro: CONICET

F.02.003

Ipsiversive turning behavior of unilateral MPTP-lesioned rats as a model of motor alterations during the early phase of Parkinson's disease

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Turning behavior observed in rats with unilateral lesion of the *substantia nigra pars compacta* (SNc) induced by 6-hydroxydopamine (6-OHDA) and challenged with dopaminergic drugs was proposed by Ungstead as a model of the end stage of Parkinson's disease (PD) in 1970. Since then, it has been one of the most popular models to study drugs potentially useful to treat this disease. The cause of the turning behavior seems to be related to a misbalance between right and left brain hemispheres to initiate movements. The purpose of this study is to show that MPTP treated rats is a good model of the early stage of PD. Data were analysed by ANOVA followed by the Newman-Keuls test. Adult male Wistar rats received 100 ug 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into the SNc. This treatment caused partial loss of tyrosine hydroxylase-immunoreactive neurons mainly restricted to the SNc and partial depletion of striatal dopamine (sham=5161.2±369; MPTP=1698.2±296.1; P<0.01). These MPTP rats presented dose-dependent ipsiversive turning behavior when challenged either with the dopamine receptor agonist, apomorphine (saline=2±0.5; apomorphine 0.25mg/kg: 65.6±13.14, 0.5mg/kg: 139.6±21.84, 1.0mg/kg: 178.7±53.36; P<0.001), or with the indirect dopamine receptor agonist, amphetamine (saline=2±0.5; amphetamine 1.0mg/kg: 40.8±5.2, 5.0mg/kg: 44.7±12.3, 10.0mg/kg: 126.2±15.8; P<0.001). This behavior differed qualitatively from that observed in 6-OHDA rats, which presented contraversive turns when challenged with apomorphine, levodopa or pramipexole. The present results validate the ipsiversive turning behavior of MPTP rats as a simple and quantitative model with predictive value for the screening of drugs potentially effective to improve motor impairments observed in the early stage of PD.

Palavras-Chave: Parkinson's disease; MPTP; 6-OHDA; rat; dopamine; turning behavior; substantia nigra; basal ganglia

Apoio Financeiro: Capes and Fundação Araucária

Effect of scopolamine during early postnatal period in the rat

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The central cholinergic muscarinic transmission has a modulatory function, and their stimulation appears to be mainly excitatory. The effect of the non selective muscarinic antagonist scopolamine in adult rats has been classically reported as amnesic; but there is not much information about its effect on neuroplasticity and in learning and memory during early postnatal period. Radioligand binding assays were carried out to evaluate muscarinic receptor changes at different ages in the rat, using selective receptor subtype muscarinic toxins. In order to investigate the effect of scopolamine (0.5 mg/kg, i.p.) in learning and memory we trained 22, 32 and 42 days old Wistar rats in a step-down inhibitory avoidance of a foot-shock task. There was a facilitatory effect at 22 days, no evident effect at 32 days and an amnesic effect at 42 days, as reported in adults. However, an effect on anxiety and/or pain sensitivity in these early stages would also be involved. Therefore, we submitted the animals to a T-maze test for anxiety-like behaviour. Scopolamine did not show any significant effect. To analyze the possible action of scopolamine on pain perception and/or transmission, we performed a tail-flick test at 22, 32 and 42 postnatal days. Although there was a significant difference in sensitivity (tail-withdrawal latency) at some ages, there were not any significant difference concerning scopolamine effect compared to every control group. These results suggest that central muscarinic transmission would have an inhibitory function at early postnatal stages.

Palavras-Chave: learning , muscarinic , scopolamine , rat

F.02.005

The role of the muscarinic M1 receptor in context-shock association.

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Objectives: Previous data have shown that dicyclomine, a muscarinic antagonist with high affinity for M1 receptors, impairs the acquisition of context fear conditioning (CFC). However, the role of the muscarinic M1 receptor in context-shock association during CFC may be obscured by the typical training paradigm, in which acquisition of context representation and context-shock association occurs almost simultaneously within a single training session. The objective of this study was to verify the effects of dicyclomine specifically on acquisition of the context-shock association, by using the paradigm of the context preexposure facilitation effect, in which the learning about the context is separated temporarily from the learning about the context-shock association.

Methods: Wistar male rats were treated with saline (i.p.) 30 min before being placed into two different contexts (A or B), for 5 min (preexposure session). The training session was conducted 24h after the context preexposure. The animals were treated with saline or dicyclomine (16mg/Kg, i.p.) and after 30 min, they were placed in context A and one footshock (0.6mA, 1s) was delivered after 15s. The CFC test was performed 48h after training. Animals were individually placed in context A and the freezing time was measured for 5 minutes, and used as an index of conditioning.

Results: Within the control group, which received saline before both, the preexposure and the training sessions, animals which were preexposed to the same context of the training (context A) displayed more freezing time than those which were preexposed to a different context (context B) (ASAL: 35.4 ± 4.6 ; BSAL: 17.1 ± 5.0 ; $p < 0.05$, data represented as mean freezing time $\pm$ SE). The treatment with dicyclomine before the training session impaired the conditioned freezing response of animals preexposed to the context A (ASAL: 35.4 ± 4.6 ; ADICI: 15.9 ± 3.5 ; $p < 0.05$).

Conclusion: These results suggest that dicyclomine impaired the acquisition of CFC, possibly by impairing the learning about the context-shock association. Another possibility is that dicyclomine has impaired the retrieval of the context memory during the training session. More studies are needed to elucidate this issue.

Palavras-Chave: Memory, Dicyclomine, Contextual fear conditioning, M1 receptors

Apoio Financeiro: CAPES, AFIP

F.02.006

VITAMINS E AND C PRETREATMENT PREVENTS MEMORY DEFICIT CAUSED BY OVARECTOMY IN RATS

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Objectives: In the present study we investigated the influence of the pretreatment with vitamins E (alpha-tocopherol) and C (ascorbic acid) on memory impairment in inhibitory avoidance task caused by ovariectomy in rats.

Methods: Female adult Wistar rats (21 day-old) were assigned to one of the following groups: sham saline, sham vitamins, ovariectomized saline and ovariectomized vitamins. Animals were treated for 60 days with a single intraperitoneous injection of vitamins E (40 mg/kg) and C (100 mg/kg), or saline (Neurochem Res. 27:1685, 2002). Twelve hours after the last injection animals were submitted to surgery (Experimental and Surgical Technique. In The rat. 276, 1992). Thirty days after ovariectomy, rats were trained in a step-down inhibitory (Neurobiol. Learn. Mem. 68:285, 1997).

Results: Ovariectomized rats presented an impairment in the step-down inhibitory avoidance task. Pretreatment with vitamins E plus C significantly prevented the effect of ovariectomy on memory.

Conclusions: We concluded that ovariectomy impairs learning/memory and that pretreatment with vitamins E and C prevented such effect. Assuming that ovariectomy-induced memory impairment might mimic, at least in part, the cognitive deficit sometimes present in the human condition of lack of reproductive hormones, our findings support a novel therapeutic strategy, based on vitamins E and C, to prevents cognitive impairments in post-menopausal women.

Palavras-Chave: Ovariectomy, memory, vitamins E and C

Apoio Financeiro: CNPq, PROPESQ/UFRGS, FINEP (IBN-Net 01.06.0842-00).

F.02.007

Role of anxiety levels in the performance of rats in an appetitive discrimination task

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The plus-maze discriminative avoidance paradigm has been used to study the relationship between aversive memory and anxiety. The present study aims to verify if the elevated plus-maze can provide information about appetitive memory and anxiety, through a task motivated by food reward. Animals were allowed to explore an elevated plus-maze and received reinforcement in one of the enclosed arms. In a test session performed 24h later, in the absence of reward, rats showed preference for the previously rewarded enclosed arm (163.0 ± 16.52 s) (mean $\pm$ EP)* over the neutral enclosed arm (89.00 ± 13.34 s). The administration of diazepam and pentylenetetrazole before training induced, respectively, anxiolytic and anxiogenic effects (as evaluated by open-arm exploration). Both drugs induced amnesic effects, i.e., lack of preference for the rewarded arm in the test session: diazepam (rewarded: 105.66 ± 14.62 s and neutral: 127.58 ± 19.13 s); and pentylenetetrazole (rewarded: 100.41 ± 26.65 s and neutral: 129.10 ± 30.88 s). The results suggest that appetitive memory can be influenced by anxiety levels as well. The plus-maze appetitive discrimination task seems to be a useful model to investigate the relationship between memory and anxiety.

Palavras-Chave: Memory; Appetitive discrimination; Anxiety; Diazepam; Pentylenetetrazole; Behavior

Apoio Financeiro: CNPQ, CAPES

The Hippocampus and Dentate gyrus of the Cebus Monkey (*Cebus apella*): Cyto-, Myelo-, and Histochemical Architecture

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Behavioral, electrophysiological, and anatomical assays in non human primates have shown substantial evidence that the hippocampus and dentate gyrus are essential for memory consolidation. In New World primates, there are no detailed anatomical investigations into these regions to complement those assays. The hippocampus of the Cebus monkey, a New World primate very much used in cognitive studies is located in the middle temporal lobe, on the floor of the lateral ventricle. Immunohistochemistries for neuron and glial fibrillary acid protein (GFAP), histochemistries for NADPH-diaphorase and biotin labeled *Wisteria floribunda* lecithin, Nissl and Gallyas staining were used to describe the morphological organization of the hippocampus and dentate gyrus of the *Cebus apella*. All the hippocampal fields and the dentate gyrus show typical three laminar structures that include: a superficial plexiform layer; a deeper, well-stained cellular layer with a high density of granular cells in the dentate gyrus and less dense pyramidal cells in CA1, CA2 and CA3; and an underlying polymorphic layer with low density polymorphic neurons. After NADPHdiaphorase histochemistry and lecithin labeled extracellular matrix, conspicuous limits between the subicular region, CA1, CA2 and CA3 were revealed. The neuropil of CA2 was entirely labeled by *Wisteria floribunda* lecithin and the *stratum lucidum* of CA3 was highlighted by the absence of NADPH diaphorase histochemical activity. In comparison with CA1, the subicular region presented smaller and quite distinct perineuronal nets. Dentate gyrus and hilar region of the *Cebus apella* were quite similar to previous description in the Rhesus monkey (Cerebral Cortex, Jones, E. G. & Peters A., ed. (6): 345, 1987) except by the fact that the CA4 was not a distinct subfield in the hilar region in the Cebus apella. Immunolabeled astrocytes were most concentrated under the granular cell layer of dentate gyrus in the margin of the polymorphic layer. The relatively small differences between Rhesus and Cebus monkey hippocampi are phylogenetically consistent with hippocampus being part of the archicortex. **Palavras-Chave:** hippocampus, Cebus Monkey, anatomical assays.

Apoio Financeiro: Cnpq, Capes, Finep e CWPD financiado por IBNnet

F.02.009

Accumulated Entropy: an alternative model for explaining sequence learning

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The temporal organization of behavior, memory for stimuli associations, and predictions of future events have been evaluated in sequence learning tasks such as serial reaction time (RT) task. One of the main factors determining performance in this task is the sequence complexity. Presently, this is expressed by the conditional probability of transitions between different elements of the sequence which may involve relationships of first, second and third orders.

In this study we present and test an alternative model for explaining performance on sequence learning tasks, relying on the Accumulated Entropy based on the Shannon's informational entropy concept. Thus, this study aimed at evaluating the predictive power of both (1) a model relying on Conditional Probability and (2) a model of Accumulated Entropy, for explaining results of serial RT tasks.

RTs of human volunteers in a serial RT task were measured; the volunteers had to press one out of 4 keys as soon as a target stimulus was presented on a screen; each key corresponded to one stimulus location. Two different sequences of locations were used, one with repeated elements at every 8 items (R) and the other non-repetitive (N) such that some transitions had the same probability of first, second and third orders on both sequences.

Contrary to the Conditional Probability model's prediction, the RTs exhibited by the subjects in these transitions were shorter in the R sequence as compared to the N sequence. Differently, the Accumulated Entropy model precisely predicted performance in specific transitions and also in the complete series of R and N sequences. In addition, this model allowed providing a parsimonious explanation of a plethora of literature data on serial reaction time tasks, and the generation of testable predictions on this issue.

Palavras-Chave: accumulated entropy, sequence learning, serial reaction time task

Apoio Financeiro: CNPq, FAPESP

F.02.010

HIPPOCAMPAL NMDA RECEPTORS ARE DIFFERENTIALLY INVOLVED IN MEMORY CONSOLIDATION DEPENDING ON THEIR NR2 COMPOSITION.

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The hippocampus is involved in consolidation of various different long term memories (LTM). The N-Methyl-D-Aspartate receptor (NMDAR) is involved in activity-dependent synaptic plasticity and in related central functions like learning and LTM. It always consists of the essential NR1 and different NR2 (A-D), and sometimes NR3 subunits. NR2B has been postulated as responsible of facilitating learning and memory, and LTP. To further analyze its role in memory, adult Wistar rats were stereotactically cannulated in both hippocampus under anesthesia. Either the NMDAR non-selective antagonist MK801 or a NR2B subunit relatively selective antagonist ifenprodil (NR2C also), were infused through the cannulae immediately after training in various behavioral tasks. The rats were tested for retention 24 h later (LTM). We have shown that hippocampal NMDARs are involved in habituation to an open field and in an step-down inhibitory avoidance to a foot-shock. Ifenprodil infused into the hippocampus had no evident effect on any of those tasks. However, when an underthreshold foot-shock was applied, ifenprodil improved performance, while control animals did not appear to remember the task. Neither MK801 nor ifenprodil affected the performance in an object recognition task, suggesting that early steps of consolidation of this task are independent of NMDAR in the dorsal hippocampus. However, ifenprodil also exerted facilitatory effects in an object location task. Hence, our results suggest that hippocampal NR2B containing NMDA receptors would act as negative modulators on the early consolidation of specific memories, with strong aversive and/or spatial content rather than those mainly concerning novelty.

Palavras-Chave: NMDA receptor, hippocampus, memory

Apoio Financeiro: FONCyT (PICT 05-14346), Univ. of Bs. As. (M040), CONICET (PIP 6086), CNRS/CONICET.

F.02.011

Emotional memory impairment induced by low doses of reserpine in rats

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Objectives

We have recently verified that the monoamine depleting drug reserpine – at doses that do not modify motor function - impairs memory in a rodent model of aversive discrimination. In this study, we verified the effects of low doses of reserpine on the performance of rats in object recognition, spatial working memory (spontaneous alternation) and emotional memory (contextual freezing conditioning) tasks.

Methods and Results

After subcutaneous reserpine (0.1-0.5 mg/kg) or vehicle administration, male Wistar rats were tested in object recognition, spontaneous alternation or contextual freezing conditioning tasks. While object recognition and spontaneous alternation behavior were not affected by reserpine treatment, contextual fear conditioning was impaired. Freezing duration (mean $\pm$ SE) of vehicle, 0.25 and 0.5 reserpine-treated rats in the test session were 72 ± 60.19 , 29 ± 6.85 and 19 ± 4.44 , respectively.

Conclusions

Together with previous studies, these results suggest that low doses of reserpine would preferentially induce deficits in tasks involved with emotional contexts.

Palavras-Chave: reserpine, Parkinson Disease, memory, emotion, animal model

Apoio Financeiro: CNPq, PROAP/UFRN

On the participation of mTOR in recognition memory

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Evidence indicates that activation of the neuronal protein synthesis machinery is required in areas of the brain relevant to memory for consolidation and persistence of the mnemonic trace. Here, we report that inhibition of hippocampal mTOR, a protein kinase involved in the initiation of mRNA translation, immediately or 180min but not 540min after training impairs consolidation of long-term object recognition memory without affecting short-term memory retention or exploratory behavior. When infused into dorsal CA1 after long-term memory reactivation in the presence of familiar objects the mTOR inhibitor rapamycin (RAP) did not affect retention. However, when given immediately after exposing animals to a novel and a familiar object, RAP impaired memory for both of them. The amnesic effect of the post-retrieval administration of RAP was long-lasting, did not happen after exposure to two novel objects or following exploration of the training arena in the absence of other stimuli, suggesting that it was contingent with reactivation of the consolidated trace in the presence of a behaviorally relevant and novel cue. Our results indicate that mTOR activity is required in the dorsal hippocampus for consolidation of object recognition memory and suggest that inhibition of this kinase after memory retrieval in the presence of a particular set of cues hinders persistence of the original recognition memory trace.

Palavras-Chave: Memory; Object recognition; Consolidation; Reconsolidation; mTOR; Rapamycin; Hippocampus; Protein synthesis; Learning

Apoio Financeiro: CNPq, CAPES, PRONEX

F.02.013

Posttraining activation of CB1 cannabinoid receptors in the CA1 region of the dorsal hippocampus impairs long-term object recognition memory

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Evidence indicates that brain endocannabinoids are involved in memory processing. However, the participation of CB1 and CB2 cannabinoid receptors in recognition memory has not been yet conclusively determined. Therefore, we evaluated the effect of the posttraining activation of hippocampal cannabinoid receptors on the consolidation of object recognition memory. Rats with infusion cannulae stereotaxically aimed to the CA1 region of the dorsal hippocampus were trained in an object recognition learning task involving exposure to two different stimulus objects. Memory retention was assessed at different times after training. In the test session, one of the objects presented during training was replaced by a novel one. When infused in the CA1 region immediately after training, the non-selective cannabinoid receptor agonist WIN-55,212-2 and the endocannabinoid membrane transporter inhibitor VDM-11 blocked long-term memory retention in a dose-dependent manner without affecting short-term memory, exploratory behavior, anxiety state or the functionality of the hippocampus. The amnesic effect of WIN-55,212-2 and VDM-11 was completely reversed by co-infusion of the CB1 receptor antagonist AM-251 and mimicked by the CB1 receptor agonist ACEA but not by the CB2 receptor agonists JWH-015 and palmitoylethanolamide. Our data indicate that activation of hippocampal CB1 receptors early after training hamper consolidation of object recognition memory. **Palavras-Chave:** object recognition memory, consolidation, hippocampus, cannabinoids, CB1, CB2, WIN-55,212-2, ACEA, AM251, VDM-11, JWH-015, palmitoylethanolamide

Apoio Financeiro: CNPq, CAPES, PRONEX

On the role of hippocampal histaminergic receptors in the extinction of aversive memories.

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It is widely known from literature that fear-related or aversive memories are solidly stored in the brain, allowing animals to properly respond to threatening situations. Parallel to the importance of forming these memories, the ability to extinguish memories related to learned fear when they are no longer relevant is crucial. Difficulties in that inhibition process constitute the basis of anxiety-related psychiatric disorders. Extinction of memories constitutes a new learning that involves neuroanatomic, cellular, and molecular substrates, besides requiring gene expression and protein synthesis in distinct brain areas, as like as the hippocampus, that it is known to play a crucial role in that process. Based on those facts, it is necessary to find ways to improve extinction of aversive memories, as well as to study which signaling pathway it takes place through. The role played by the histaminergic system at the CA1 region of the dorsal hippocampus was examined in the extinction of memories related to the step-down inhibitory avoidance task. We have used three-month-old male Wistar rats trained in a one-trial, step-down inhibitory avoidance task (IA). In that task, during the first day, the animal placed in the avoidance box exhibited an inborn exploratory response. After it stepped down from the platform inside the box towards the metallic floor, it received a light footshock applied to its four legs. In the second day, the animal was allowed to explore the box for a maximum of 600 seconds. Afterwards, it was taken from the box and histamine receptor H1, H2, and H3 agonists and antagonists were infused into bilateral cannulas placed at the CA1 region of the dorsal hippocampus. Immediately after that the animal was returned to its living box. On the third day, the animals were once again placed in the avoidance box. The difference between the times the animal remained over the platform in the test session and in the training session was the value used as a learning measure. We have found that histamine improves the extinction of aversive memories, and such effect seems to be mediated by the H₂ receptor.

Palavras-Chave: memory, histamine, hippocampus, extinction, inhibitory avoidance

Apoio Financeiro: CNPq, CAPES, PRONEX

CaMKII activity in dorsal CA1 is necessary for persistence but not for reconsolidation of spatial memory after non-reinforced retrieval

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Memory consolidation involves a sequence of temporally defined and highly regulated changes in the activation state of several signaling pathways that leads to the lasting storage of an initially labile trace. Despite appearances, consolidation does not make memories permanent. It is now known that upon retrieval well-consolidated memories can become again vulnerable to the action of amnesic agents and in order to persist must undergo a protein synthesis/mRNA transcription– dependent process named reconsolidation. Moreover, long-term memories (LTMs) have as its characteristic attribute the persistence over time, and little is known about the molecular mechanisms that underlie this process. Experiments with genetically modified animals suggest that calcium/calmodulin dependent protein kinase II (CaMKII) are important for spatial memory and earlier studies indicate that several CaMKII substrates are activated following spatial learning. Nevertheless, none of the reports published so far analyzed pharmacologically the role played by CaMKII during spatial memory processing after retrieval. Using the CaMKII inhibitor autocamtide-2-related inhibitory peptide (AIP) and utilizing the Morris water maze learning task (MWM), we found that the activity of this kinase in the CA1 region of the rat dorsal hippocampus is not required for reconsolidation but is for maintenance of spatial memory immediately after the first non-reinforced retrieval test, as verified in a second retrieval test carried out 24h after the first retrieval, when the animals showed to remember the spatial map learned in the MWM training, or in a second test carried out 5d after the first retrieval, when the animals showed to have become amnesic. These findings suggest that post-retrieval activation of hippocampal CaMKII is essential for persistence of spatial memory, even though it is not for spatial memory reconsolidation. Furthermore, these results raise the possibility of we modulate the persistence of a memory trace after its retrieval, and through a distinct mechanism those engaged with memory reconsolidation.

Palavras-Chave: memory, CaMKII, hippocampus, water maze, persistence

Apoio Financeiro: CNPq, CAPES, PRONEX

F.02.016

Aversive but not spatial or recognition memory deficit induced by maternal deprivation is reverse by physical exercise

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We studied the effects of physical exercise on memory deficits induced by maternal deprivation in male Wistar rats. The rats were divided in four groups: control, deprived, physical exercise, and deprived + physical exercise. Pups were deprived from their mother for 3 hour/day during the firsts 10 of life. At postnatal day 45 (PND-45) exercise groups started forced physical exercise in a treadmill, during 8 weeks, 5 times per weeks, for 50 min/day, with an intensity corresponding to 60-75% of the maximum VO₂ of each animal. At 100 days of age animals were submitted to memory tests: Morris water maze (MWM), object recognition test (OR), or inhibitory avoidance (IA). Maternal deprivation disrupts long-term aversive, spatial and recognition memory. Physical exercise per se does not alter memory retention. However, in deprived animals physical exercise reverses the aversive memory deficit, but not the spatial and the object recognition memory deficits. Our data suggest that physical exercise may reverse some of the adverse cognitive outcomes caused by maternal deprivation.

Palavras-Chave: treadmill running, memory tests, learning and memory, maternal deprivation.

Apoio Financeiro: CNPq, CAPES

Early postnatal maternal deprivation in rats induces cognitive impairment in adult life; the deficit is reversed by donepezil and galantamine

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Maternal deprivation is known to result in long-lasting neuroendocrinological, behavioral and structural disorders. Here we investigated whether the cognitive aspects of these deficits might be related to a disruption of the cholinergic system. Pregnant Wistar rats were individually housed and maintained on a 12:12 h light/dark cycle with food and water freely available. The mothers were separated from their pups for 3 h per day from postnatal day 1 (PND-1) to PND-10. To do that, the dams were moved to a different cage and the pups maintained in the original home cage, which was transferred to a different room kept at 32°C. After they reached 120-150 days of age, maternal-deprived and control animals were trained in an object recognition task and a social recognition task as described (Rossato et al, 2007; Lévy et al, 2003). Maternal deprivation induced a clear impairment in object recognition as measured 2 or 24 hours after training and social recognition memory. This change was accompanied by an increase of acetylcholinesterase activity in hippocampus and perirhinal cortex. However, deprived rats that received donepezil or galantamine (1mg/kg) 30 min before training showed normal retention. Our results suggest that maternal deprivation affects memory processing at adulthood and that this impairment can be mediated by modification of the cholinergic system. **Palavras-Chave:** Maternal deprivation, object recognition, social recognition, galantamine, donepezil

Apoio Financeiro: CNPq

O EFEITO DA CLORFENIRAMINA NA APRENDIZAGEM, MEMÓRIA E ANSIEDADE EM *Carassius auratus* SUBMETIDOS À ABLAÇÃO TELENCEFÁLICA.

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Objetivo: verificar o papel da clorfeniramina (CPA), antagonista H₁, na aprendizagem, memória e ansiedade de teleósteos submetidos à ablação telencefálica.

Método: 32 peixes entre 3 e 7,3g foram distribuídos em 5 grupos: Lesão Fictícia (LF), ablação não injetado (ABLA-NI), ABLA-SAL, ABLA-CPA e ABLA-MDZ. O procedimento comportamental, conduzido em 10 dias (T) consecutivos, teve início 10 dias após a cirurgia. Utilizou-se 13 aquários de vidro transparente (10,5L) que possuíam em uma das arestas um tubo de acrílico para direcionar o alimento até o fundo e uma divisória opaca para delimitar a área de alimentação. 30 s após a retirada da divisória, o alimento foi fornecido pelo tubo e o tempo de entrada na área de alimentação (latência) cronometrado. Após 10 min a divisória foi recolocada. Após 10 min, os animais dos grupos ABLA-SAL, ABLA-CPA e ABLA-MDZ receberam injeção (i.p.) com salina (0,9%) ou CPA (16 µg/mg) ou Midazolam (1 µg/mg) em T1, T3, T5, T7 e T9.

Resultados: Os dados referentes às latências foram submetidos à ANOVA (two way) para medidas repetidas, seguido do Teste Student-Newman-Keuls ($p < 0,05$). LF (n=7; T1: 407,57±82,54; T9: 107,29±83,98; T10: 91,43±84,77); ABLA-NI (n=9; T1: 487,89±74,16; T10: 409±95,58); ABLA-SAL (n=5; T1: 460±73,33; T10: 246±144,52); ABLA-CPA (n=5; T1: 600±0; 600±0; T10: 507,4±92,6) e ABLA-MDZ (n=6; T1: 526,33±73,67; T6: 209,67±93,63; T7: 217,33±121,09; T8: 235,67±117,24; T9: 173,83±104,91; T10: 111,67±97,75). A ANOVA aponta uma diferença significativa da latência entre os dias (ANOVA; $p < 0,0001$), o que não ocorre entre os grupos (ANOVA; $p = 0,103$) e não há interação entre os fatores (ANOVA; $p = 0,0695$). O grupo LF apresentou redução da latência que foi significativa a partir de T9. O mesmo ocorreu com o grupo ABLA-MDZ que apresentou redução significativa a partir de T6. Assim sugere-se aprendizagem. Os grupos ABLA-NI, ABLA-SAL e ABLA-CPA não apresentaram queda significativa nos valores de latência, sugerindo que não houve aprendizagem.

Conclusão: O modelo experimental, utilizado para avaliar a metodologia, foi sensível em diferenciar a velocidade de entrada na área de alimentação. A CPA, aparentemente, não possuiu efeito ansiolítico. **Palavras-Chave:** ansiedade, aprendizagem, *Carassius auratus*, clorfeniramina, midazolam

Apoio Financeiro: FAPESP, CNPq

INTERAÇÃO ENTRE O FATOR DE CRESCIMENTO SEMELHANTE A INSULINA (IGF) E A FUNÇÃO COGNITIVA EM IDOSOS SAUDÁVEIS

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Introdução: Estudos têm sugerido que com o envelhecimento ocorre diminuição nas funções cognitivas e nos níveis plasmáticos de IGF-1. É possível que esses eventos estejam relacionados, uma vez que o IGF-1 é conhecido por sua capacidade de promover um sinal neuro-ativo periférico agindo não apenas como protetor contra doenças cerebrais, mas atuando também no funcionamento normal dessa estrutura (Aleman et al, 1999; Van Dam e Aleman, 2004; Arwert et al, 2005; Aberg et al, 2006; Trejo et al, 2007). Corroborando com isso, um estudo que envolveu exercício físico verificou que após o treinamento de força houve um aumento nos níveis séricos de IGF-1 e que este estava associado a uma melhora na função cognitiva dos voluntários (Cassilhas et al, 2007).

Objetivo: O presente estudo teve como objetivo verificar se existe correlação entre a função cognitiva e os níveis de IGF-1 e IGF BP-3 séricos.

Métodos: Participaram do estudo 47 idosos saudáveis do gênero masculino, com a média $\pm$ desvio-padrão de idade, massa corporal, estatura e IMC de $69,4 \pm 4,229$, 73.796 ± 11.666 , 1.698 ± 0.068 , 25.557 ± 3.521 , respectivamente. Com o intuito de avaliar a função cognitiva desses idosos, foi aplicado o questionário Mini- Exame do Estado Mental (MEEM), além de análises sanguíneas para a verificação dos níveis de IGF-1 e IGF BP-3 séricos. Este estudo foi aprovado pelo Comitê de Ética em Pesquisa da UNIFESP – CEP- 1338/06.

Resultados: Os resultados revelaram que houve uma correlação positiva e significativa entre IGF-1 e MEEM ($r=0,35$) e entre IGFBP-3 e MEEM ($r=0,42$).

Conclusão: O presente estudo sugere que há uma associação entre altos níveis de IGF-1 e IGF BP-3 séricos com um melhor desempenho cognitivo, em idosos saudáveis. O que endossa a hipótese de que os níveis sérico de IGF-1 está relacionado com a função cognitiva.

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Palavras-Chave: IGF-1; IGFBP-3; IDOSO; ENVELHECIMENTO; COGNIÇÃO

Apoio Financeiro: FAPESP, AFIP, CEPE, CNPQ e CAPES

EFFECTS OF FLUOXETINE ON MEMORY AND ANXIETY IN RATS

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Depression is the most common mood disorder and patients diagnosed with depression may have cognitive deficits, so that a link between depression and memory loss has been observed. Additionally, depression presents comorbidity with anxiety, and antidepressant drugs, particularly selective serotonin reuptake inhibitors (SSRI), have been used for anxiety disorders. Considering a causal relationship between memory and anxiety has been reported (*J. Neurosci Met* 102:117,2000), this study aims at evaluating the effects of repeated treatment with the SSRI fluoxetine on anxiety and memory in rats. For the simultaneous evaluation of memory and anxiety, we used the plus-maze discriminative avoidance task. Wistar female rats were treated with fluoxetine (20mg/kg/day ip in volume of 1ml/kg) or saline. After treatment, the animals were placed individually in the center of the plus-maze, which had two open arms and two closed arms, in one of them an aversive stimulation (light and noise) was produced each time the animal entered the arm (in the training session). We observed that fluoxetine promoted improvement in learning since fluoxetine-treated animals presented decreased percent time spent in aversive enclosed arm (6.0 ± 0.9 ; mean $\pm$ SE) when compared to controls (12.7 ± 1.9) in the training session. However, in the test session, no differences were found in aversive arm exploration, suggesting no effects on memory. Percent time in open arms was decreased (3.1 ± 0.6) compared to saline controls (7.6 ± 1.8), suggesting an anxiogenic effect. These results suggest that fluoxetine induced learning improvement in an aversive task in rats, although this effect could be related to the increased anxiety induced by the drug during conditioning. This anxiogenic effect is not in line with the reported anxiolytic effect of the drug. More studies are needed to clarify this issue.

Palavras-Chave: depression, anxiety, memory, fluoxetine

Apoio Financeiro: CNPq

F.02.021

Privação de sono paradoxal afeta a aquisição e a evocação da tarefa de condicionamento de medo ao contexto, e esse efeito não é estado-dependente.

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Objetivo: A privação de sono paradoxal (PSP) antes do treino prejudica o desempenho na tarefa de condicionamento de medo ao contexto (CMC). Entretanto, esse prejuízo não pode ser atribuído exclusivamente à aquisição ou consolidação da informação, gerando dificuldades na interpretação dos dados. Neste estudo, ratos foram submetidos à PSP após o treino com objetivo de avaliar os efeitos da PSP na consolidação da memória. A possibilidade de aprendizado estado-dependente também foi investigada neste trabalho.

Métodos: Na etapa 1, os animais foram treinados e então divididos em dois grupos: controle (C) e privado de sono (PS). Após o final da PSP os animais puderam dormir, e então foram submetidos ao teste de CMC. Na etapa 2, foi seguido o protocolo de aprendizado estado-dependente (2x2).

Resultados: Na etapa 1, o tempo de congelamento (TC) dos animais PSP não foi diferente do de animais C. Na etapa 2, os grupos PSP antes do treino tiveram prejuízo no treino e no teste em relação aos C, sendo que o mesmo ocorreu em relação aos grupos PSP antes do teste.

	Etapa 1 – PSP pós-treino	Etapa 2 – Estado-dependente		
		Treino Teste		
C	33,81± 6,2	C-C	13,63±7,0	36,37±6,2
		PS-PS	9,96±5,4*	1,55±1,1*
PS	27,82± 6,1	PS-C	5,25±3,1*	0,60±0,4*
		C-PS	4,96±3,1	5,00±2,6*

Dados expressos em TC ± e.p.; * p<0,05 em relação ao C-C

Conclusões: Quando a aquisição e a evocação foram garantidas (etapa 1), pudemos concluir que a PSP por 96h pós-treino não afetou a consolidação. Como esse achado se opõe à sugestão de alguns autores de que o sono é fundamental para a consolidação de informações, aventamos a possibilidade de que os animais pudessem exibir um padrão de aprendizagem estado-dependente. Todavia, uma vez que os grupos (etapa 2) que apresentaram o mesmo ESTADO no dia do treino e do teste (C-C e PS-PS) não apresentaram desempenho melhor do que os que encontravam-se em estado alternado, essa hipótese não foi comprovada. Nesse trabalho observamos, ainda, que tanto a PSP pré-treino quanto a pré-teste prejudicam o desempenho dos animais nesta tarefa.

Palavras-Chave: memória; condicionamento de medo ao contexto; privação de sono paradoxal; aprendizado estado-dependente

Apoio Financeiro: AFIP; FAPESP

Grelina induce retención de la memoria activando la vía de NOS/NO de la cascada bioquímica de la memoria.

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Ha sido demostrado que la administración icv o intra-hipocampal de Grelina (Gr) en ratas incrementa la retención de la memoria^[1,2]; sin embargo no se conocen el/los mecanismos moleculares por los cuales Gr media este efecto. La formación de una memoria involucra, entre otros eventos, la activación de la enzima óxido nítrico sintasa (NOS) en el hipocampo (Hi) y la síntesis de óxido nítrico (NO)^[3]; en consecuencia, estimuladores o inhibidores de la NOS podrían afectar el aprendizaje y la memoria^[4]. En el presente estudio se investigó si la inhibición de la enzima NOS en Hi modifica los efectos conductuales sobre la memoria y/o electrofisiológicos inducidos por Gr. Se utilizaron ratas Wistar machos canuladas en la región CA₁ Hi e inyectadas con: solución salina (Controles: Ctrl), dos dosis diferentes de Gr (0.3 y 3.0 nmol/μl); un inhibidor de NOS, (NG -nitro-L-arginina: NOARG) o NOARG + Gr inmediatamente post entrenamiento en el paradigma conductual de retención de la memoria (*test de step down*). Luego de que los animales fueron testeados, se utilizaron para las determinaciones de los niveles tisulares de NO o para los experimentos electrofisiológicos. Veinte min después de la inyección, los animales fueron decapitados y se procedió a la determinación de NO por conversión de ¹⁴C-Arginina a ¹⁴C-Citrulina. Gr 0.3 y 3.0 nmol/μl incrementa significativamente los niveles de NO (Gr 3.0: 3.5 ± 0.26 ; Gr 0.3: 2.51 ± 0.51 vs. Ctrl: 1.62 ± 0.14 pmol/mg prot) mientras que NOARG disminuye significativamente los niveles de NO respecto al Ctrl (NOARG: 0.42 ± 0.08 pmol/mg prot vs. Ctrl). Cuando el inhibidor fue administrado previo a Gr los valores alcanzados no difieren del grupo Ctrl. Para los experimentos electrofisiológicos los animales fueron sacrificados 24 hs después del test conductual. Los animales tratados con Gr presentan una disminución en el umbral para generar LTP respecto al Ctrl (Gr 3.0: 7 ± 1.5 ; Gr 0.3: 6 ± 1.1 vs. Ctrl: 87 ± 9.6 Hz). Cuando NOARG fue administrado previo a la inyección de Gr 0.3 nmol/μl los valores no difieren de los controles. Estas evidencias sugieren que la retención de la memoria inducida por Gr dependería de la cascada activada por NOS/NO.

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Palavras-Chave: grelina, retención de la memoria, óxido nítrico sintasa, potenciación a largo plazo (LTP)

Apoio Financeiro: Financiado por CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) y SeCyT (Secretaría de Ciencia y Tecnología-UNC).

F.02.023

Effects of Acute and Chronic Physical Exercise and Stress on Different Types of Memory in Rats

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We study the effect of acute and chronic physical exercise in a treadmill and of daily stress (because forced exercise involves a degree of stress) during 2 or 8 weeks on different types of memory in male Wistar rats. The memory tests employed were: habituation in an open field, object recognition and spatial learning in the Morris water maze. Daily foot-shock stress enhanced habituation learning after 2 but not after 8 weeks; it hindered both short- (STM) and long-term memory (LTM) of the recognition task at 2 weeks but only STM after 8 weeks and had no effect on spatial learning after either 2 or 8 weeks. Acute but not chronic exercise also enhanced habituation in the open field and hindered STM and LTM in the recognition task. Chronic exercise enhanced one important measure of spatial learning (latency to escape) but not others. Our findings indicate that some care must be taken when interpreting effects of forced exercise on brain parameters since at least part of them may be due to the stress inherent to the training procedure.

Palavras-Chave: physical activity, stress, learning and memory, forced running

Apoio Financeiro: CNPq, CAPES, PRONEX

F.02.024

Los factores que limitan el proceso de reactivación-reconsolidación de una memoria de miedo contextual determinan la vulnerabilidad al efecto amnésico de Midazolam.

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Una memoria al ser reactivada es nuevamente vulnerable al efecto de agentes amnésicos y requiere de una fase de reconsolidación para re-estabilizarse. En trabajos anteriores de nuestro laboratorio demostramos que Midazolam (MDZ), modulador positivo del receptor GABA-A, administrado post-reactivación redujo la memoria de miedo contextual. Otros estudios señalan que el tiempo de reactivación y la edad de la memoria condicionan el grado de estabilidad de la traza después del recuerdo.

El objetivo de este trabajo fue investigar la forma en que estos factores, limitantes del proceso de reconsolidación, pueden afectar el efecto de MDZ sobre esta fase.

Ratas Wistar machos fueron condicionados al contexto y para investigar si el efecto amnésico de MDZ era dependiente del tiempo de re-exposición al contexto condicionado (EC) ,24 hs después, fueron re-expuestas 1, 3, 5 o 10 min. y posteriormente inyectadas con MDZ 1.5 mg/Kg/i.p. Para estudiar la influencia de la edad de la memoria sobre el efecto de MDZ los animales fueron re-expuestos durante 3 min, 1, 7, 21 o 36 días después del condicionamiento e inmediatamente después tratados con MDZ (1.5 mg/Kg). Finalmente para analizar la efectividad de diferentes dosis de MDZ para atenuar la reconsolidación de memorias de diferente edad, otro grupo de animales fue re-expuesto 7,21 o 36 días después del condicionamiento e inyectado con diferentes dosis de MDZ (1, 1.5 o 3 mg/Kg). Al día siguiente se evaluó la respuesta de congelamiento al EC.

En memorias recientes (1 o 7 días), MDZ 1.5 mg/Kg después de 3 o 5 min de reactivación atenuó la reconsolidación de la memoria. Después de 10 min. de re-exposición bloqueó la consolidación de la memoria de extinción. La memoria de 21 días requirió de 5 min. de reactivación para ser vulnerables al efecto amnésico de MDZ, mientras que la memoria de 36 días fue bloqueada solo por MDZ 3 mg/Kg administrado después de 5 min. de reactivación.

Podemos concluir que MDZ puede disminuir o preservar la memoria de miedo contextual, según el tiempo de re-exposición al EC, el intervalo condicionamiento-re-exposición y la dosis utilizada. La interacción entre los 2 primeros factores limita el proceso de labilización-reconsolidación y la consecuente vulnerabilidad de la memoria a ser afectada por MDZ.

Palavras-Chave: memoria de miedo contextual, reconsolidación, reactivación, edad de la memoria, midazolam

Apoio Financeiro: Agencia Nacional de Promoción Científica y Tecnológica y CONICET.

ZOLANTIDINA FACILITA ESQUIVA INIBITÓRIA EM CARASSIUS AURATUS INDEPENDENTE DO CEREBELO.

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O objetivo foi investigar o efeito do antagonista H₂ (Zolantidina) na consolidação da memória de esQUIVA inibitória em *Carassius auratus* após ablação cerebelar. Foram utilizados 60 peixes, distribuídos em 4 grupos de acordo com o tipo de cirurgia realizada, ablação cerebelar (A) ou lesão fictícia (LF), e o tratamento farmacológico, injeção intraperitoneal de salina (SAL) ou Zolantidina (ZO-20 mg/kg). Utilizou-se um aquário retangular dividido em dois compartimentos (Preto/Branco) separados por uma porta. Após três dias de recuperação, o procedimento comportamental foi realizado em três dias consecutivos, sendo que no primeiro dia foi realizada a Habituação, no segundo os Treinos (T1, T2, T3, T4 e T5) e por fim o Teste (TE). Durante os treinos, o peixe era colocado no compartimento branco por 30 segundos e o animal tinha 5 minutos para passar para o compartimento preto. Quando o animal entrava no compartimento preto, um peso (45g) era solto à sua frente e o procedimento era repetido mais quatro vezes. O tratamento era realizado 10 minutos após o ultimo treino (T5). O tempo utilizado pelo animal para entrar no compartimento preto (latência) foi registrado em segundos nos treinos e no teste, sendo considerados como indicadores de aprendizagem e memória. Após o teste de homogeneidade, os dados de latência foram submetidos à ANOVA (*Two-way*) seguido pelo Student-Newman-Keuls ($p < 0,05$). Em T5, as latências dos grupos A/ZO (251,27±22,02)(15), A/SAL (277,13±19,22)(15), LF/ZO (268,07±21,93)(15) e LF/SAL (239,60±19,17)(15), obtiveram aumento significativo em relação as de T1 (A/ZO (81,87±17,94), A/SAL (78,73±21,83), LF/ZO (85,87±19,76) e LF/SAL (61,53±15,93)), demonstrando que todos os grupos foram capazes de aprender a tarefa (SNK; $p < 0,05$). No entanto, em TE apenas a latência do grupo LF/SAL (55,07±16,35) diminuiu significativamente quando comparada à de T5, indicando que tanto a ablação cerebelar quanto a Zolantidina facilitaram a consolidação de esQUIVA inibitória. Os resultados sugerem que a Zolantidina exerce efeito sobre a consolidação e/ou interpretação do valor aversivo do estímulo em esQUIVA inibitória e que este processo é mediado pelo diencefalo e/ou mesencefalo. **Palavras-Chave:** Cerebelo, EsQUIVA Inibitória, Zolantidina, Aprendizagem, Memória.

Apoio Financeiro: Processo FAPESP 2007/06041-0.

F.02.026

Corticosterone secretion parallels extinction of fear conditioning in rats

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Objective: Corticosterone plasma levels (CORT) increase during extinction (EXT) of appetitive tasks in rats, most likely due to removal of the pleasant stimulus. EXT procedure for aversive tasks, however, implies the removal of aversive stimuli and may affect the HPA axis in a different way. Given that contextual fear conditioning (CFC) is an aversive task, we reasoned that CORT levels would decrease instead of increase during EXT.

Methods: Wistar male rats were trained (T) in the CFC and distributed in two groups: Extinction (EXT) and Control (C). EXT group was re-exposed to the same environment daily for 5 consecutive days, while C only returned to the conditioning box on day 5 (D5). Animals were sacrificed either before any procedure (B), after training, after the first re-exposure (D1) or after the last re-exposure days (D5) for determination of CORT. Another experiment was carried out in order to assess the effects of habituation (H - daily placement, but no footshock) and novelty (N - acute stimulus – D5 exposure).

Results:

Exp 1 - Freezing time (FT, seconds)

	T	D1	D2	D3	D4	D5
EXT	0.5±0.05	37.1±3.3*	16.5±3.7*	9.6±2.8	8.04±2.1	9.7±3.4#
C	0±0.03					35.4±4.2

CORT (µg/ml)

	B	T	D1	D5
EXT	4.3±2.1	35.3±6.2¥	42.3±3.6¥#	16.4±2.3¥#
C	3.7±1.4	41.3±4.5¥	2.7±0.8	35.6±4.0¥

Exp 2 – FT

	T	D1	D2	D3	D4	D5
EXT	0.31±0.2	36.2±3.6*	27.4±4.1*	21.7±4.2*	19.1±3.8*	9.1±1.6†
H	0.0±0.22	6±3.6	6.7±4.1	6.8±4.2	3.8±3.8	0.9±1.6
N						0.1±0.07

CORT (sacrificed on D5)

	CORT
EXT	23.2±5.0
H	7.4±1.6†
N	25.5±8.3

* - $p < 0.05$, compared to T; # compared to C; ¥ compared to B; † compared to other groups

Discussion: FT and CORT were higher in the first re-exposure (D1) for EXT group compared to training /baseline and decreased throughout the days. C rats that returned for the 1st time to the aversive environment on D5, presented high FT and CORT, although no correlation was found between these variables. FT in acutely exposed animals (novelty) was lower, but CORT were equivalent to EXT.

Animals that were daily exposed to the environment, but never experienced footshock, presented low FT and CORT. We conclude that CORT secretion parallels FT during EXT, but the hormonal response does not reflect fear conditioning as much as the freezing behavior.

Palavras-Chave: learning; memory; HPA axis

Apoio Financeiro: CAPES, AFIP, CNPq

Papel del sistema GABAérgico sobre los procesos de memoria en el cangrejo *Chasmagnathus granulatus*.

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La consolidación es un proceso mediante el cual la memoria pasa de una forma lábil y de corta duración a una forma estable y de larga persistencia. La reactivación de una memoria previamente consolidada, puede inducir un nuevo proceso de estabilización, denominado reconsolidación. La memoria reactivada es nuevamente lábil y pasible de disrupción, y es gracias a ésta nueva fase, por la que pasa nuevamente a una forma estable. En el caso de aprendizajes asociativos, la memoria es generalmente reactivada por la presentación del estímulo condicionado (CS) en ausencia del estímulo incondicionado (US). Es ampliamente conocido que compuestos que modulan receptores del neurotransmisor ácido γ -aminobutírico (GABA) tienen un claro efecto sobre los procesos mnésicos. La administración sistémica o localizada de agonistas impide la formación de la memoria en distintas tareas, mientras que el suministro de antagonistas la favorece.

El objetivo de nuestra investigación fue analizar el papel del sistema GABAérgico sobre las fases de consolidación y reconsolidación de la memoria contexto-señal (MCS) del cangrejo *Chasmagnathus granulatus*.

Realizando experimentos farmacológicos-comportamentales variando la droga utilizada, sus concentraciones, los tiempos de administración y fase de la memoria bajo estudio, demostramos que un agonista de los receptores de GABA_A, el muscimol, impide tanto la consolidación como la reconsolidación de la MCS; que la bicuculina, un antagonista del mismo receptor, produce una facilitación de las fases de consolidación y reconsolidación. Así mismo, encontramos que la bicuculina revierte el efecto amnésico del muscimol, cuando las dos son co-administradas. Finalmente encontramos que las benzodiazepinas, moduladores positivos del receptor de GABA_A, no poseen la capacidad de modular el tono GABAérgico, y que la picrotoxina, un antagonista no competitivo del receptor, no produce ningún efecto mnésico.

En este trabajo demuestra que el sistema GABAérgico juega un papel importante en la formación de las distintas fases de la MCS aquí analizadas. **Palavras-Chave:** Memoria, GABA, *Chasmagnathus*

Apoio Financeiro: CONICET - Agencia Nacional de Promoción Científica y Tecnológica

Dynamics of the formation of new associations during memory consolidation in *Chasmagnathus granulatus*

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Objectives

In the Context-Signal Memory (CSM) model in *Chasmagnathus*, a Visual Danger Stimulus (VDS) is associated with the training context. As a result of a strong training protocol (STP), MCS is expressed at least for five days. A single presentation of the VDS, usually insufficient for building a long-term expression CSM, can generate it if it happens inside a time coincident with MCS consolidation. This time also coincides with short-term memory expression which, at difference of long-term expression, is context-independent.

This work explores whether what *Chasmagnathus* have learned from a single presentation of the VDS, after a STP in a different context, leads to the formation of a new memory or whether it is a modification of the original memory produced by the STP. For this, we have evaluated if a consolidation process, defined in terms of protein synthesis requirement, is necessary for this new learning, after the STP or after the single presentation of the VDS in the second, different context. We also have evaluated whether long-term expression of the MCS in the second training context is due to new association learning or whether it is due to a process of generalization, i.e.: a loss in context discrimination. Finally, using reconsolidation as a tool, we have studied whether reactivation of what was learned in one context produces reactivation of the information acquired in the other context.

Results

Our results show that for hours after a STP, a single presentation of the VDS in a different context is enough to generate a new association, which requires protein synthesis after this single presentation, but not after the initial STP. The unreinforced presentation of any of the two training contexts triggers labilization of the association learned in that context, but not the one learned in the other training context.

Conclusions

This behavioral re-arranged process is not a modification of the original memory as it has been originally proposed. We propose that two different memory traces are involved in the formation of these memories.

Palavras-Chave: Consolidation, Memory Trace

The role of the medial-external parabrachial nucleus in acquisition of concurrent and delayed-sequential flavor avoidance learning induced by hypertonic NaCl

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Objetives

The external area of the medial parabrachial nucleus (PBNme) has been neuroanatomical and neurobehaviorally related with the convergent processing of the gustatory and the visceral information which is apparently required for the flavor aversion learning (FAL) formation (Am J Physiol Regul Integr Comp Physiol 281:1581-93; Brain Res 957:193-206; Brain Res 866:144-51; Behav Neurosci, 107: 1005-17; Behav Neurosci, 112: 160-71). Therefore, this area may play an important role both in the sensorial (gustatory and visceral) processing and in the gustatory-visceral integrative processes implicated in the FAL acquisition. The present study was designed with the aim of exploring the role played by the PBNme in the formation of the FAL.

Methods

This study used a lesion approach in male rats. In a first experiment, PBNme lesioned rats were subjected to a concurrent hypertonic NaCl-induced FAL. In this modality both, the gustatory stimulation and the visceral, take place simultaneously. In a subsequent experiment, rats with lesions similarly located were subjected to a sequential-delayed FAL induced by the same chemical agent.

Results

The results found show a clear disruptive effect of the external-medial located PBN electrolytic lesion on the acquisition of a hypertonic NaCl-induced concurrent FAL (experiment 1), as well as on the acquisition of a delayed-sequential FAL induced by the same chemical agent in animals with lesions similarly located (experiment 2).

Conclusions

These results seem to indicate that the PBNme is essential for the formation of a hypertonic NaCl-induced FAL, independently of the paradigm used. The underlying nature, sensorial or associative, of the deficits showed by PBNme lesioned animals in the acquisition of each modality of FAL is considered. The findings of the present study suggest that there is further proof to add to that of previous studies (reviewed in Brain Res Bull 48: 239-254) of the existence of a central circuit, in which the external region of the PBNm may be crucial for the association between gusto-olfactory and visceral stimuli with independence of the temporal requirements of the task imposed to the animals.

Palavras-Chave: concurrent flavor aversion learning, delayed-sequential flavour aversion learning, electrolytic-lesion, visceral malaise, flavor.

Apoio Financeiro: Junta de Andalucía (CTS283)

F.02.030

A LINHAÇA INFLUENCIA A HABITUAÇÃO NO TESTE DE *OPEN-FIELD*.

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Objetivos: Uma vez que o DHA influencia a habituação de animais no teste de *Open-field*, este estudo se propôs a analisar o efeito da linhaça (*Linum usitatissimum*) nesta situação. **Métodos:** Utilizou-se *Rattus norvegicus*, linhagem *Wistar*, machos (n=36) recém-desmamados, divididos em três grupos com n=12. Controle a base de caseína (GC); Linhaça como fonte protéica à caseína suplementada com linhaça (GL); e Grupo caseína modificado, a base de caseína (GCM), adicionado de óleo e fibras para se igualar ao GL, ao qual foi mantido em *pair feeding*. Todas as rações com 10% de proteína. Foram mantidos durante 56 dias em ambiente com temperatura de 22°C ± 2°C e ciclo claro-escuro de 12/12h. Ao final deste período, uma vez ao dia, durante três dias consecutivos, por 4 minutos, mensurou-se em segundos suas atividades na Arena *Open-field* com o programa para registro comportamental X-Plo-Rat 2005 para *Windows* (versão 1.1.0), sendo: alta (correr e andar), baixa (sentar-se, com pequenos movimentos da cabeça e das patas) e inatividade (“freezing” e “resting”). Foi estabelecido que quanto menor era a movimentação, maior era a habituação. Analisaram-se os resultados por dia de teste, utilizando o *Kruskal-Wallis rank sum test*, para aferir diferenças, com nível de significância de $p < 0,05$. **Resultados:** Dos três dias, somente o 3º dia não teve resultados significativos. No 1º dia, só a inatividade foi significativa, com GL (10±8) o maior valor, seguido do GC (6±18) e GCM (3±4). No 2º dia, a ordem para inatividade se repete, com GL (19±20) sendo o maior valor, e na sequência o GC (8±22) e GCM (5±10). Neste dia, há diferenças também na alta atividade, com GL (36±20) o de menor valor, GCM (54±17) intermediário e GC (58±19) o maior. **Conclusão:** A linhaça influencia a habituação no *Open-field*.

Palavras-Chave: Linhaça, Open Field e Ratos

Apoio Financeiro: FAPERJ e ARMA ZEN Ltda

F.02.031

Early postnatal ethylmalonic acid administration provokes behavioral deficit in rats

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Objective: High concentrations of ethylmalonic acid (EMA) occur in tissues and biological fluids of patients affected by ethylmalonic encephalopathy and other illnesses characterized by development delay with neurological and muscular symptoms. The pathophysiological mechanisms responsible for the brain damage in these diseases are virtually unknown. Therefore, in the present work we investigated whether chronic exposure of EMA during early development could alter the cognitive performance of adult rats in behavioral tasks.

Material and Methods: EMA was administered to rats from the 5th to the 28th day of life. Control rats were treated with saline in the same volumes. After 30 days of recovery, their performance in the Morris water maze (WM) and elevated plus maze tasks was evaluated.

Results: EMA treatment impaired spatial performance in the WM since adult rats pretreated with EMA were not able to remember the previous location of the platform spending significantly less time in the training quadrant ($28\% \pm 11.9$) as compared to controls ($46\% \pm 9.5$) and more time in the opposite quadrant (controls: $18\% \pm 8.9$; EMA-treated: $27\% \pm 9.5$). EMA-treated animals also presented a lower number of correct annulus (controls: 4.2 ± 1.6 ; EMA-treated: 1.37 ± 1.7) and a higher latency to cross over the platform position (controls: 12.43 ± 2.40 s; EMA: 40.0 ± 24.1 s). In contrast, EMA chronic administration did not affect rat performance in the elevated plus maze tasks, indicating that EMA treatment was not anxiogenic.

Conclusion: The results provide evidence that early chronic EMA treatment induces long-lasting spatial behavioral deficit.

Apoio Financeiro: Financial support: CNPq, FAPERGS, PRONEX and the FINEP research grant Rede Instituto Brasileiro de Neurociência (IBN-Net) # 01.06.0842-00.

SLEEP DEPRIVATION (BUT NOT SLEEP REBOUND) IMPAIRS ACQUISITION, CONSOLIDATION AND RETRIEVAL OF A DISCRIMINATIVE AVOIDANCE TASK IN RATS: EVALUATION OF THE INVOLVEMENT OF CONCOMITANT MODIFICATIONS IN ANXIETY AND MOTOR ACTIVITY

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The aim of the present study was to investigate the effects of paradoxical sleep deprivation (PSD) for 96h on the learning/memory processes in rats submitted to the plus-maze discriminative avoidance task (PM-DAT), which simultaneously evaluates learning, memory, anxiety and motor function. Four experiments were performed in which rats were submitted to: 1) post-training combined with pre-test PSD; 2) post-training or pre-test PSD; 3) pre-training PSD or paradoxical sleep (PS) rebound (24h) and 4) pre-test PS rebound. Concerning experiment I, post-training PSD combined with pre-test PSD induced memory deficits, an anxiolytic-like behavior and an increase in locomotor activity. In experiment II, both post-training PS-deprived and pre-test PS-deprived groups showed memory deficits per se. However, only the pre-test PS-deprived animals presented anxiolytic-like behavior and increased locomotor activity. In experiment 3, pre-training PS-deprived rats showed learning and memory deficits, anxiolytic-like behavior and increased locomotor activity. A 24h sleep recovery period after the PSD abolished the learning and memory deficits but not anxiety and locomotor alterations. Finally, sleep rebound did not modify acquisition (experiment III) and retrieval (experiment IV). This study strengthened the critical role of sleep (but not sleep rebound) in all the steps of learning and memory formation. In addition, it suggests that PSD effects on acquisition and consolidation do not seem to be related to other behavioral alterations induced by this procedure.

Palavras-Chave: sleep, rebound, learning, memory, anxiety, motor function, rats

Apoio Financeiro: Associação Fundo de Incentivo à Psicofarmacologia (AFIP), FAPESP (no 06/58274-5 to T.A.F.A., n° 06/14606-9 to C.L.P., and CEPID no. 98/14303-3 to S.T.) and CNPq

F.02.033

Memoria olfativa a largo término en hormigas carpinteras

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Las hormigas del género *Camponotus* pueden utilizar distintas claves olfativas para orientarse durante la recolección de alimento e incluso aprender a asociar un olor a una recompensa alimentaria (Anim Behav. 72:1081, 2006). El aprendizaje olfativo fue estudiado mediante un paradigma de condicionamiento que consistía en entrenar a las hormigas a discriminar entre dos olores puros en un laberinto en Y, uno asociado a una solución de sacarosa (+) y el otro a una solución de quinina (-). En el presente trabajo evaluamos la duración y robustez de la memoria olfativa establecida en *C. fellah* y *C. mus*. El entrenamiento consistió en 16 y 24 visitas al laberinto para *C. fellah* y *C. mus* respectivamente. Los olores utilizados fueron Limoneno y Octanal. La retención se evaluó mediante 5 visitas sin refuerzo a las 24 horas (*C. mus*) y a las 24 o 72 horas (*C. fellah*) post-entrenamiento. Durante 2 min registramos la primera elección (brazo correcto o incorrecto) y el tiempo en cada brazo.

En los tests de retención a 24hs, ambas especies mostraron tener una memoria a largo plazo reflejada tanto en la primera elección como en el porcentaje del tiempo pasado en el brazo correcto (para el 1^{er} test, *C. fellah*: 79% 1^a elección correcta y 72% del tiempo en brazo correcto; *C. mus*: 88% 1^a elección correcta y 76% del tiempo en brazo correcto). Sin embargo, mientras que *C. mus* presentó cierta extinción luego de la 2^{da} visita sin recompensa (1^a elección correcta: 83, 76, 59, 64 y 58% en los 5 test), *C. fellah* no mostró extinción aún después de 5 visitas de retención (1^a elección correcta: 79, 79, 84, 79 y 79% en los 5 test). Más aun, *C. fellah* mostró una buena retención también a las 72 horas (para el 1^{er} test: 76.2% de elecciones correctas y 66.5% del tiempo en el brazo correcto). Estos resultados muestran que las memorias olfativas a largo término son estables y robustas en las hormigas carpinteras. Las diferencias entre especies del mismo género podrían responder a diferencias en biología de cada especie.

Palavras-Chave: aprendizaje asociativo, condicionamiento olfativo, hormigas, memoria a largo término

Apoio Financeiro: PIP 5986; CONICET-CNRS; IBRO Travel Grant

F.02.034

Clozapina, antagonista D₂/D₄, administrada no córtex pré-frontal medial bloqueia os prejuízos de memória operacional espacial produzidos pelo Δ^9 -Tetrahydrocannabinol (Δ^9 -THC).

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Objetivo: Este estudo investigou o envolvimento de receptores dopaminérgicos D₂/D₄ pré-frontais nos efeitos de prejuízo da memória operacional espacial de duração prolongada produzidos pela administração intracortical (IC) no córtex pré-frontal medial (CPFm) de Δ^9 -THC.

Métodos: Ratos Wistar machos (250-300 g), treinados no labirinto radial de 8 braços (LR-8) e com cânulas bilaterais implantadas no CPFm (bregma: + 2,5 mm AP; $\pm$ 1 mm L; - 2,7 mm V) foram submetidos a administrações IC de Clozapina (CZP – antagonista D₂/D₄) 3,2 μ g ou seu veículo HCl 0,05 N (HCl) seguidas, após um intervalo de 10 min, por administrações IC de Δ^9 -THC (32, 100 ou 180 μ g) ou seu veículo (VEH). Após um período de 5 min os animais foram submetidos a testes com retardos de 1 h no LR-8.

Resultados: O Δ^9 -THC prejudicou o desempenho no pós-retardo de 1 h, sendo estatisticamente significativo para as doses de 32 ($2,5 \pm 0,5$, $P < 0,01$), 100 ($2,7 \pm 0,4$, $P < 0,01$) e 180 μ g comparados ao controle HCl seguido de VEH. A administração prévia de CZP diminuiu significativamente ($p < 0,01$) o número de erros produzidos pelo Δ^9 -THC IC nas doses de 32 μ g ($0,5 \pm 0,2$) e 100 μ g ($0,3 \pm 0,1$).

Conclusões: Os receptores D₂/D₄ do CPFm estão envolvidos nos efeitos de prejuízo de memória operacional espacial de duração prolongada produzidos pelo Δ^9 -THC.

Palavras-Chave: Δ^9 -tetrahydrocannabinol, córtex pré-frontal medial, memória operacional, labirinto radial.

F.02.035

BASIC EFFECTS DURING SPATIAL LEARNING ON THE TOAD *Bufo arenarum*

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Spatial learning allows amphibia, as well as other vertebrates, to localize shelter and food, remember this information and return to those places months later. Now it is time to analyse the biological mechanisms that rule this learning, looking for common patterns with other vertebrates, potentially present in a common ancestor. The objective of this work is to determine if two basic effects: blocking and overshadowing, well documented in mammals and birds, are present during spatial learning in amphibia (toad *Bufo arenarum*). Two experiments were conducted where animals were trained to localize a reinforcement (water) using visual cues placed on the wall of an open field. On the first experiment it was observed that the presence of a second visual cue can cause animals to use less a first one than they would have used if trained on it alone, phenomena called overshadowing. On the second one a blocking effect was observed, the prior presentation of one element of a compounded visual cue reduces or blocks the learning about the second component of the cue. These results are demonstrating that blocking and overshadowing are generalized basic phenomena present in a wide variety of vertebrates, including mammals, birds and amphibians.

Palavras-Chave: spatial learning - amphibia

Apoio Financeiro: CONICET

F.02.036

Wakefulness induced after Inhibitory Avoidance training is not sufficient to assure performance of sleep deprived rats.

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Objective: In previous study we observed that pre-training paradoxical sleep deprived animals (PSD) presented impairment in both acquisition and retention on the multiple trial inhibitory avoidance task (MTIA). In addition, sleep architecture has shown to be altered in PSD compared to control group (C), i.e., there was a substantial paradoxical sleep (PS) rebound in detriment of wakefulness (W) and slow wave sleep (SWS). On the other hand, immediately after training, C animals presented increased W, lasting approximately one hour, followed by SWS increase, compared to baseline. In the present study we tried to induce wakefulness in PSD animals immediately after training, in order to verify whether the wakefulness itself would allow them to retain the information acquired in the MTIA.

Methods: male Wistar rats were submitted to 48h of PSD. Immediately after the deprivation period animals were trained on the MTIA and 72h after training they were tested. Immediately after training they received: corticosterone (CORT; 3mg/kg), modafinil (MODA; 100mg/kg) or vehicle (V) injections; or total sleep deprivation (TSD) or control.

Results: One way ANOVA revealed that all PSD animals were impaired in the acquisition session compared to all C ones. Two way ANOVAs revealed that in all 3 conditions, the manipulations did not reverse the retention impairment cause by pre-training paradoxical sleep deprivation.

	Training session	Test session
C-V	15,8 $\pm$ 3,6	138,5 $\pm$ 46,5
C-CORT	11,4 $\pm$ 2,9	64,2 $\pm$ 28,5
PSD-V	20,5 $\pm$ 4,8	53,7 $\pm$ 36,5
PSD-CORT	17,6 $\pm$ 3,4	64,4 $\pm$ 37,4
C-V	9,1 $\pm$ 1,7	104,9 $\pm$ 42,7
C-MODA	13,3 $\pm$ 1,9	159,1 $\pm$ 54,6
PSD-V	26,1 $\pm$ 7,5	174,4 $\pm$ 40,7
PSD-MODA	16,3 $\pm$ 3,9	86,3 $\pm$ 36,0
C	16,9 $\pm$ 3,6	198,1 $\pm$ 38,2
C-TSD	17,8 $\pm$ 2,8	209,5 $\pm$ 43,2
PSD	15,4 $\pm$ 2,1	112,6 $\pm$ 41,7
PSD-TSD	13,6 $\pm$ 2,5	70,5 $\pm$ 38,3*
	Trials to criterion	
C	2,3 $\pm$ 0,1	
PSD	3,0 $\pm$ 0,2*	

Values are mean $\pm$ S.E. *p<0,05 compared to respective C.

Conclusion: Wakefulness induced by gentle handling did not improve PSD animals' performance, and since EEG recording was not performed in drug administered animals, it is not possible to guarantee that these manipulations altered sleep pattern, but we conclude that the possible wakefulness induced after the MTIA training was not sufficient to assure performance of sleep deprived rats.

Palavras-Chave: Sleep deprivation, Learning, Memory, Inhibitory avoidance

Apoio Financeiro: Afip, Fapesp, Cnpq, Capes

FLEXIBILIDADE DO CONHECIMENTO IMPLÍCITO AVALIADO PELA TAREFA DE TEMPO DE REAÇÃO SERIAL.

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Objetivos

Considera-se que a aprendizagem implícita é gradual, requer treinamento repetitivo e resulta em conhecimento pouco flexível (isto é, traria pequeno benefício em contexto diferente). O objetivo do presente estudo foi avaliar a flexibilidade do conhecimento implícito na tarefa de tempo de reação serial.

Métodos

Os voluntários foram instruídos a responder aos estímulos 1, 2, 3 ou 4 mostrados na tela de um computador apertando um botão correspondente. Cada estímulo / resposta corresponde a uma tentativa. O tempo de reação (TR) i.e., tempo decorrido entre o aparecimento do estímulo e a resposta, foi registrado. Após uma fase de adaptação motora em que o desempenho assintótico na tarefa foi atingido, todos os voluntários passaram por uma etapa de **exposição** a uma sequência cujas probabilidades das transições foram controladas. Na etapa de **teste**, os voluntários executaram uma nova sequência que poderia ter uma estrutura parecida (grupo A) ou uma estrutura diferente (grupo B) daquela presente na etapa de exposição.

Resultados

Apesar do desempenho dos grupos A e B não diferir na etapa de teste ($F(1, 16)=1.25$, $p=0.28$), análises adicionais revelaram que transições raras durante a etapa de exposição apresentavam tempo de reação mais lento na fase de teste no grupo A, mas não no grupo B (efeito grupo x probabilidade: $F(1, 16)=6.97$, $p=0.02$)).

Conclusões

Esses resultados indicam que o conhecimento implícito apresenta flexibilidade. O conhecimento prévio das estruturas é mantido (grupo A) ou descartado (grupo B) em função de semelhanças ou diferenças entre as condições de exposição e teste; assim, os voluntários otimizam o desempenho em cada condição.

Palavras-Chave: aprendizagem implícita, conhecimento implícito, flexibilidade, tarefa de tempo de reação serial

CORELAÇÃO ENTRE MEMÓRIA DE TRABALHO E APRENDIZAGEM DA LEITURA

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OBJETIVOS: Correlacionar os resultados em tarefas de memória de trabalho com a leitura, no início do processo e após uma ano de experiência com a aprendizagem formal.

MÉTODO: A amostra envolveu 45 crianças estudantes do CAP-UFRJ, submetidos à mesma metodologia de alfabetização (idade inicial M 80.29 DV=3,40). Foram avaliados no 1º ano do ensino fundamental, e após 12 meses, no 2º ano. Habilidades de memória de trabalho foram avaliadas através do subteste Memória Sequencial Auditiva, do ITPA (Bogossian e Santos, 1977), e pela prova de Repetição de Pseudopalavras (Kessler, 1997). Foi utilizado como teste de leitura de palavras isoladas, as listas adaptadas por Capovilla e Capovilla (2000).

RESULTADOS: Em relação às correlações entre as variáveis, verificou-se, na primeira coleta, significância de $p < .005$ entre a leitura de palavras reais e inventadas; $p < .005$ entre a leitura de palavras reais e o resultado no *span* de dígitos; $p < .013$ entre a leitura de palavras reais e a repetição de não palavras; $p < .017$ entre a leitura de pseudopalavras e o *span* de dígitos; $p < .025$ entre a leitura de pseudopalavras e a repetição de não palavras; $p < .000$ entre o *span* de dígitos e a repetição de não palavras. Já na avaliação realizada no ano seguinte, as correlações significativas foram: $p < .002$ entre a leitura de palavras reais e inventadas; $p < .002$ entre a leitura de palavras reais e o resultado no *span* de dígitos; $p < .000$ entre a leitura de palavras reais e a repetição de não palavras; $p < .015$ entre a leitura de pseudopalavras e a repetição de não palavras; $p < .000$ entre o *span* de dígitos e a repetição de não palavras.

CONCLUSÃO: Foi altamente significativa a relação entre as modalidades de memória de trabalho nas duas coletas realizadas. A correlação entre memória de trabalho e leitura foi confirmada em ambas as fases, logo no início da aprendizagem formal da leitura e um ano depois, tanto em palavras reais quanto em pseudopalavras.

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Palavras-Chave: memória aprendizagem leitura

Spermidine and other ligands of the polyamine binding site at the NMDA receptor have modulatory effects on fear extinction

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Polyamines, such as spermidine, are a group of aliphatic amines that modulate NMDA receptor (NMDAR). Microinjections into hippocampus of the agonist of the polyamine binding site at the NMDAR spermidine improve the performance of rats in memory tasks. Arcaine, an antagonist of the polyamine site, reverses the facilitatory effect of spermidine on the memory of fear. However, the role of polyamines in fear extinction was not investigated to date. Extinction requires NMDAR activation and protein synthesis in memory-relevant areas of the brain, such as hippocampus. In this study, we investigated whether polyaminergic agents alter the extinction of pavlovian fear conditioning.

Adult male Wistar rats were implanted with 27 gauge cannulas aimed 1 mm above the CA1 region of the hippocampus. After recovery, the animals were conditioned with 3 footshocks (unconditioned stimulus, US, 0.6 mA, 2 sec). 24 hours later they were submitted to extinction training where the conditioned stimulus (context) was presented lacking the US. Immediately after training, all rats were infused with PBS (control) or polyaminergic agonist: spermidine (0.02 - 2 nmol) or polyaminergic antagonists: traxoprodil (0.02 - 2 nmol) or ifenprodil (0.2 - 20 nmol) bilaterally into hippocampus. On the next four days retention tests were carried out at 24 hours intervals. Freezing behavior was assessed as the measure of fear memory. Statistical analysis (two-way ANOVA) revealed that spermidine (2 nmol) decreased freezing responses during retention tests when compared with PBS group ($F(4,72) = 2.65$; $p < 0.05$), indicating enhanced extinction memory. Accordingly, traxoprodil (0.2 nmol, $F(12,76) = 2.85$; $p < 0.01$) and ifenprodil (20 nmol, $F(3,18) = 5.15$; $p = 0.01$) increased freezing responses when compared with PBS group, indicating an impairment of extinction. These data suggest a role for polyamines in the extinction of fear, since while antagonists of the polyamine binding site at the NMDAR impair, an agonist facilitates fear extinction.

Palavras-Chave: Polyamines, fear extinction, NMDA receptor, memory

Apoio Financeiro: CCNE, CNPQ, CAPES

F.02.040

Rapid eye movement sleep expression after an object recognition task in the rat

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There is a growing set of evidences supporting the notion that sleep stages play a central role in memory processing among mammals. A central prediction of this hypothesis is that the timing of Rapid Eye Movement sleep (REMs) expression is critical for the memory consolidation. It has been described in mice that REMs deprivation (RSD) installed after an object recognition task (ORT), impairs novel object recognition. Here, we explore the changes in REMs expression after an ORT, and the effect of RSD in ORT performance in the rat.

Male Sprague Dawley rats were EEG and EMG recorded under a 12:12 light-dark cycle in isolation chambers, with water and food *ad libitum*. During the acquisition phase (AQP), animals were exposed during 5 minutes to a set of 4 objects in five sessions. AQP was performed during the last hour of the dark phase. Immediately after AQP, animals were subjected to two different delay phase conditions: (i) undisturbed sleep-wake (UD; n=7) and (ii) 4 hours of RSD (4RSD; n=5). Test phase was applied 24 hours later and consisted of two 10 minute sessions, during which two of the familiar objects were replaced by two novel objects. A third group correspond to naïve rats (NR; n=15). All the rats submitted to exploration were handled and habituated 7 days before.

Compared to naïve rats, wakefulness of UD rats is augmented in 53.2% during the first four hours after AQP. This enhancement occurred at expenses of Non Rapid Eye Movement sleep (NREMs) and REMs, in a period that corresponds to initial rest phase. The REMs is significantly enhanced in the UD group in the middle of the rest phase at expenses of wakefulness. The enhancement of REMs is reflected in a 71.3% increase in the number of REMs episodes. No differences were observed between UD and 4RSD groups in the performance during test phase of ORT.

REMs enhancement occurs after at least four hours respect to the end of the ORT. RSD applied during the first four hours after an ORT does not interfere with object recognition performance. Our results suggest that REM sleep occurring immediately after memory acquisition phase plays no significant role in memory consolidation in the rat.

Palavras-Chave: memory, object recognition task, REM sleep

Apoio Financeiro: FONDECYT 1061089

F.02.041

The bilateral lesion of the pedunclopontine tegmental nucleus (PPTg) impairs learning of the two-way active avoidance in rats.

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The pedunclopontine tegmental nucleus (PPTg) of adult male Wistar rats was lesioned by a bilateral infusion of 4.23 micrograms of ibotenic acid (PPTg group, N = 7). The control group consisted of unoperated rats (N = 10). The animals were given a daily session of two-way active avoidance task with 50 tone-footshock pairings each, for 3 consecutive days. The results were analyzed by one-way ANOVA, taking the days as repetitive measures, followed by the Newman-Keuls test. The number of active avoidance increased progressively and significantly along the training days in the control ($d1 = 20.6 \pm 4.11$; $d2 = 37.4 \pm 3.08$; $d3 = 43.7 \pm 2.31$; $p < 0.01$), but not in the PPTg group ($d1 = 13.7 \pm 4.37$; $d2 = 9.86 \pm 3.47$; $d3 = 13.29 \pm 2.84$; $p > 0.57$). In addition, the scores of avoidances in days 2 and 3 were higher in the PPTg, compared to the control group ($p < 0.001$). No significant difference was found between the groups in relation to the intertrial crossings (control: $d1 = 53.2 \pm 16.1$; $d2 = 47.6 \pm 13.5$; $d3 = 50.5 \pm 14.7$; PPTg: $d1 = 70.1 \pm 20.1$; $d2 = 50.6 \pm 15.8$; $d3 = 47.4 \pm 10.8$; $p = 0.79$), latencies of the conditioned responses to the tone (control: 2.16 ± 0.15 s; PPTg: 1.77 ± 0.34 s; $p = 0.25$), and latencies of the unconditioned response to the footshock (control: 1.17 ± 0.09 s; PPTg: 1.50 ± 0.14 s $p = 0.30$). These results suggest that the PPTg does not modulate the sensitivity or motor reactions to the tone and footshock, but that it is necessary for the association between the unconditioned and conditioned stimuli that occurs during the learning of the two-way active avoidance. **Palavras-Chave:** PPTg, active avoidance, learning

Apoio Financeiro: CAPES, CNPq, Institutos do Milênio

F.02.042

l-histidine facilitates learniNG in stressed *Danio rerio*

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Objective: Study the effect of the histamine precursor l-histidine on stressed *Danio rerio* (zebrafish) submitted to a learning procedure.

Methods: Zebrafish received intraperitoneal injection of l-histidine 100mg/kg (n=18) or saline (n=18) and five minutes later were submitted to confinement stress for 1 hour. One hour later, the aquarium air supply was turned off and after 30 seconds food was offered in a feeder. Animals were expected to associate both events. This procedure was repeated for five consecutive days. Time to approach the feeder was measured (in seconds) and considered indicative of learning. **Results:** The animals that were submitted to the stress procedure treated with saline presented a decrease to enter the feeding area (D1:217,5±25,66; D2:162,81±25,66; D3:151,31±27,49; D4:147,69±32,46; D5:110,25±26,79; Friedman, P=0.0002, DF=4, $\chi^2=21,8316$), however the multiple comparisons test of Dunn could not indicate differences between the days of training. Animals treated with LH presented a significant decrease of latencies to enter the feeding area, with statistical difference between the days 1 and 5 (D1:157,11±19,2; D2: 92,44±20,86; D3:81,83±16,71; D4:71,83±18,33; D5:62±14,66; Friedman, P=0.0012, DF=4, $\chi^2=17.9889$; Dunn, P<0.05).

Conclusions: l-histidine facilitated learning in stressed zebrafish.

Palavras-Chave: Danio rerio, learning, stress, l-histidine, histamine, memory

Apoio Financeiro: Financial Support: Fapesp 04/10659-0.

F.02.043

Zolantidine inhibits learning in stressed and non-stressed zebrafish

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Objective: Study the effect of the H₂ histaminergic antagonist zolantidine on stressed and non-stressed zebrafish submitted to a learning procedure.

Methods: Zebrafish received intraperitoneal injection of zolantidine 20mg/kg (n=21) or saline (n=18) and five minutes later were submitted to confinement stress for 1 hour. One hour later, the aquarium air supply was turned off and 30 seconds later food was offered in a feeder. Animals were expected to associate both events. This procedure was repeated for five consecutive days. Time to approach the feeder was measured and considered indicative of learning. Animals not submitted to the stress procedure received saline (n=16) or zolantidine (n=21) after training. **Results:** The animals treated post-training with saline presented a significant decrease of latencies to enter the feeding area after day 4 (D1:94,81±13,32; D2:33,06±6,44; D3:42,44±10,55; D4:31±5,66; D5:27,19±5,94; Friedman, P<0.0001, DF=4, $\chi^2=27,23$; Dunn, P<0.05). Animals that received zolantidine (D1:88,58±15,32; D2:44,16±8,62; D3:56,42±17,27; D4:38,58±7,44; D5:41,68±10,52; Friedman, P=0.0314, DF=4, $\chi^2=10,6$), also presented a decrease in the latencies however the test of Dunn could not indicate differences between the days of training. The animals that were submitted to the stress procedure treated with saline presented a decrease of the latencies to enter the feeding area (D1:154,83±23,46; D2:136,17±29,17; D3:107,67±23,63; D4:83,89±20,32; D5:54,28±13,13; Friedman, P=0.0002, DF=4, $\chi^2=22,2111$). Animals that received zolantidine did not present a decrease to enter the feeding area (D1:129,10±24,47; D2:126,05±24,01; D3:141,62±25,67; D4:138,52±27,37; D5:194,38±29,21; Friedman, P=0.7533, DF=4, $\chi^2=1,9048$). **Conclusions:** Animals treated with saline learned the task despite the stress procedure. However, a delay on learning was observed in this group. Zolantidine inhibited learning in non-stressed and stressed zebrafish.

Palavras-Chave: zolantidine, learning, stress, H₂ receptor

Apoio Financeiro: Fapesp 04/10659-0

F.02.045

Is there interaction between the hippocampus and the striatum during the learning Morris water maze task?

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Memory is not a unitary process, but consists of different systems relying on separate brain structures. Evidence for declarative and procedural memory systems operating in parallel has been reported in animals and humans. These systems are centered on the hippocampus and dorsal striatum, respectively. However, it remains controversial how these two memory systems interact to support behavior. The present study investigated the effects of lesions in the dorsal hippocampus and/or dorsal striatum on learning of two versions of the Morris water maze task. Adult male Wistar rats were divided into sham-operated (SHAM), hippocampus- (HIP), striatum- (STR) and hippocampus+striatum-lesioned (H+S) groups. Seven days after surgery the animals were given 4 trials per day training in the spatial version (1st-5th days) followed by the cued version (7th-8th days) of the water maze task. Data were analyzed by two-way ANOVA, taking the days as repeated measures. Except for the H+S group, all animals learned the spatial version, as evidenced by the progressive and significant decrease in the latencies (s) to find the platform (1st day: SHAM= 43.2 ± 4 , HIP= 55.5 ± 2 , STR= 46.4 ± 5 , H+S= 58.5 ± 1 ; 5th day: SHAM= 9.0 ± 2 , HIP= 15.3 ± 3 , STR= 16.2 ± 6 , H+S= 49.9 ± 6) ($p \leq 0.001$). In addition, the HIP group presented worse scores from the 1st to the 3rd day ($p \leq 0.05$). All groups, except the H+S, also learned the cued version (1st day: SHAM= 13.0 ± 1 s, HIP= 12.6 ± 2 s, STR= 14.7 ± 3 s, H+S= 34.2 ± 8 s; 2nd day: SHAM= 7.2 ± 1 s, HIP= 7.8 ± 1 s, STR= 9.2 ± 2 s, H+S= 26.8 ± 7 s) ($p \leq 0.001$). These results are in agreement with previous findings that the hippocampus is important for the learning of the spatial version of the water maze task. However, they also suggest that the striatum cooperates with the hippocampus to solve both the spatial and cued versions of that task.

Palavras-Chave: memory; learning; striatum; hippocampus; water maze

Apoio Financeiro: CAPES, CNPq, Institutos Milênio

Atuação da L-histidina sobre os comportamentos emocionais e a atividade locomotora de camundongos no labirinto em cruz elevado.

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O objetivo do presente trabalho foi analisar a atuação da L-histidina sobre a ansiedade e memória emocional em camundongos, re-expostos ao labirinto em cruz elevado (LCE). Os sujeitos experimentais foram camundongos da linhagem Suíço-albino, machos, com peso entre 25 e 35 gramas. O equipamento utilizado foi o LCE que consiste em dois braços abertos e dois braços fechados (30,0×5,0×0,25cm) com paredes de vidro (30,0×5,0×15,0cm), unidos a uma plataforma central (5,0cm × 5,0cm) e elevado a uma altura de 38,5cm do solo. No primeiro dia de teste (T1), os animais receberam injeção intraperitoneal (i.p) de salina (SAL) ou L-histidina (LH) na dose 1000mg/kg e volume de 5ml/kg e foram divididos em dois grupos: SAL (n=22) e LH (n=24). Após 40 minutos da injeção, os animais foram colocados no centro do labirinto com a face voltada para o braço aberto. Durante o teste de cinco minutos, foram registrados através de filmagem os parâmetros convencionais: número de entradas e tempo total nos braços abertos e fechados (EBA, EBF, TBA e TBF); tempo total na plataforma central (TC); porcentagem de entradas e de tempo nos braços e no centro (%EBA, %EBF, %TBA, %TBF e %TC); total de entradas nos braços (TE); e os comportamentos etológicos: esticar, mergulhar, levantar e imobilidade. Em T2, 24 horas após T1, os animais de cada grupo receberam injeção i.p de SAL ou LH formando quatro grupos experimentais: SAL-SAL (n=11), SAL-LH (n=11), LH-SAL (n=13) e LH-LH (n=11) e foram re-expostos ao LCE conforme os procedimentos acima citados. O teste *t* para amostras independentes mostrou que em T1 o grupo LH apresentou uma diminuição das EBF (SAL=10,95 ± 2,93; LH= 7,37 ± 3,98, *t* (44) = 3,48, *p* <0,05) e TE (SAL=16,45 ± 5,17; LH=9,92 ± 5,78, *t* (44) = 4,04, *p* <0,05) em relação ao grupo SAL. A análise dos comportamentos etológicos mostrou que o grupo LH apresentou em T1 um aumento do tempo de imobilidade (SAL= 2,56 ± 8,50; LH= 8,88 ± 20,42, Mann-Whitney U-Test, *p*=0,0195) em relação ao grupo SAL. A LH na dose de 1000mg/kg prejudica a atividade locomotora de camundongos expostos ao LCE, impossibilitando análises conclusivas sobre os efeitos da droga na ansiedade e na memória emocional.

Palavras-Chave: L-histidina; labirinto em cruz elevado; ansiedade; memória emocional; camundongo.

Apoio Financeiro: CNPq

F.02.047

Inhibition of protein synthesis in the entorhinal cortex disrupts consolidation but not reconsolidation of object recognition memory

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Several studies indicate that formation and persistence of long-term memory entail the induction of protein synthesis in specific areas of the brain. Here we show that microinfusion of the protein synthesis inhibitors anisomycin (160µg/µl) and emetine (10µg/µl) in the entorhinal cortex immediately but not 3 h or 6 h after training rats in an object recognition learning task hinders long-term memory retention without affecting short-term memory or behavioral performance. When given into the entorhinal cortex after a memory reactivation session involving familiar objects or a combination of familiar and novel objects neither anisomycin nor emetine affected persistence of the consolidated trace. Our data suggest that protein synthesis in the entorhinal cortex is necessary early after training for consolidation of object recognition memory. However, reconsolidation of the recognition trace after retrieval does not seem to require protein synthesis in this area of the brain. **Palavras-Chave:** entorhinal cortex, consolidation, object recognition, memory.

Apoio Financeiro: CNPq / CAPES

Modelo animal de exercício físico resistido e memória: implicação no estudo dos mecanismos de melhora da memória via exercício físico.

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Objetivo: Desenvolver um modelo animal de exercício resistido (em ratos) que seja capaz de testar melhora do desempenho em tarefa relacionada com memória hipocampo dependente. **Métodos:** 20 ratos EPM-1 Wistar adultos, foram distribuídos em dois grupos (experimental/ n=10) e (controle/ n=10). Durante oito semanas o grupo experimental foi submetido a treinamento progressivo resistido em aparato de escalada em escada, somando-se 40 sessões de treinamento. Após o período de intervenção, os dois grupos foram submetidos à tarefa na caixa de esQUIVA inibitória. Na fase de treino da tarefa, mediu-se a latência em segundos da passagem de uma câmara à outra. 24 horas depois, realizou-se a fase de teste em que, novamente o tempo de latência em segundos de passagem de uma câmara à outra foi mensurado. 48 horas após a tarefa, os animais foram sacrificados e o músculo estriado esquelético flexor digitorum longus (FDL) da perna direita foi retirado e congelado imediatamente em nitrogênio líquido. Posteriormente, o FDL foi cortado em criostato a -20°C (8µm), submetido a ensaio histológico corado com hematoxilina e eosina e fotografado com um aumento de 40 vezes. Todas as fibras das fotografias tiveram a sua área mensurada em (µm) e as médias receberam tratamento estatístico. **Resultados:** A latência no teste da esQUIVA inibitória do grupo experimental foi maior (460,30 ± 154,56) quando comparado ao grupo controle (275,50 ± 214,62) p<0,05. Observou-se que após a intervenção, o músculo FDL do grupo experimental mostrou um aumento da área seccional transversal (50,62 ± 7,78) em comparação ao grupo controle (37,58 ± 3,44) p<0,001. Essa diferença no tamanho transversal do músculo corresponde a uma hipertrofia de 34,5%. **Conclusões:** Este modelo em animal se mostrou adequado para se estudar os mecanismos que modulam a melhora da memória via exercício físico resistido, tendo em vista que os animais treinados mostraram uma hipertrofia de 34,5%, além de um melhor desempenho em teste de screening para verificar possíveis manipulações que tem efeito na memória hipocampo dependente. Serão conduzidos experimentos controles de atividade locomotora e de sensibilidade ao choque.

Palavras-Chave: Exercício; Memória; EsQUIVA inibitória;

F.02.049

Estudio del gen de la proteína precursora del péptido amiloide en la formación de la memoria en el cangrejo *Chasmagnathus granulatus*

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La proteína precursora del beta-amiloide (amyloid-beta precursor protein, APP) humana genera el péptido amiloide, que es el componente principal de las placas neuríticas en pacientes con la enfermedad de Alzheimer y se lo propone como causante del déficit mnésico y de la degeneración neuronal en dicha enfermedad. Los miembros de esta familia son proteínas sinápticas y han sido asociadas con sinaptogénesis y plasticidad neuronal, tanto en vertebrados como en invertebrados. Recientemente ha sido secuenciado un fragmento del gen homólogo en el cangrejo *Chasmagnathus granulatus*.

Con el objetivo de caracterizar la expresión de este gen y de su proteína en la formación de la memoria de largo término, se utilizó un modelo de memoria contextual asociativa en esta especie de cangrejo, que se encuentra muy bien caracterizado en sus aspectos comportamentales y mecánicos. Se observó una distribución ubicua del mensajero, tanto en los tejidos nerviosos como en el músculo y las branquias. En cuanto a la localización de la proteína, se encontró la presencia de dos proteínas de distinto tamaño, presentes en todos los tejidos analizados. Sin embargo, en el tejido muscular se encontró sólo la proteína de menor tamaño, sugiriendo la existencia de un proceso de “splicing” alternativo. Posteriormente, por medio de la técnica “real time PCR” se estudió el curso temporal de expresión del gen durante la consolidación de la memoria de largo-término, observándose cambios en la expresión del gen. A su vez, por ensayos de Western Blot se estudió el curso temporal de los niveles de la proteína chAPPL, encontrando una marcada correlación con los niveles de expresión del gen. Además, por inmunofluorescencia se encontró que la proteína estaba localizada en áreas fundamentalmente de función asociativa y de integración sensorial. Los resultados obtenidos sugieren la participación de chAPPL en la formación de la memoria de largo término.

Palavras-Chave: APP - Memoria - Chasmagnathus

Apoio Financeiro: IFIByNE - CONICET/ UBA/ Agencia Nacional de Promoción Científica y Tecnológica

SHORT- AND LONG-TERM EFFECTS OF CANNABINOIDS ON THE EXTINCTION OF CONTEXTUAL FEAR MEMORY IN RATS.

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Objective: Previous results from our group (Psychopharmacology. 188:641, 2006) pointed to a facilitation of contextual fear memory extinction by a low dose of a cannabinoid agonist, with unclear suggestion of within-session effects. Hence, the aim of this study was to investigate the effects of cannabinoid drugs in the short- and long-term extinction of conditioned fear using an extended extinction protocol.

Methods: Male Wistar rats were placed in a conditioning chamber and after 3 min received a footshock (1.5 mA, 1s). On the next day, they were injected i.p. with a cannabinoid agonist (WIN55212-2, 0.25 mg/kg), an inhibitor of eCB uptake/metabolism (AM404, 10 mg/kg), an antagonist of CB₁ cannabinoid receptors (SR141716A, 1 mg/kg) or control solution (10% DMSO + 0.1% Tween80) and 20 min later were re-exposed to the conditioning chamber for 30 min (extinction training). No-Extinction groups received the same drug treatment, but were exposed for 3 min to the conditioning chamber. A drug-free test of contextual memory (3 min) was performed 7 days later. Time (s) of freezing behavior was used as fear memory index.

Results: WIN55212-2 and AM404 facilitated short-term extinction, whereas the SR had a minimal effect during extinction training (*WIN*: 53±12; *AM404*: 38±14; *SR*: 92±16 vs *control*: 89±13). WIN55212-2 or AM404 groups, and the SR group showed less and more freezing, respectively, than the control group in the drug-free test (*WIN*: 51±7; *AM404*: 43±13; *SR*: 134±10 vs *control*: 85±10). The long-term consequences of pharmacological treatments were in line with the effects observed during extinction training ($r=0.66$, $p<0.0001$, Pearson's correlation) and were dependent on the extinction protocol, since no alteration in freezing time was observed in No-Extinction controls (*WIN*: 113±9; *AM404*: 117±4; *SR*: 116±8 vs *control*: 124±8).

Conclusion: Our present findings show that acute treatments with cannabinoids drugs facilitate fear memory extinction, with long-term consequences in mnemonic processes underlying defensive freezing behavior.

Palavras-Chave: cannabinoid, fear conditioning, extinction, WIN55212-2, AM404 and SR141716A

Apoio Financeiro: CNPq/CAPES

F.02.051

Possible participation of the melanin-concentrating hormone innervation of the hippocampal formation in the regulation of food-seeking behavior.

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Objectives: Melanin-concentrating hormone (MCH) is a hypothalamic peptide involved in the energy balance regulation, controlling food intake and energy expenditure in order to conserve energy stores. However, the anatomical distribution of MCH suggests that this peptide is more related to the motor activity, emotional responses and memory processes associated to feeding than hunger sensation itself. In this study, we analyzed the MCH role in the food-seeking behavior using spatial memory.

Methods: Adult young male Wistar rats (n=12, 200-220g) were submitted to bilateral cannula implants in the CA3 field of the hippocampus to receive, in the last day of the experiment, a bilateral infusion of 1ml of anti-MCH or saline. After surgery recuperation period, the animals were food deprived until their weight fell to 85% of the ad-lib level, habituated to the apparatus and then trained to find buried food in a dry version of the water maze using spatial working memory. Training and test trials were recorded using a video camera and the videos were analyzed using ANY-maze tracking program. Data collected (latency, distance travelled, speed, percent time spent in the target area and percent time spent in the previous target area) were submitted to analysis of variance (ANOVA) for repeated measures followed by Duncan's test.

Results: The ANOVA performed during training phase showed a significant difference between training days, but not between experimental and control groups, suggesting that performance improved over the training phase without significant differences between the groups. On the other hand, ANOVA revealed significant ($p<0.05$) treatment effect on latency in the test day, with reduced time to find the food in the group injected with anti-MCH.

Conclusions: Hippocampal MCH blockade improved the ability of food seeking. This result suggests that, even though MCH is an orexigenic peptide, in states of negative energy balance (food deprivation) MCH can decrease motor activity in order to conserve energy stores.

Palavras-Chave: melanin-concentrating hormone, hippocampus, spatial memory, feeding behavior, energy balance.

Apoio Financeiro: FAPESP, CAPESP, CNPq.

Análise do efeito da idade no desempenho de idosos em duas tarefas de evocação livre baseado no paradigma de aprendizagem auditiva-verbal de Rey e no paradigma de memória de figuras concretas.

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Objetivo: Avaliar o efeito da idade em duas tarefas de memória de longo-prazo: 1) evocação livre tardia do Teste de Aprendizagem Auditivo-Verbal de Rey (RAVLT30") e 2) evocação livre tardia do Teste de Memória de Figuras da Bateria Cognitiva Breve (BCSB-MT5") em um grupo de idosos normais.

Método: Foram avaliados 149 sujeitos com idades acima de 60 anos, subdivididos em duas faixas de escolaridade (fundamental incompleto e completo). Essa amostra clínica foi selecionada em ambulatórios especializados nos atendimentos de idosos de hospitais universitários do Rio de Janeiro e São Paulo. O RAVLT (A Compendium Neurop. Tests 149, 1991; Rev. Bras. Neurol. 36:79, 2000) consiste em duas listas (LA e LB) de 15 palavras. Na etapa 1) a lista A é lida em voz alta, seguido de uma evocação livre imediata. Este procedimento é repetido 5 vezes; 2) apresenta-se a lista B, seguida de uma evocação livre; 3) evocação livre da lista A após interferência da LB; 4) evocação livre da lista A após 30 minutos (RAVLT30"); 5) reconhecimento da LA. O BCSB (Arquivos de Neuropsiquiatria 52:457, 1994) consiste na apresentação de 10 figuras concretas. 1) nomeação das figuras seguida de uma evocação livre incidental; 2) nomeação seguida de evocação livre. Este procedimento é repetido 2 vezes; 3) evocação livre tardia após um intervalo de 5 minutos (MT5"); 4) reconhecimento das figuras. **Resultados:** Foi observado uma correlação negativa entre idade e número de estímulos evocados no RAVLT30" ($r=-0,270$, $p<0,05$) e no MF5" ($r=-0,217$, $p<0,05$) para idosos com ensino fundamental completo. No entanto, para idosos com ensino fundamental incompleto, foi observado uma correlação negativa significativa apenas no RAVLT30" ($r=-0,293$, $p<0,05$). **Conclusão:** Idosos normais com ensino fundamental completo demonstram uma perda do número de estímulos evocados após um intervalo de tempo, tanto em tarefas auditivo-verbais bem como em tarefas de figuras concretas. Nos idosos com ensino fundamental incompleto, o efeito da idade foi observado apenas na tarefa auditivo-verbal.

Palavras-Chave: Idosos, memória, aprendizagem, avaliação neuropsicológica

F.02.053

Nociceptin/orphanin FQ mRNA brain expression is related to individual variability observed in contextual fear conditioning

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Objective: Nociceptin, also known as orphanin FQ (N/OFQ), has been identified as the endogenous ligand for the NOP receptor, a recent member of the opioid receptor family. Several studies have implicated the N/OFQ – NOP system in learning and memory processes. Exogenous application of N/OFQ is known to impair some learning tasks, including contextual fear conditioning (CFC). The purpose of this study was to examine mRNA expression of the N/OFQ precursor (ppN/OFQ) during the conditioning process, in areas related to CFC (amygdala, hippocampus and bed nucleus of the stria terminalis).

Methods: Wistar rats were subjected to CFC training for 4 days (one trial per day), and exposed or not to the conditioning context (test) 1 week after the last trial. In situ hybridization was performed using 35S-labeled riboprobes.

Results: Within animals submitted to the test, those animals which received footshocks during training (CFC group) displayed more freezing time than those which were only exposed to the context, without footshocks, during training (Habituation group). The mean freezing time (+ SE) of these groups during the test session were: CFC: 38.1 + 3.2; Habituation: 3.5 + 1.0; $p < 0.01$). N/OFQ precursor mRNA levels in animals subjected to conditioning training or test did not differ from those that were not tested or from subjects exposed to a familiar environment. However, within the CFC group, there was a negative correlation between the conditioned freezing response to the context during the test session and the ppN/OFQ mRNA levels in the central nucleus of the amygdala ($r = -0.88$; $p < 0.05$) and bed nucleus of the stria terminalis ($r = -0.82$; $p < 0.05$).

Conclusions: These results suggest that the ppN/OFQ mRNA expression induced by the conditioning test may be related to individual variability observed during retrieval and/or expression of conditioned fear responses to the context.

Palavras-Chave: emotional memory, nociceptin, orphanin FQ, opioid

Apoio Financeiro: FAPESP, CNPq, AFIP

POSSIBLE DISSOCIATION AFTER DORSAL STRIATUM LESION IN SOMATOMOTOR AND NEUROENDOCRINE RESPONSE ON TONE FEAR CONDITIONING

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Conditioned behavioral and autonomic emotional responses are separately disrupted by destruction of different amygdala targets. Previous studies from our laboratory showed that dorsal striatum (DS) is involved in tone fear conditioning (TFC). It would be important to investigate whether lesion of the DS - a structure that receives direct and indirect projections from the amygdala - selectively interferes with the somatomotor response of freezing, leaving hormonal fear response intact. The aim of this study was to evaluate the hormonal response (by assessing the release of corticosterone) and somatomotor response (freezing) in animals with DS bilateral lesions submitted to the TFC. In experiment 1, we sought to standardize the hormonal response in conditioned (C) and pseudo-conditioned (PC) animals. Freezing response was different between (C) and (PC) groups. However, corticosterone levels did not differ between the groups. Another set of animals were submitted to electrolytic DS lesion and TFC training and test. (experiment 2). Behavioral performance was impaired in lesioned animals (L) in TFC when compared with sham animals (S) but corticosterone plasma levels were not different between groups.

Exp 1 - Freezing time -seconds (Mean +/- SE)

	Min 1	Min 2	Min 3	Min 4
C	0,37 +/- 0,18	13,6 +/- 3,56	43,0 +/- 7,16	49,5 +/- 6,70
PC	0,00 +/- 0,186	3,00 +/- 3,55	18,5 +/- 7,16	20,3 +/- 6,70

Exp 1 - Corticosterone - ng/ml - (Mean +/- SE)

C	252,2 +/- 21,52
PC	269,6 +/- 42,81

Exp 2 - Freezing time - seconds - (Mean +/- SE)

	Min 1	Min 2	Min 3	Min 4
L	0,66 +/- 1,10	1,66 +/- 4,94	10,11 +/- 6,43	10,22 +/- 6,13
S	3,63 +/- 1,0	17,0 +/- 4,47	30,9 +/- 5,81	30,63 +/- 5,54

Exp 2 - Corticosterone - ng/ml - (Mean +/- SE)

L	355,4 +/- 54,0
S	243,0 +/- 6,40

These results suggest that corticosterone levels does not reflect associative features of conditioned fear response and confirm that DS mediates the somatomotor freezing response. The present observations are also in line with other studies showing that different amygdala targets are involved in mediating fear conditioned responses.

Palavras-Chave: amygdala, emotional memory, corticosterone, freezing

Apoio Financeiro: AFIP, FAPESP, CNPq, FADA.

Efeitos ambientais sobre a relação mãe-filhote e sobre comportamentos dos ratos na idade adulta.

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Em condições naturais, ratas prenhas constroem seus ninhos em buracos ou locais protegidos, cuidando dos filhotes nos primeiros dias de vida. Todavia, em laboratórios, as prenhas não possuem material para construir tocas ou refúgios. O presente trabalho visou aprofundar o entendimento do impacto causado por alterações ambientais, através da adição de uma caixa-ninho dentro das caixas-residência. Analisando, se esta alteração modifica o comportamento maternal e o comportamento dos filhotes na vida adulta. Ratas prenhas Wistar foram divididas em: grupo controle, sem presença de caixa-ninho (n=9); grupo caixa-ninho do 1º ao 10º dia pós-parto (n=11) e grupo caixa-ninho do 11º ao 21º dia pós-parto (n=12). Em todos os grupos foi realizada a observação do comportamento maternal durante os 21 dias pós-parto. Com 90 dias, os filhotes machos foram submetidos aos testes de campo aberto e de labirinto em cruz elevado. Os resultados foram expressos através de média ($\pm$ EPM). O comportamento maternal foi comparado por uma ANOVA de medidas repetidas, seguida de Newman Keuls ($p \leq 0,05$). Para os testes comportamentais dos filhotes, foi utilizada ANOVA de uma via, seguida pelo pós-teste Newman-Keuls ($p \leq 0,05$). Os resultados demonstraram que o comportamento maternal não apresentou diferenças significativas entre os grupos.

Entretanto, no teste de campo aberto, os animais do grupo caixa-ninho dia 11 ao 21 apresentaram aumento significativo no tempo de locomoção total ($155,4 \pm 9,2$) em comparação ao grupo caixa-ninho dia 1 ao 10 ($118,7 \pm 9,1$). No teste de labirinto houve um aumento significativo no número de entradas no braço aberto e frequência de colocar a cabeça para fora do braço aberto nos grupos caixa-ninho dia 1 ao 10 ($9,4 \pm 1,2$) e caixa-ninho dia 11 ao 21 ($9,1 \pm 1,9$), quando comparados ao controle ($3,4 \pm 0,7$). Concluimos que a caixa-ninho age diretamente sobre o desenvolvimento dos animais, sem intermédio do comportamento maternal. A caixa-ninho proporciona um enriquecimento ao ambiente neonatal, mas não altera a relação mãe-filhote. Dessa forma, a diminuição da ansiedade parece ter sido causada somente pela alteração ambiental proporcionada pela introdução da caixa-ninho.

Palavras-Chave: caixa-ninho, ambiente, comportamento.

Apoio Financeiro: CNPq

Immediate early gene *zif-268* expression during acquisition, retrieval and extinction of an aversive memory

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Objective:

On the behavioral paradigm of the discriminative avoidance task, short and long-term memories have been extensively studied both behaviorally and pharmacologically. (J. Neurosci. Meth. 102: 117, 2000). the objective of the present study is to evaluate, using the cited model, the hippocampal expression of *zif-268* – a calcium dependent immediate early gene, implicated on synaptic plasticity – across the many steps of memory formation, such as acquisition, retrieval and extinction.

Methods:

The behavioral apparatus consists of a modified elevated plus-maze, with its enclosed arms disposed in “L”. A pre-exposure to the maze was made with the animal (n=3/group) using all four arms enclosed, lasting 30 min, followed by a training and a test, each 10 min long. The interval between sessions was 24 hours. During training, aversive stimuli (intense light and sound) was activated whenever the animals entered one of the enclosed arms (the aversive arm). Acquisition, retrieval and extinction of the memory were evaluated through the percentual total time exploring the aversive arm. The investigated parameters (time in the arms and distance traveled) were recorded by an animal tracking software (Anymaze, Stoelting, USA). Learning during training was estimated by the decrease in the time spent exploring the aversive arm. One hour after the start of each the session, animals were anesthetized with a thiopental (i.p.) overdose and perfused with heparinized 0.9% saline and 4% paraformaldehyde. The brains were cryoprotected in 20% saccharose, divided into three blocks and frozen. The middle block, containing the hippocampus, was sectioned at 20 µm on the coronal plane and resulting sections were then submitted to the immunohistochemistry against Zif-268.

Results:

Our results show an augmented expression of Zif-268 on the dentate gyrus (DG) during the retrieval and extinction phases.

Conclusion:

There is a distinct participation of the dentate gyrus during memory retrieval and extinction, but not during memory acquisition. Furthermore, all hippocampal areas (CA1, CA3 and DG) show an elevated expression of Zif-268 during the extinction process.

Palavras-Chave: Discriminative Avoidance Task, Zif-268, Hippocampus, Memory, Extinction, Rats

Apoio Financeiro: AASDAP, CNPq

Exercise increase the sensitivity to insulin in mice hippocampus: is a neuroprotective or neurotoxicity mechanism?

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Objective: Insulin and insulin receptors (IRs) are found in the central nervous system, their role(s) in brain function has been a subject attracting many researchers' interest. Thus, the role of insulin is still far to be determined. Diabetes and obesity affects the central nervous system once epidemiological studies have indicated that insulin resistance is a risk factor for dementia. Exercise training has multiple beneficial effects on SNC, including modulation of glutamatergic system. Overstimulation of glutamate receptors by glutamate has been involved in neural damage in ischemic events. The main objective of this work was to evaluate the effects of exercise and insulin on memory, ischemia as well as glutamate uptake.

Materials and methods: we used one month of voluntary exercise in CF1 mice. After that we injected intracerebroventricular (icv) insulin in different concentrations (0-5mU) and it was evaluated the long term memory in inhibitory avoidance and Morris's water maze tasks (MWM). Glutamate uptake in slice of hippocampus and a model of ischemia ex vivo were used to evaluate neuroprotection and neurotoxicity. **Results:** Insulin at 0-5mU did not improve learning in sedentary group in both tasks.

Exercise group had have improve in cognition in MWM ($p < 0.05$), but insulin at 5mU caused a distinct pattern. The same dose was amnesic to exercise in inhibitory avoidance task ($p < 0.05$). Glutamate uptake in slices of hippocampus was increased in exercise compared to sedentary (0.59 ± 0.12 x 0.43 ± 0.08 nmol/ μ g/min respectively, $p < 0.05$). However insulin caused decreased in glutamate uptake with 0.05mU insulin in exercise group. The same pattern occurred in sedentary group only with 0.5mU. Exercise protected cells from ischemia (20%) compared to sedentary ($p < 0.05$), this effect was abolished by insulin at 5mU. In this dose insulin was neuroprotective in sedentary group. **Conclusion:** Our results showed that exercise was neuroprotective and insulin (icv) altering this effect. We proposed that exercise training increased insulin sensitivity in hippocampus and when we overstimulate this system with more insulin it may cause neurotoxicity.

Palavras-Chave: exercise, insulin, inhibitory avoidance , Morris's water maze , Glutamate uptake , ischemia, central nervous system

Apoio Financeiro: CNPQ, CAPES, FAPERGS

On the participation of the hippocampal dopaminergic system in consolidation and persistence of spatial memory

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Dopamine (DA) acts as a key neurotransmitter in brain, and numerous studies have shown its regulatory role for motor and limbic functions, such as memory, attention and cognitive reinforcement. Regarding memory, several evidences suggest that brain dopamine is involved in memory consolidation, but the knowledge about the actual contribution of the hippocampal dopaminergic system to this process needs still to be more detailed. Specifically, is the dopaminergic system required for spatial memory consolidation as it is for other types of memory, like aversive memory? And if so, which are its consolidation time window? Furthermore, little is known about the participation of the hippocampal dopaminergic system in the mechanisms underlying the memory persistence.

Here, we show that the infusion of SCH23390, a D1-D5 dopaminergic receptors antagonist, into the CA1 region of the dorsal hippocampus immediately after training in the Morris water maze learning task (MWM), but not 3h later, impairs the consolidation of memory for this task, as verified at the test carried out 24 hours post-training.

Moreover, we show that the infusion of SKF38393, a D1-D5 agonist, into the CA1 region of the dorsal hippocampus immediately after training in MWM in a sub-training version, although doesn't enhance the consolidation of memory for this task, does promote the persistence of the mnemonic trace along the subsequent days.

Together, these results point to the participation of the hippocampal dopaminergic system in the consolidation of spatial memory as well to a potential positive modulator of the persistence of a spatial memory trace.

Palavras-Chave: memory, dopamine, persistence, water maze, hippocampus

Apoio Financeiro: CNPq, CAPES

F.02.059

Olfactory memories acquired during trophallaxis affect the odor choice at the food source in carpenter ants

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Introduction: A few social insect species are known to establish olfactory memories at the food source associating an odour cue with the reward stimuli (nectar). *Camponotus mus* ants are able to use this memory to localize the nectar at the food source in absence of other cues (Anim. Behav. 72:1081- 2006). We studied whether this carpenter ant is able to learn an odour *during the mouth-to-mouth food exchange (trophallaxis)* and tested the role of this memory in a Y-shaped food source.

Methods: Pairs of ants were isolated under controlled conditions. After a given period, one of them (donor ant) was fed with aromatised sucrose solution and then put back with her partner (receiver ant). After the first trophallaxis, the response of receiver ant was evaluated on a Y-shaped food source without any reward by presenting two different odours: in one branch, the solution odour (correct arm) and in the other, a new scent (incorrect arm). Two pairs of odours were used: Pair 1, Rose-Teebaum and Pair 2, Sandalwood-Cypress. We recorded the first choice and the time spent in each arm during two minutes.

Results: In non-reinforced tests, receiver ants consistently chose the branch with the odour presented in the sucrose solution (80 and 75% for both odour pairs) and spent more time searching therein (Pair 1: 36,9±2.7 s in the correct arm, 17.0±1.5 s in the incorrect arm. Pair 2: 32.9±2.5 s in the correct arm, 17.5±1.6 s in the incorrect arm). Olfactory learning occurred independently of the duration of trophallaxis (2 to 156 seconds) (Spearman correlation: for Pair 1, p=0.6 and for Pair 2, p=0.8).

Conclusions: These results thus show for the first time that individual nectivorous ants perceive and learn odours during trophallaxis and that this memory influences the subsequent search behaviour at the food source. An only trophallactic event was enough for learning even when it was very short. According to these findings, during trophallaxis a worker might access information of a new source visited by a nestmate. In this sense, it might also play an important role in the recruitment and group foraging organization.

Palavras-Chave: social learning, ants, trophallaxis, olfactory memory

Apoio Financeiro: PIP 5986, CONICET-CNRS

Enhancement of spatial and recognition memory in both middle-aged rats by the anti-amyloidogenic compound 2,4-dinitrophenol

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Low concentrations of 2,4-dinitrophenol (DNP) are known to upregulate neuronal cAMP and tau levels and promote neurite outgrowth and neuronal differentiation (FASEB J, 2005, 19: 1627-1636). Furthermore, DNP has been shown to block the oligomerization and neurotoxicity of A β (Curr. Alzheimer Res, 2007, 4:263-71). Here, we have evaluated the effects of DNP in cognition in both middle-aged mice and rats, using the Morris Water Maze (MWM) and Object Recognition (OR) tasks. Animals were trained in a circular pool to find, by swimming, a circular platform submerged the water surface. Both eight month old balb/c mice (control n=14, treated n=15) and 10 month old Wistar rats (control n=11, treated n=11) were administered (via daily intraperitoneal (IP) injections) with 5 mg/Kg/animal DNP or vehicle (control group) for 14 days. After treatment period, animals were trained in the MWM task and their performances were evaluated. DNP-treated mice showed a better performance in the MWM task when compared to the control group. The average latency time for the control group was 30.6 seconds, while for the treated group it was 21.2 seconds ($p<0.001$). The probe test showed that mice treated IP with DNP had a significant preference for the platform quadrant ($p<0.03$). In addition, rats that received DNP presented a better performance in the MWM in the day 5 when compared to rats injected IP with vehicle ($p<0.03$). DNP-treated rats spent more time exploring the novel object indicating recognition of the familiar object, whereas control rats spent the same amount of time exploring the novel and the familiar objects ($p<0.0001$). Importantly, we found enhanced levels of tau and phosphorylated CREB in the hippocampi of rats treated with DNP. Our results indicate that DNP has a beneficial effect in cognition in both middle-aged rats and mice. Taken together, these and previous results showing neuroprotective and neuritogenic actions of DNP reinforce its potential as a lead compound for the development of novel therapeutic approaches for a disease of memory such as Alzheimer's disease.

Palavras-Chave: 2,4 dinitrophenol, learning, memory, morris water maze, object reconition,

F.02.061

Efeitos da exposição ao fluoreto de sódio durante o desenvolvimento na memória/aprendizado de camundongos

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Estudos anteriores sugerem que a exposição ao flúor está associada a alterações cognitivas. Para verificar esta hipótese, o presente estudo avaliou os efeitos da exposição ao fluoreto de sódio durante o desenvolvimento na memória/aprendizado de camundongos. Camundongos suíços adultos fêmeas foram expostos a 120ppm de fluoreto de sódio (n=8) ou somente a água (n=4) na garrafa de água *ad lib* 2 meses antes do acasalamento, durante o acasalamento, o período gestacional e a lactação. Após o desmame (PN21), as ninhadas foram expostas à mesma dose até PN28. A partir de PN22, filhotes tratados com fluoreto de sódio (NaF, n=53) ou água (VEH, n=28), de ambos os sexos, foram submetidos aos testes comportamentais descritos a seguir. De PN22 a PN26, utilizamos o Labirinto aquático de 8 braços para avaliação da memória espacial: cada animal deve encontrar uma plataforma submersa 1 cm em água opaca. A posição da plataforma é modificada a cada dia de teste (4 testes/dia por 5 dias consecutivos). A latência para encontrar a plataforma é utilizada como medida de memória/aprendizado. Em PN27 e PN28, utilizamos o teste da Esquiva passiva para avaliação da memória operacional: o animal é colocado numa câmara clara e espera-se por 5 minutos que entre na câmara escura. Ao entrar, a porta se fecha e é administrado um choque (0,2 mA/2s). Após 24 horas, os animais são testados novamente. A latência para entrar na câmara escura no 2º dia de teste é usada como medida de memória/aprendizado. Nossos resultados indicaram um efeito do tratamento ($F=5,0$; $gl=1$; $P=0,028$) no labirinto aquático, que pode ser explicado pelo fato do grupo NaF apresentar pior performance cognitiva quando comparado ao grupo VEH no 5º dia de teste (NaF: $46,9 \pm 2,7$; VEH: $33,8 \pm 3,2$; $P=0,029$). Houve, também, uma tendência à significância no 1º (NaF: 59 ± 3 ; VEH: $49,1 \pm 3,7$; $P=0,087$) e 4º (NaF: $52,6 \pm 2,9$; VEH: $42,5 \pm 3,5$; $P=0,081$) dias de teste. O teste da esquiva passiva não indicou diferenças significativas entre os grupos. Assim, a exposição ao flúor durante o desenvolvimento pré e pós-natal de camundongos causa danos à memória, que parecem ser específicos para memória visuo-espacial, não afetando a memória operacional.

Palavras-Chave: Flúor, cérebro, camundongo, cognição, memória visuo-espacial, memória operacional

Apoio Financeiro: FAPERJ e SR-2

Neural basis of the sensory cortex area integration on object coding

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Objectives: One of the tenets of modern neuroscience is the specialization of sensory areas in the cerebral cortex according to different modalities. Here we present preliminary results revealing the presence of tactile information in neuronal ensembles recorded from the rat primary visual cortex (V1) during novel object exploration in the dark.

Methods: Adult male rats (n=6) were surgically implanted with multielectrode arrays of teflon-coated tungsten microwires (35 μ m, 1.0–1.2 MOhm at 1 KHz). The targeted brain areas comprised the hippocampus (HP), the primary somatosensory cortex (S1) and V1. After a 1-week recovery period, extracellular recordings of neuronal action potentials (spikes) were performed across the sleep-wake cycle before and after novel object exploration in the dark (4 different objects). Behaviors were recorded with an infrared video camera. Data from 450 single units were used as input of populations of Artificial Neural Networks (ANN) to perform pattern classification. We used the Area Under Receiver Operating Characteristic (AUROC) curves to estimate the quality of pattern classification by ANN populations. The analyses were implemented on a computational grid of 67 machines.

Results: For most objects, satisfactory pattern classification (mean AUROC > 0.75) was obtained with V1 data. Results were comparable to those derived from S1 neuronal ensembles. A neuron-dropping analysis was implemented to investigate the minimum amount of neurons required for adequate object classification. We found that ensembles as small as 8 single units allow for accurate object classification, both in S1 and V1. A preliminary classification using Self-Organizing Maps (SOM) showed good agreement between the quality of the ANN classification and the SOM separation of object-related neuronal data according to object classes.

Conclusions: The results suggest that, it is possible to perform object classification based on V1 neuronal ensemble activity when rats perform object exploration in the dark.

Palavras-Chave: object coding, artificial neural networks, area integration, pattern classification

Apoio Financeiro: CAPES, AASDAP, Finep, CNPq, MCT

BEHAVIOURAL AND MOLECULAR EVIDENCE OF STANDARDIZED EXTRACT *Ginkgo biloba* L. (EGb 761) ON FEAR MEMORY.

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Studies regarding the effects of standardized *Ginkgo biloba* L. (EGb 761) on the central nervous system have lead to the suggestion of a beneficial effect of this extract on memory and the generation of conflicting evidence. Therefore, the characterization of the putative neuromodulatory effects of EGb 761 on learning behaviour and the molecular mechanisms underlying this process will help to understand this issue. The purpose of the present study was to examine the effects of different doses of EGb 761 in chronic treatment on rats submitted to a conditioned emotional response (CER). In addition, we investigate whether genes previously associated with classical conditioning (*CREB-1* and *GAP-43*) and candidate genes (*GFAP*) are modulated after EGb 761 treatments. To this end, eighty adult, male, *Wistar* rats ($\pm$ 250-300 g) were fear conditioned in an operant chamber while drinking behavior in an off-baseline procedure. *CREB-1*, *GAP-43* and *GFAP* expressions were evaluated using both quantitative PCR (qPCR) and immunohistochemical. We here observed that the rats submitted to chronic EGb 761 treatments had acquisition of fear conditioning. Indeed, the results reported herein provide evidence for an effect of a standardized extract of EGb 761 in regulation of the expression of fear conditioned. Additionally, we showed, for the first time, that GAP-43, CREB-1 and GFAP mRNAs and protein expressions are modulated in the prefrontal cortex, amygdala and hippocampus after treatment with EGb 761. All together, our findings not only provide new evidences for a role of EGb 761 on memory but also identify molecular changes that underlie the fear memory consolidation. **Palavras-Chave:** Fear conditioning, CREB-1, GAP-43, GFAP, *Ginkgo biloba*

Apoio Financeiro: FAPESP- CAPES

F.02.064

Participação do Córtex Pré-frontal na aprendizagem da esQUIVA discriminada em ratos testados no labirinto em cruz elevado.

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Introdução: A porção medial do córtex pré-frontal (CPFm) tem sido associada à aquisição e à emissão de respostas aversivas condicionadas. Neste trabalho avaliamos os efeitos da lesão no CPFm na esQUIVA discriminada de ratos testados no labirinto em cruz elevado modificado (LCEM).

Método: Foram utilizados 40 ratos *Wistar* machos, pesando em média 350g, distribuídos em 4 grupos, a saber: 1) Grupo lesão-aprendizagem, (GLA), 2) Grupo sham-aprendizagem (GSA), 3) Grupo não lesão-aprendizagem (GA), 4) Grupo controle manipulação-sem cirurgia-sem aprendizagem (GM). A lesão do CPFm foi realizada com ácido quinolínico (Sigma-Aldrich, 50 η M, 0,5 μ l, pH = 7,4), nas regiões pré-límbica (PrL) e infralímbica (IL), 14 dias antes do treino. O procedimento comportamental consistiu na exposição dos animais ao LCEM, que tem um braço fechado aversivo (BFAV). Durante o treino com duração de 10 minutos, a apresentação de som e de luz branca era contingente à resposta de entrar e permanecer no BFAV. No teste, realizado 24 horas após, o animal era exposto ao LCEM por 3 min, sem apresentação de estímulos. Foi analisada a porcentagem do tempo de permanência nos braços abertos (BA) e BFAV durante os 5 min iniciais (TR5) e finais (TR10) do treino e no teste. Para comparações intra-sessões usamos o teste de Friedman, seguido do teste de Dunn. Comparações entre sessões e entre grupos foram realizadas pelo teste de Kruskal Wallis, seguido do teste de Bonferroni.

Resultados: Durante o treino (TR5 vs. TR10) houve (i) redução na porcentagem de tempo de permanência no BFAV para os grupos GLA, GSA e GA ($p < 0.05$) (ii) redução na porcentagem de permanência nos BA para os grupos GSA e GM e GA ($p < 0.05$) e (iii) aumento para o grupo GLA nos BA ($p < 0.05$). No teste, o grupo GLA manteve-se por maior tempo no BA em relação aos demais grupos ($p < 0.05$).

Conclusão: Em conjunto os dados sugerem a participação do CPFm na modulação das respostas de esQUIVA condicionada ao BFAV. Um efeito ansiolítico da lesão é sugerido pela maior porcentagem de tempo de permanência nos braços abertos observado nos ratos com lesão.

Referências: 1- Psychopharmacology 48:192, 2007. 2- J Neurosci Methods 102:117, 2000.

Palavras-Chave: córtex pré-frontal, memória, esQUIVA discriminada, labirinto em cruz elevado

The role of dorsal hippocampus in an episodic-like memory task in rats

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Objectives

Human episodic memory refers to the capability of recollect what, when and where a specific event occurred. There are many studies relating the hippocampus in the formation of the episodic memory, however most of the non-human mammals protocols used in these studies do not involve at the same time these aspects of the episodic memory. Dere et al (Neurobiol Learn Mem 84:214, 2005) have developed a three-trial object exploration task in rodents that simultaneously access what, when and where an event occurred. The goal of this study was to evaluate if pharmacological inactivation of dorsal hippocampus would impair the acquisition of this object exploration task.

Methods and Results

The task consisted of two sample trials and a test trial. In the first sample trial, the rat was placed in the open-field with four copies of object A for five minutes. After 1 h, the animals received the second sample trial. The animals were exposed to four copies of object B in a different spatial configuration. After 24 h, the animals received the test trial. In this phase, 2 copies of each sample trial were present. One object of the first trial was in a different spatial location. The other objects were in the same positions of the sample trials. Pretraining infusions of muscimol in the dorsal hippocampus (0,250 mg/mL/side) did not affect the total distance moved and the total object exploration throughout the sample trials. In the test, saline rats showed increased exploration of objects A compared to objects B (exploration rates (mean + SE): 0,3 + 0,01; 0,19 + 0,1; respectively), reflecting the episodic components what and when. However, saline rats explored equally the displaced and the stationary objects A, indicating an absence of the episodic component where (0,3 + 0,04; 0,3 + 0,04). Muscimol-treated group explored the four objects in the same proportion (0,28 + 0,03; 0,22 + 0,05; 0,25 + 0,03; 0,23 + 0,02).

Conclusions

Pretraining infusions of muscimol in the dorsal hippocampus impairs at least the acquisition of when and what components of episodic memory. More studies are needed to determine why the saline rats did not show the spatial episodic memory component

Palavras-Chave: memory, episodic-like memory, hippocampus

Apoio Financeiro: CAPES, CNPq, PROAP/UFRN

EFFECTS ON MEMORY AND ANXIETY PRODUCED BY CHRONIC LOW-DOSE ETHANOL TREATMENT IN ADOLESCENT RATS..

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OBJECTIVES – Chronic ethanol consumption is common among adolescents. Studies have suggested that this consumption during adolescence causes cognitive impairments. On the other hand, ethanol effects on memory tasks can be modified by the anxiolytic action of this drug. The aim of the current study was to investigate the effects of chronic ethanol administration on memory and anxiety of adolescent rats evaluated concomitantly in the plus maze discriminative avoidance task.

METHODS AND RESULTS – In this experiment 12 female adolescent (1 Month old) Wistar rats received chronic ethanol (0.18 g/kg) or saline i.p. treatment during thirty days. The animals were tested in the plus-maze discriminative avoidance task five days after the end of the treatment. This paradigm is an elevated plus-maze adaptation able to evaluate anxiety and memory simultaneously. The experiment consisted of training and test sessions. The animals were placed individually in the center of the plus-maze, which had two open arms and two closed arms, in one of them an aversive stimulation (light and noise) was produced each time the animal entered the arm. The duration and the number of entries in each arm were registered. In the test session, ethanol-treated animals presented increased aversive-arm exploration when compared to saline controls (mean±SE were 85.8±75.5s and 32.9±19.2s, respectively), indicating a memory deficit. No differences were found in open arm exploration (9.9±4.9 and 9.4±4.6 for saline and ethanol, respectively), suggesting no differences in anxiety-like behavior.

CONCLUSION – Data showed that rats chronically treated with ethanol presented memory deficits, while no changes were seen in anxiety among the groups, suggesting that the cognitive effects of ethanol on adolescent rats are not related to anxiety alterations.

Palavras-Chave: Memory, Anxiety, Ethanol, Adolescence, Rats

Apoio Financeiro: CNPQ, CAPES (PROAP - UFRN)

Role of octopamine in an appetitive and an aversive conditioning in the crab

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An intriguing issue in the study of associative memories is to determine which compounds underlie these memory representations in the nervous system. Octopamine (OA) is a biogenic monoamine structurally related to noradrenaline that acts as a neurohormone neuromodulator and neurotransmitter in invertebrates. Results obtained in insects as the honeybee, the fruitfly and the cricket have shown that OA would mediate the reinforcement in appetitive learning. Here, we attempt to study the participation of OA in two associative learning paradigms in the crab *Chasmagnathus granulatus*: one appetitive and one aversive. Our basic hypothesis is that the action of an endogenous bioamine as OA would not be restricted to one type of learning. We use an aversive learning paradigm which promote the formation of the context-signal memory (CSM), extensively characterized from a behavioral and a neurobiological point of view. It consists in the association between the learning context (conditioned stimulus CS) and a visual danger stimulus, the negative reinforcement (unconditioned stimulus US). In addition, we developed a new appetitive learning paradigm using food as a positive reinforcement (US) and the same visual learning context (CS) as the one in the aversive paradigm. We explored the role of OA in these two conditioning paradigms, using injections of OA and its antagonists. The results demonstrated that OA is an amnesic agent of consolidation and reconsolidation of the CSM when injected temporally close to the end of training and reactivation respectively. In contrast, OA facilitates the appetitive learning, and an OA antagonist impairs the appetitive memory when applied previous to training. We propose, in agreement with the findings in insects, that OA could act as a positive signal that enhances appetitive learning, but inverts its effects in an aversive learning.

Palavras-Chave: aversive learning, appetitive learning, octopamine

Apoio Financeiro: Agencia Nacional de Promoción Científica y Tecnológica and Universidad de Buenos Aires

RECOGNITION AND WORKING MEMORY IMPAIRMENTS FOLLOWING LOW DOSE CHRONIC ETHANOL IN ADOLESCENT RATS

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Objectives

Ethanol consumption is common among adolescents, and studies on animal models suggest increased sensitivity to acute ethanol effects in this phase compared to adulthood (*Psychopharmacology*, 191:311, 2007). This study aims to investigate the effects of repeated low dose ethanol treatment on adolescent rats submitted to working and recognition memory tasks.

Methods and Results

In this experiment, 12 five-week-old female Wistar rats (control and ethanol groups) were injected I.P. with 0.18g/kg of ethanol or saline once a day for thirty days. Plus maze spontaneous alternation and open field novel object recognition tasks were performed 4 and 7 days after the last injection, respectively. Percent of alternation among the four enclosed arms of the plus-maze was decreased in ethanol-treated animals ($35.1 \pm 1.4\%$; mean $\pm$ SE) compared to controls ($40.3 \pm 1.8\%$). Saline controls ($40.7 \pm 5.2s$), but not ethanol-treated rats ($30.6 \pm 5.7s$), showed increased novel object exploration time compared to the time spent exploring a previously presented object in the open-field arena. Locomotor activity parameters (distance moved in both apparatuses) were not affected by ethanol treatment.

Conclusion

Low-dose chronic ethanol treatment affected not only the working memory, impairing the exploratory activity's efficiency in the plus maze spontaneous alternation task, but also recognition memory, decreasing novel object exploration in the open field object recognition task. The dose used was lower than the one that usually induces memory impairment when administered chronically to adult rodents (0.6 g/kg, *Psychopharmacology*, 192:39, 2007), suggesting an increased sensitivity to chronic ethanol impairing effects in adolescent rats.

Palavras-Chave: Working Memory, Recognition Memory, Adolescence, Ethanol, Rats

Apoio Financeiro: CNPQ, CAPES (PROAP - UFRN)

Long-term masticatory reduction induces spatial memory decline, anxiety-like behavior and decreases the number of neurons in the polymorphic layer of dentate gyrus in adult swiss mice.

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Objectives: We investigated the impact of masticatory reduction induced by a model of soft (powder) in comparison with hard (pellet) food, from 21st postnatal day until adulthood, on cognitive decline, extracellular matrix perineuronal nets (PN) and neuronal numbers of the polymorphic layer of dentate gyrus (PODG).

Methods: Two experimental groups were made: powder soft food (**PO**, n=9) and hard pellet food (**PE**, n=5), from 21^o postnatal day until 6 months old. After behavioral tests 4 subjects of each group were fixed and had their brains processed for *Wisteria floribunda* histochemistry (**PO**, n=3; **PE**, n=3) and immunohistochemistry for Neu-N (**PO**, n=4; **PE**, n=4). *Water maze* (WM), episodic-like memory (EM), dark/light box (DLB) and elevated plus maze (EPM) were video recorded and analyzed by Any Maze (Stoelting®). Optical fractionator (Microbright Systems Inc) was used to estimate neuronal population and ImageJ software was used for densitometric analysis. In all statistical tests (T-test or ANOVA one-way, Bonferroni *a priori*) the threshold for significance was set at p<0.05.

Results: Learning rate in WM was significant at 2nd day of training in **PE** (0.33±0.18) whereas in **PO** (0.32±0.1) only in the 4th training day. In retention trial (without platform) the entries in the platform region for **PE** (3.80±1.02) was lower than **PO** (8.11±0.99), in the working memory test **PE** learned platform new position in the 2nd trial (1st 7.28±1.22/ 2nd 2.0±0.61), and **PO** in the 5th (1st 2.47±0.46/ 5th 1.37±0.19). Both groups distinguished old and recent objects in EM. **PO** spent more time in the dark zone of DLB (82.50±3.4) and closed arm in EPM (71.18±3.47) than the **PE** (68.2±1.22 and 63.87±3.04 respectively). A significant reduction in the number of neurons of PODG in **PO** (374.13±25.69) comparing with **PE** (481.73±28.54) but no difference was found in PN densitometric measurements.

Conclusion: Taken together the results indicate that hippocampus is a target area of neurodegenerative processes related to masticatory stress and neuronal reduction seems to be an important neuropathological feature of the polymorphic altered layer.

Palavras-Chave: Masticatory reduction; dentate gyrus; memory; number of neurons; anxiety-like behavior; swiss mice.

Apoio Financeiro: FINEP, CNPq, PROPESP-UFPA, IBN net

F.02.070

No cualquier evocación desencadena la fase de labilización-reconsolidación: especificidad en la estructura del recordatorio en una memoria declarativa en humanos.

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La reconsolidación de la memoria postula que una memoria consolidada puede devenir nuevamente lábil y susceptible a la acción de agentes amnésicos o facilitadores por un período de tiempo luego de la reexposición a un recordatorio. Basados en resultados previos obtenidos con nuestro modelo invertebrado, propusimos dos características diagnósticas para evaluar el proceso de reconsolidación: la labilización de la memoria reactivada y la estructura específica del recordatorio. El objetivo de este trabajo fue evaluar si en nuestro paradigma de memoria declarativa en humanos (Learn. Mem. 14: 295, 2007) se cumplen estas características. El diseño experimental utilizado consistió en el aprendizaje de una lista de sílabas sin sentido en un contexto determinado durante el primer día, la presentación de recordatorios con diferente estructura para los distintos grupos seguido por el aprendizaje de otro listado durante el segundo día, y finalmente la evaluación de ambas tareas en el tercer día.

Los resultados demuestran que nuestro paradigma cumple con los requerimientos que definen esta fase, evidenciando que una simple evocación no desencadena el fenómeno de labilización-reconsolidación. Por el contrario, el recordatorio requeriría de una estructura específica para permitir el gatillado de dicho proceso, incluyendo como relevante la discordancia entre el evento esperado y el que realmente ocurre.

Palavras-Chave: reconsolidación, estructura del recordatorio, memoria declarativa

Apoio Financeiro: FONCYT (grant PICTR 00349), UBACYT (grant X326), FUCIBA

Enriched-environment counteract the age-related spatial learning impairments in rats

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Introduction: Epidemiological studies suggest that individuals with greater education, or more cognitively demanding occupations, have diminished risk of developing dementia. In order to address this issue in laboratory animals, the present study sought to investigate whether a similar effect is found in old rats exposed to environmental enrichment, a well documented paradigm to attenuate behavioral and cognitive deficits induced by various pathological insults.

Methods and Results: Male Wistar rats at 12 months of age were placed into the enriched or standard cages and tested for cognitive behavior after 6 months of differential housing. The rat was placed in the water at one of the four starting points and the time elapsed until it had located and climbed onto the platform was recorded. A total of five consecutive trials were performed following the same order N, S, E, and W. The test session was carried out twenty-four hours after the training session. The session consisted of a 60 s probe trial in which the platform was removed from the pool. The time spent in the correct quadrant was recorded and the percentage of the total time was analyzed. One-way repeated measured analysis of variance followed by Duncan test ($p < 0.05$) showed that enrichment environmental significantly improved the learning performance in the spatial version of the water-maze since the time spent finding the hidden platform on training session was reduced [standard cage (mean $\pm$ E.P.M) = 282 ± 20 s, enriched cage = 215 ± 25 s] and the time swimming in the correct quadrant on test session was increased [standard cage (mean $\pm$ E.P.M) = 20 ± 11 s, enriched cage = 27 ± 9 s] when compared to controls.

Conclusion: Our results substantiate the proposition that environmental enrichment can counteract the age-related cognitive impairments in rats.

Palavras-Chave: Learning, memory, enriched-environment

Apoio Financeiro: Univali

Participación del sistema noradrenérgico de la amígdala y de los glucocorticoides estriatales en la consolidación de la memoria.

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Diversos estudios indican que las hormonas glucocorticoides mejoran la consolidación de la memoria cuando son administradas inmediatamente después del entrenamiento de tareas de tipo emotivo. Estos efectos son bloqueados cuando la amígdala basolateral (BLA) es lesionada o cuando el sistema noradrenérgico de este núcleo es bloqueado por la administración de un antagonista β -adrenérgico. Existen estudios que demuestran que el mejoramiento de la memoria inducido por glucocorticoides depende de la interacción de la BLA con otras regiones cerebrales. Evidencias de nuestro laboratorio indican que la administración de glucocorticoides en el estriado antero-dorsal también produce mejoría en la consolidación de la memoria. El presente trabajo tiene como objetivo conocer si existe una interacción entre el sistema noradrenérgico de la BLA y el estriado antero-dorsal. Se estudiaron cinco grupos de ratas de la cepa Wistar que fueron implantados unilateralmente con una cánula en el estriado dorsal y otra en la BLA. Los grupos fueron entrenados 7 días después de la cirugía en una tarea de evitación inhibitoria utilizando un estímulo nociceptivo de 0.45 mA. Inmediatamente después, un grupo recibió 10 ng de corticosterona (CORT) en el estriado y salina en la BLA; los otros cuatro grupos recibieron 10, 20, 30 ó 60 ng de CORT en el estriado, en combinación con la infusión de 0.5 μ g de atenolol en la BLA. Los volúmenes de los fármacos en el estriado fueron de 1 μ l para el estriado y de 0.2 μ l para la BLA. Cuarenta y ocho horas después se midió la retención de la tarea. Los resultados obtenidos indican que la activación del sistema noradrenérgico de la BLA actúa como modulador sobre los efectos de los glucocorticoides estriatales en la consolidación de la memoria ante un evento aversivo.

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Palavras-Chave: consolidación, estriado, amígdala

Apoio Financeiro: PAPIIT-UNAM IN211608 e IN216708, CONACYT 46754-Q. y UC-MEXUS CN-06-77

Avaliação do papel das repetições de palavras na aquisição da memória verbal.

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Objetivo: Este estudo avaliou as repetições de palavras nas séries do teste de memória e aprendizagem auditiva verbal de Rey (RAVLT), considerando duas hipóteses: 1) serem utilizadas como estratégias para aumentar a aprendizagem ou 2) erros na recuperação da memória.

Método: Foram avaliados 14 sujeitos, de ambos os sexos, idade de 25,0±5,3 anos, com nível superior. Além do RAVLT, foram aplicados diferentes testes neuropsicológicos nos 20 min entre a série que avalia a interferência imediata (A6) e a interferência tardia (após 20 min, A7). Após 2 semanas, foi realizado re-teste do RAVLT, com uma nova lista de palavras e com a instrução para que o sujeito evite a repetição de palavras durante a verbalização da lista.

Resultados: A dinâmica da memória e do aprendizado verbal nas 5 séries iniciais do RAVLT foram ajustadas por equação de hipérbole, com dois parâmetros determinados: Ap_{max} (máximo de aprendizado) e vel_{ap} (velocidade do aprendizado). Os valores de Ap_{max} obtidos foram 14,7±0,4 e 14,8±0,5, e os valores de vel_{ap} foram 1,0±0,1 e 1,4±0,15 para os que realizaram o RAVLT sem restrição e com restrição para não repetirem, respectivamente. Portanto, não foram observadas diferenças significantes no aprendizado máximo nem na velocidade de aprendizagem. Porém, observando a média e o desvio padrão em cada ponto, nota-se que todos os pontos da aplicação com restrição de repetições, encontram-se abaixo quando comparados com a primeira aplicação (sem restrições). O efeito das interferências imediata e tardia na memória e no aprendizado verbal também não apresentou diferenças estatisticamente significantes, porém os valores obtidos na aplicação com restrição de repetições foram inferiores aos da aplicação sem restrições. Esses resultados indicam tendência da restrição da repetição em diminuir a eficiência da memória e do aprendizado no RAVLT.

Conclusões: Esses dados não demonstraram que a repetição de palavras está envolvida no processo de aprendizagem.

Palavras-Chave: Memória Verbal, Repetição de palavras, Aprendizagem, RAVLT.

Apoio Financeiro: Departamento de Ciências Fisiológicas da FCMSCSP.

WORKING MEMORY IMPAIRMENT AFTER LONG-TERM FEMALE HORMONES DEPRIVATION INDEPENDS OF BRAIN ACETYLCHOLINE LEVELS

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The estrogen-induced effects in brain areas not originally involved in reproduction can be mediated by acetylcholine. Interestingly, depletion of female hormones at menopause is a risk factor for the development of Alzheimer's diseases, a condition with memory impairments and cholinergic neurons loss. **Objective:** investigate working memory in Vesicular Acetylcholine Transporter knock-down heterozygous (KD^{HET}) female mice, a model of central hypocholinergic function, after short and long-term ovarian hormones deprivation. **Methods:** 7 months wild-type (WT) and KD^{HET} mice were ovariectomized (OVX) or sham-operated. After 1 and 12 weeks, mice were placed in the y maze and allowed to move freely through the maze during 8 minutes. The total numbers of arm choices and alternations were recorded. To determine if alternation scores were significantly above the chance (50%), we used one-sample t test. To compare genotypes we used two-way ANOVA with Bonferroni's post-hoc. **Results:** There was no difference in time [F(1,30)=2.143, p=0.1536] or genotype [F(1,30)=2.669, p=0.1128] when sham groups were compared. WT and KD^{HET}, 12 weeks post-OVX, were unable to alternate above the chance (p=0.1004 and p=0.3042, respectively). After 12 weeks, WT OVX group presents working memory deficits (p<0.05) comparing to 1 week OVX group. KD^{HET} mice 12 weeks after OVX, shown a trend to impairment compared with 1 week after OVX. There were no differences in number of entered arms. **Conclusions:** Our results showed that both WT and KD^{HET} present working memory impairments after long-term female hormone deprivation. Then, less brain acetylcholine release did not increase the susceptibility to working memory deficits induced by OVX, since were no differences between genotypes.

Palavras-Chave: memory, acetylcholine, hormone, female

Apoio Financeiro: FAPEMIG, CNPq

EFFECTOS DE LA RESTRICCIÓN CALÓRICA EN RATAS EXPUESTAS A ESTRÉS AGUDO ANTES DE LA RECUPERACIÓN DE UNA TAREA DE APRENDIZAJE ESPACIAL

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Reportes recientes de la literatura muestran que el ayuno intermitente aparentemente mejora la ejecución en tareas de aprendizaje espacial, aunque dichos efectos han sido asociados a incrementos en la actividad locomotora más que a verdaderas consecuencias benéficas sobre la memoria. Resultados obtenidos en nuestro laboratorio muestran que la exposición a un único periodo breve de estrés mejora la recuperación de tareas de memoria espacial. El presente estudio tiene como objetivo determinar los efectos del ayuno intermitente desde el final de la lactancia hasta la edad adulta en la recuperación de una tarea de aprendizaje espacial en ratas expuestas a estrés agudo. Se utilizaron 20 ratas Wistar distribuidas en dos grupos, el primero sometido a ayuno intermitente desde el final de la lactancia y estrés agudo antes de la recuperación de la tarea y el segundo con libre acceso a comida y estrés agudo antes de la recuperación de la tarea. En el grupo ayuno intermitente se observa disminución en la latencia de llegada al agujero meta durante la fase de adquisición mientras en el número de errores de exploración de agujeros los dos grupos se comportan de manera similar. En la fase de recuperación la exposición simultánea a los tratamientos tiene un efecto acumulativo. En la recuperación de la tarea animales estresados que han tenido una historia previa de ayuno intermitente tienden a tener una mejor ejecución en el laberinto de Barnes.

Palavras-Chave: Ayuno Intermitente, Estrés, Memoria Espacial

Amnésia induzida pela privação de sono paradoxal: participação do fenômeno de dependência de estado

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Objetivos: Evidências experimentais sugerem que o sono, incluindo o sono paradoxal, tem um papel crítico nos processos de aprendizado e memória. Paralelamente, a privação de sono paradoxal (PSP) promove diversas alterações comportamentais, como o aumento da atividade motora e alterações na ansiedade que poderiam estar relacionados a tais déficits. Assim, a dependência de estado poderia contribuir para os déficits de memória induzidos pela PSP. Nesse sentido, avaliamos a participação da dependência de estado sobre os déficits de memória induzidos pela PSP em camundongos submetidos à esquiwa discriminativa em labirinto em cruz elevado (ED), um modelo animal que avalia concomitantemente o aprendizado/memória, ansiedade e atividade motora.

Métodos: Foi empregado o método das plataformas múltiplas no procedimento de PSP. Camundongos Swiss, machos, com 3 meses de idade foram mantidos em suas gaiolas moradia (CONT) ou submetidos à condição de PSP por 72h. Os animais foram distribuídos em 4 grupos (n=12): CONT-CONT (controle), CONT-PSP (PSP pré-teste), PSP-CONT (PSP pré-treino), PSP-PSP (PSP pré-treino e pré-teste). O intervalo entre as sessões de treino e teste foi de 7 dias.

Resultados: Na sessão de treino, a PSP induziu déficits de aprendizado (porcentagem de tempo no braço aversivo: CONT:2,3±0,6; PSP:10,5±3,3), efeito ansiolítico (porcentagem de tempo nos braços abertos: CONT:7,58±1,95; PSP:25,72±4,95) e aumento da atividade motora (número de entradas: CONT:12,54±1,21; PSP:19,54±1,97). Na sessão de teste, a PSP pré-treino *per se* promoveu déficits de retenção (tempo nos braços aversivo e não-aversivo, respectivamente: CONT-CONT:32±13/88,9±17,8; PSP-CONT:44,1±7,9/70,2±9,9). Ainda, tal déficit foi revertido pela PSP pré-teste (PSP-PSP:13,4±4,9/52,1±8). Por fim, o grupo PSP-PSP apresentou um aumento da porcentagem de tempos nos braços abertos (CONT-CONT:5,3±2,4 e PSP-PSP:37,5±5,6).

Conclusão: Tomados em conjunto, os dados sugerem que há a participação do fenômeno de dependência de estado nos déficits de memória induzidas pela privação de sono e que a emocionalidade dos animais poderia contribuir para tal fenômeno.

Palavras-Chave: memória, ansiedade, privação de sono e dependência de estado

Apoio Financeiro: FAPESP, CEPID e AFIP.

On the role of the hippocampal/VTA dopamine loop in fear memory persistence

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To understand the behavioral and molecular mechanisms controlling storage of information is fundamental to develop pharmacological and therapeutic strategies for the treatment of neurological illnesses associated with cognitive decline.

We have recently demonstrated that persistence of inhibitory avoidance (IA) memory requires a BDNF-dependent phase in the hippocampus beginning 12 h posttraining^{1,2}. Using molecular and pharmacological tools, here we show that activation of D1 dopamine receptors in the dorsal CA1 region during this critical phase controls BDNF expression through a PKA-dependent mechanism and promotes persistence of the avoidance trace without affecting memory formation. IA memory persistence, BDNF expression and hippocampal dopamine signaling are impaired by reversible inactivation of the ventral tegmental area (VTA), the main dopaminergic afferent to the hippocampal formation in the rat, 12 h after training. Conversely, bilateral intra-VTA infusions of NMDA and picrotoxin prolong IA memory retention, enhance expression of hippocampal BDNF and induce the PKA-mediated phosphorylation of the dopaminergic markers tyrosine hydroxylase and DARPP-32 in dorsal CA1. Importantly, the amnesia induced by activation of NMDAR in the VTA is hindered by function-blocking anti-BDNF antibodies given in the CA1 region 12 h posttraining, while intra-CA1 administration of BDNF reverses the amnesic effect of AP5 given in the VTA.

Our results indicate that activation of the hippocampal/VTA dopamine loop around 12 h after IA training is essential to induce BDNF expression and signaling in the dorsal CA1 region and suggest that the VTA plays a key role in the mechanisms controlling persistence of aversive memories.

Palavras-Chave: memory, persistence, dopamine, BDNF, PKA, hippocampus, protein synthesis

Efecto de la exposición perinatal a una mezcla de bifenilos policlorinados, el Aroclor 1254, sobre la actividad de la sintasa del óxido nítrico y su repercusión sobre el aprendizaje.

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El óxido nítrico (NO) tiene un papel importante en diferentes funciones biológicas, tales como el aprendizaje y la memoria. Se reportó que la exposición a los bifenilos policlorinados (PCB) durante el desarrollo inhiben a la sintasa del óxido nítrico (NOS). Los PCB son tóxicos ambientales que se utilizaron ampliamente en la industria, no son biodegradables y son bioacumulables en las cadena alimenticia. El objetivo de este trabajo fue estudiar el efecto de la exposición a la mezcla comercial de los PCB, Aroclor 1254 (A1254), sobre la actividad de la NOS en diferentes regiones cerebrales (corteza prefrontal, hipocampo y tálamo dorsal) de crías de rata tratadas *in útero* y analizar su repercusión sobre el aprendizaje de la evitación inhibitoria. Se utilizaron ratas hembra Wistar gestantes, se les dio de comer fritura de maíz (chetos) con una dosis de A1254 de 30 mg/kg/día, o solo aceite de maíz (vehículo) del día 10 al 19 de la gestación. Se formaron dos grupos de crías: Grupo I: crías tratadas y controles de 10 días postnatales (dPn), se fijaron con paraformaldehído al 4%, se obtuvieron cortes coronales de cerebro (40µm) y se aplicó la técnica histoquímica para la NADPH-d. Grupo II: se dejaron crecer hasta los 3 meses y se sometieron al aprendizaje de evitación inhibitoria probándose posteriormente a 1h, 24h y 144h. Posteriormente las crías se procesaron para la NADPH-d. Los cortes de los núcleos estudiados se analizaron y tomaron fotomicrografías en un microscopio óptico para cuantificar la densidad óptica. Los resultados mostraron una reducción significativa de la actividad de la NOS en todas las regiones cerebrales estudiadas de las crías tratadas comparadas con las controles. En la prueba de evitación inhibitoria, se observó un decremento significativo del aprendizaje. Estos resultados sugieren que la exposición perinatal al A1254, inhibe la actividad de la NOS y como consecuencia se afectan funciones en la que el NO participa, como es el aprendizaje y la memoria. Agradecimientos: Téc. Feliciano Camacho García por su asistencia técnica. **Palavras-Chave:** PCB, desarrollo, óxido nítrico, aprendizaje

Apoio Financeiro: UCMEXUS CONACYT, Miguel Alemán y al Fondo de Apoyo a la Investigación INPRFM.

Distúrbio de Aprendizagem e desempenho no teste WISC-III

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Distúrbio de aprendizagem (D.A.) é um problema escolar e social, sendo uma das principais causas de encaminhamento de crianças para avaliação psicológica. O estudo caracterizou-se como uma pesquisa exploratório-descritiva, uma vez que procurou conhecer e proporcionar uma visão geral sobre as características de um grupo clínico com D.A.. Neste estudo foram avaliadas 263 crianças de escolas públicas de duas cidades do Rio Grande do Sul, que por apresentarem dificuldades na aprendizagem foram encaminhadas para avaliação psicológica com o teste de inteligência WISC-III. A amostra constituiu-se de alunos entre 6 e 16 anos, com média de idade de 9 anos ($dp=1,99$), sendo 69,2% do sexo masculino. Problemas na leitura e déficit de atenção, no relacionamento e hiperatividade foram identificados como fatores mais comuns no grupo estudado. Os subtestes Aritmética, Código, Informação, Dígitos, Arranjo de Figuras e Semelhanças foram os que apresentaram menores escores (resultados ponderados abaixo de 7 pontos), demonstrando prejuízo nas funções relacionadas à atenção e concentração, velocidade de processamento, memória, à organização visual e ao raciocínio abstrato. Quanto à capacidade intelectual geral do grupo, a média do QI Total foi classificada como limítrofe. Os resultados evidenciaram uma discrepância significativa ($p=0,000$) entre o raciocínio verbal (QI Verbal = 82) e não-verbal (QI Execução = 80), mostrando maior facilidade para inteligência funcional no que se refere a assuntos que necessitam de treinamento, educação, cultura prévia, e experiências de vida. Entretanto, a dicotomia QI Verbal/QI Execução pareceu revelar-se mais útil para saber se a criança apresentou um déficit que apenas afeta as capacidades lingüísticas, ou perceptivo-espaciais do que para fins de diagnóstico clínico. Questionar de que forma o desenvolvimento das crianças com D.A. difere dos demais permite identificar se elas usam as mesmas funções cognitivas que as crianças sem o distúrbio ou quais as diferenças, auxiliando os profissionais envolvidos com a aprendizagem a desenvolver e reforçar as habilidades essenciais desse processo, orientando no planejamento da intervenção.

Palavras-Chave: Distúrbio de Aprendizagem, Inteligência, WISC-III

ACUTE STRESS DIFFERENTLY AFFECTS COGNITIVE FUNCTIONS IN MALE AND FEMALE MICE

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Introduction: Stress involves a group of reactions and stimulus able to promote several alterations in the organism, including harmful effects in the cognitive functions, mainly in learning and memory. However, some studies suggest that male and female showed different responses to stressful events, with different alterations in the cognitive functions. The aim of the present study is to investigate the effects of restraint stress in male and female mice evaluate in the plus-maze discriminative avoidance task.

Materials and Results: Eighteen male (25-40g) and sixteen female mice (30-40g) were allocated to the following groups: males (MS) and females stress (FS) and control males (MC) and control females (FC). All animals were placed in cylindrical restraint tubes during 4 hours for three consecutive days. Afterwards, the animals were placed in the center of a plus maze with two open arms and two closed arms. In one of the closed arms, aversive stimuli (light and noise) were presented every time the animal entered in the aversive arm. The duration and the number of times that each animal entered in each one of the arms were registered. In the test (in the absence of stimuli), FS presented increased percent time in aversive arm ($69.1 \pm 9.8\%$; mean $\pm$ SE) when compared to FC ($43.1 \pm 11.9\%$), while decreased (though not statistically significant) aversive arm exploration was found in MS ($15.5 \pm 2.8\%$) compared to MC ($27.6 \pm 6\%$). Additionally, female mice of both groups presented decreased percent time in open arms (FC= $1.4 \pm 1.1\%$; FS= $3.5 \pm 0.8\%$) when compared to males (MC= $9.1 \pm 3\%$, MS= $7.2 \pm 3.4\%$).

Conclusion: The increased exploration in the aversive arm in the test session by females submitted to stress suggests a memory deficit, which was not presented by males. The decreased open-arm exploration by females demonstrates an increase in the anxiety levels compared to males. We suggest that the different basal anxiety levels would be related to the opposite stress effects on memory of male and female mice.

Palavras-Chave: stress, learning, memory

Apoio Financeiro: CNPq/PIBIC

EXTINCTION OF AVERSIVE MEMORY BY THE ANTIDEPRESSANT MIRTAZAPINE IN RATS

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Depression is usually accompanied by cognitive deficits. This link between depression and memory loss is reaffirmed by the fact that antidepressant treatment usually attenuates these deficits. Mirtazapine is a novel antidepressant drug that acts by selective inhibiting noradrenaline and serotonin reuptake, and the effects of this drug on memory is poorly understood. This work aimed at evaluating the effects of repeated treatment with mirtazapine on memory in rats. Additionally, the effectiveness of the treatment schedule in the forced swimming test (an animal model of depression) was verified. Wistar female rats were treated with Mirtazapine (10 mg/kg/day i.p. for 19 days) or saline. Afterwards, the animals were placed individually in the center of a modified plus-maze apparatus, in which an aversive stimulation (light and noise) was produced each time the animal entered on of the enclosed arms during the training session. In a 10 min-long test session, performed 24h later, no aversive stimulation was produced. Both groups presented task retrieval: low duration of aversive arm exploration in the beginning of the test (mean±SE in the 1st minute: 13.9±4.7s and 9.3±4.4s for saline and mirtazapine, respectively). However, while control rats continue to avoid the aversive arm, mirtazapine-treated animals progressively increased aversive arm exploration throughout the session (39.1±10.7s in the 10th minute). In the forced swimming test, performed 24h after the test, mirtazapine-treated rats showed decreased immobility duration (35.7±8.8s) compared to controls (75.6±14.2s), consistent with its known antidepressant action. Although not modifying acquisition or retrieval of the task, antidepressant treatment with mirtazapine favored the occurrence of learning extinction of the aversive arm avoidance. Although more studies are needed to clarify the relationship between depression and memory, our data suggest a relationship between antidepressant action and extinction of aversive memories.

Palavras-Chave: depression, serotonin, memory, antidepressant

Apoio Financeiro: CNPq

Progress in an auditory word recognition task with Down syndrome subjects.

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Studies demonstrate that stimuli processes in Down syndrome individuals occur more slowly. There is indication of slowing in the auditory stimulus recognition process and motor response organization, resulting in a latency period longer than expected. This study explores the progress made in a word recognition task using nine individuals with Down syndrome aged between 15 and 30. The auditory recognition test consists of three series (S1, S2 and S3), each including a learning phase, where a ten target-word list is presented, and a test phase in which a 20 word list is classified (target-words plus distractors). Individuals answer YES for items on the list and NO for distractors during the test phase with an adapted keyboard. The tasks were executed with the help of SuperLab Pro software and the use of headphones.

During the task a significant reduction in the response time process occurred for all items, target-words and distractors, and a learning curve can be observed between the series: S1 = 5.856,6 ms (SD: 3.507,9); S2 = 3.789,4 ms (SD: 2.941,9) and S3 = 3.270,7ms (SD: 1.929,7). The reduction time from S1 to S2 was statistically significant ($t = 2,63$; $gl = 8$; $p = 0,030$). The same results occurred between S1 and S3 ($t = 2,63$; $gl = 8$; $p = 0,030$), but not with S2 and S3 ($t = 0,885$; $gl = 8$; $p = 0,402$). These results may represent a learning development process, facilitating the recognition test choice decision by subsequently lessening stimulus processing time and answer latency.

Palavras-Chave: Down syndrome, auditory word recognition test, response time

Apoio Financeiro: Financial Support: Dept. of Physiology; Psychobiology PGP; Leite, R. C. B is a PIBIC/CNPq scholarship recipient.

Insight of functional value of Memory Reconsolidation: Modulation of declarative memory in humans during this phase.

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During reconsolidation, a consolidated memory become labile again for a discrete period of time after memory reactivation. The universality of this phenomenon its strongly supported by experiments in different species. Previous studies from our lab show the existence of reconsolidation in both crab's context-signal memory and declarative memory in humans. However, its functionality remain to be disclosed. One interesting hypothesis argues that reconsolidation would be a mean for integrating new information on the background of the past; but this idea does not have direct experimental evidence yet. We postulate that a functional value of reconsolidation would be to open the possibility of changing long-term memory expression. According, we previously demonstrate that crabs can build long-term memories that remain unexpressed unless an enhancing treatment is given during reconsolidation. Our experiments in course are designed to test this hypothesis in humans. The first step was to develop a training protocol that allow us to disclose facilitatory memory effects in the memory paradigm previously employed to study reconsolidation of declarative memory in humans. The use of weak training protocols to achieve this objective demonstrated not to be useful. Finally, we found a protocol that allows us to test facilitatory effects during reconsolidation by delaying the reactivation and testing sessions. Preliminary results support the original hypothesis about a functional value of this memory phase.

Palavras-Chave: Memory modulation - Reconsolidation - Declarative Memory

MEMORY WITH EMOTIONAL ACTIVATION IN DEPRESSION: WHAT IS REMEMBERED? WHAT IS LOST?

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OBJECTIVES: Much evidence indicates a strong link between memory and emotional arousal. Many of the central nervous pathways involved in memory and emotional arousal are believed to be altered in depression. Thus, there is a possibility that alterations in the formation or retrieval of emotionally arousing memory may be inherent to, or be affected by depression. This study investigated performance of emotional memory of depressed patients. **METHOD:** Forty adult women (24 with clinical depression as 16 normal age-matched controls) were submitted to the test of emotional memory described by Cahill et al. (1994) as modified by van Stegeren et al. (1998), which consists of the presentation of eleven slides that tell a story with emotional content. Twenty-four of the subjects were referred to us by clinicians and suffered from clinical depression according to DSM-IV. The diagnosis was ascertained by us using the standard diagnostic scales (Beck Depression Inventory, Montgomery-Asberg and Hamilton-D).

RESULTS: Overall, the memory tested was diminished in depressive states ($p = 0,02$). In a statistical analysis focusing the slides one by one, the recall of only one of the slides (10) was significantly lower in the depressed group. In another analysis focusing the slides by the phase of the story (neutral or emotionally arousing), as it was used by Cahill et al. (1994), the recall of the most emotional part of the story has been the same in both groups. Although, the first and the last parts of the story, both neutral, showed a significant statistically difference between the groups ($p=0,02$ and $p = 0,03$).

CONCLUSIONS: Therefore, depressed patients seem to function with a near-normal emotional memory, especially when we evaluate them with an emotional-arousing material. We discuss the relation of emotional arousal and memory in depressed and healthy subjects, as well as possible psychotherapy techniques that can be applicable to the former.

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Palavras-Chave: memory; declarative memory; implicit memory; emotional arousal; depression; depressive humor depressive symptoms.

Immediate Early Gene Modulation During Post-novelty Sleep: Preliminary Results

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Objectives: Although the relationship between sleep and learning is well described in the literature, the molecular mechanisms underlying cognitive role of sleep remain unclear. Here, we set out to assess the immediate early gene expression program triggered by post-novelty sleep.

Methods: Hippocampal local field potentials were recorded in 11 adult male rats before, during and after exposure to novel spatio-sensory stimuli. This consisted of four novel objects, designed to maximize tactile and behavioral differences, placed in the recording box for 20 min. After exposure, animals were prevented from sleeping for 60 minutes, and were then allowed to sleep freely while behaviors and local field potentials from hippocampus and somatosensory cortex were recorded. Just after sleep deprivation (waking groups) or thirty minutes after entering sleep (slow wave sleep and REM sleep groups), animals were euthanized, and their brains were dissected into frozen samples of frontal, somatosensory and visual cortices, thalamus, cerebellum and hippocampus. Finally, plasticity-related genes *Egr1* and *Arc* had their expression levels analyzed by real time PCR in somatosensory cortex of one animal per group (total n=6) with two technical replicates per animal. Given the small number of animals we decided perform just descriptive statistics.

Results: In control animals, we observed increased expression levels of both *Arc* and *Egr1* during waking, with a decrease during slow wave sleep and REM sleep. In addition, we found an increase in *Egr1* expression of exposed versus non-exposed groups during waking (1.9 fold) and REM sleep (2.5 fold). This increase was not observed in slow wave sleep, as previously reported. We also observed an increase in *Arc* expression in exposed versus non-exposed groups during waking (2.3 fold). This effect was not observed in the REM sleep and slow wave sleep groups.

Conclusion: Our work suggests that *Egr1* expression during REM sleep depends on previous waking experience. Given the association between *Egr1* expression and neural plasticity, this phenomenon could represent a window of increased plasticity during REM sleep that follows novel salient experience.

Palavras-Chave: Sleep; Memory; Learning; Immediate Early Gene

Apoio Financeiro: FAPESP; Hospital Sírio Libanes; Finep; AASDAP; Abadhs

Deficiência grave de tiamina em ratos causa déficit na aquisição de tarefas de navegação espacial correlacionado com parâmetros serotoninérgicos no tálamo.

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Em estudos anteriores, nosso grupo e outros autores mostraram que modelos experimentais animais da Síndrome de Wernicke, doença neurodegenerativa causada pela deficiência de tiamina (vitamina B12), apresentam como os seres humanos prejuízos irreversíveis de aprendizado e memória espacial. Diferenças no desempenho de animais em tarefas cognitivas que envolvem estímulos aversivos podem ser influenciadas por aspectos relacionados a componentes emocionais, que por sua vez poderiam estar associados a disfunções no sistema serotoninérgico. Um dos objetivos do presente trabalho foi verificar se os efeitos da deficiência de tiamina sobre o desempenho dos animais no Labirinto Aquático de Morris (LAM), no qual a água é um estímulo aversivo, estariam correlacionados com alterações no sistema serotoninérgico central. Utilizamos ratos Wistar machos, de três meses de idade, divididos em dois grupos, submetidos ou não a um episódio grave de deficiência de tiamina: (i) Controle (C, n=16) e (ii) Deficiente de Tiamina (DT, n=16). As concentrações de serotonina (5-HT) e do metabólito ácido 5-hidroxi-indolacético (5-HIAA) foram determinadas no tálamo, região predominantemente afetada pela deficiência de tiamina, utilizando o método de cromatografia líquida de alto desempenho (HPLC). Análise de variância com medida repetida no último elemento mostrou um efeito significativo ($p < 0,05$) da deficiência de tiamina na aquisição da tarefa de navegação espacial. No entanto, apesar da diferença no desempenho, os animais de ambos os grupos foram capazes de aprender a tarefa. Observamos que os animais deficientes apresentaram um aumento significativo na [5-HIAA] em ng/g e na taxa de renovação ($[5-HIAA]/[5-HT]$) do sistema serotoninérgico no tálamo ($C = 855,3497$ e $DT = 1154,25$, $p = 0,012677$ e $C = 6,596393$ e $DT = 15,1122$, $p = 0,026747$ respectivamente). Verificamos também uma correlação significativa positiva entre a [5-HIAA] e o desempenho dos animais na terceira sessão do LAM. Concluimos que uma disfunção serotoninérgica no tálamo, causada por deficiência de tiamina, pode levar a um prejuízo na aquisição de uma tarefa cognitiva espacial. **Palavras-Chave:** Deficiência de tiamina, sistema serotoninérgico, memória espacial, tálamo, rato

Apoio Financeiro: FAPEMIG

Pré-exposição ao contexto do treino previne o déficit de memória causado pela arcaína na tarefa de medo condicionado.

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Objetivos: A arcaína (Arc) é uma poliamina sintética que atua como um antagonista do sítio das poliaminas no receptor NMDA. A arcaína piora a memória de ratos nas tarefas de esquiva inibitória e de medo condicionado. Têm sido descrito que a pré-exposição ao contexto do treino previne o déficit de memória induzido por antagonista NMDA. Este trabalho tem como objetivo investigar se o prejuízo da memória induzido pela arcaína é prevenido pela pré-exposição do animal ao mesmo contexto do treino.

Métodos: No dia 1 (pré-exposição) ratos Wistar machos adultos foram divididos em 3 grupos: grupo I, sem pré-exposição; grupo II, pré-exposição a um ambiente diferente do contexto do treino (caixa redonda com 34 cm de diâmetro); grupo III, pré-exposição a caixa de treino para o medo condicionado (caixa retangular com o assoalho composto de barras de metal ligadas na corrente elétrica). No dia 2 todos os animais foram submetidos ao treino do medo condicionado utilizando três pareamentos de tom (2.000 Hz, 90 dB) e choque (0,6 mA). Imediatamente após o treino, receberam veículo ou arcaína (10 mg/kg i.p.). No dias 3 e 4 os animais foram testados para o medo condicionado contextual e ao tom respectivamente. A percentagem de imobilidade foi o índice do medo condicionado.

Resultados: A arcaína piorou o desempenho dos animais dos grupos I e II tanto no medo condicionado contextual (grupo I: veículo 63,6±4,65, Arc: 23,00±4,72; grupo II: veículo 55,82±5,49, Arc 31,95±6,21) como no medo condicionado ao tom (grupo I: veículo 62,80±6,07, Arc 27,30±5,00; grupo II: veículo 52,63±5,56, Arc 24,03±3,72). Porém, a arc não piorou o desempenho dos animais do grupo III no medo condicionado contextual (veículo: 63,26±4,23, Arc: 64,85±5,77) e no medo condicionado ao tom (veículo: 48,77±4,76, Arc: 40,56±6,07). Os resultados são expressos como média de porcentagem de freezing ± erro padrão, n=12-13 por grupo. Portanto, quando os animais são pré-expostos ao mesmo contexto do treino (grupo III) a arcaína não é mais amnésica.

Conclusões: Estes resultados sugerem que o efeito amnésico da arcaína depende da novidade.

Palavras-Chave: memória, arcaína, poliaminas, medo condicionado, pré-exposição ao contexto.

Apoio Financeiro: CNPq (processos 477836/2007-0, 301558/2007-8).

INVOLVEMENT OF AN INDIRECT CENTRAL AMYGDALOID NUCLEUS-STRIATAL PATHWAY IN TONE FEAR CONDITIONING: EVIDENCE FROM MUSCIMOL MICROINJECTIONS

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Objectives: Bilateral electrolytic dorsal striatum (DS) lesions or functional disconnection between DS and central nucleus of amygdala (CeA), using electrolytic lesions, disrupted the tone fear conditioning task (TFC), suggesting that other routes, aside the well-established CeA projections to the periaqueductal gray, may contribute to the TFC (Neurosci. 153:1, 2008). A major problem of this approach is the damage of fibers of passage coursing through the lesioned area. Moreover, with this approach it is not possible to differentiate which phase (acquisition, consolidation or retrieval) of TFC is affected. The purpose of the present study was to investigate whether DS or an indirect CeA-DS pathway are involved in TFC, using reversible inactivation of these structures with muscimol (a GABA A agonist) microinjections.

Methods: Male Wistar rats received muscimol microinjections (0,5 µg/0,5 µl) in DS and CeA and 2 minutes later were submitted to TFC training. After 24 hours, the animals were tested in a drug-free condition. **Results:** Asymmetrical contralateral injections (Contra CeA-DS), consisting of muscimol injection in CeA in one hemisphere combined with a muscimol injection in DS in the contralateral hemisphere, impaired TFC, as compared with animals receiving a unilateral injection in CeA and DS in the same hemisphere (Ipsi CeA-DS) (Table 1). Bilateral muscimol injections in DS also impaired TFC (Table 2).

Table 1 - Freezing time expressed in seconds (Mean±SE)

Group	Min1	Min2	Min3	Min 4	Min 5	Min 6
Contra CeA-DS	0.0±0.0	1.5±1.5	6,75±5.76	11.25±3.52*	11.0±6.98*	2.25±1.43
Ipsi CeA-DS	0.0±0.0	3.25±2.38	6.75±3.47	33.25±3.94	33.75±5.80	20.5±6.76

*p<0.05

Table 2 - Freezing time – expressed in seconds (Mean±SE)

Group	Min1	Min2	Min3	Min 4	Min 5	Min 6
DS	0.0±0.0	3.87±1.04	6.62±3.38	17.75±3.27	18.75±4.46*	13.37±5.04*
Control	0.08±0.08	3.0±1.03	5.0±1.68	27.41±4.12	38.08±5.09	34.83±5.11

*p<0.05

Conclusions: These data confirm previous effects observed with pretraining asymmetrical electrolytic lesions and, in conjunction with other studies, suggest a role for an indirect CeA-DS pathway in discrete classical conditioning.

Palavras-Chave: emotional memory, amygdala, dorsal striatum, reversible inactivation

SEX DIFFERENCES IN AVERSIVE MEMORY IN RATS: ROLE OF EMOTIONAL FACTORS

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Goals

Many studies have shown differences between male and female rats in acquiring memories, suggesting a better spatial memory in males but a better emotional memory in females. We investigated these findings comparing spatial performances between genders in tasks with or without emotional contexts.

Methods and Results

Male and female Wistar rats (250-300g) were tested in four tasks. (1) Plus-maze discriminative avoidance, containing two open arms and two enclosed arms, one of which presenting aversive stimuli. In the training, for 10 minutes, every time the rodent enters the aversive arm the aversive stimuli are given until the animal leaves it. In the test, the stimulus is no longer applied. Our data showed a significant sex difference in the percent time in the aversive arm. In training and test, female presented higher (18.35 $\pm$ 3.63) and lower (36.31 $\pm$ 5.61) aversive arm exploration, respectively, when compared to males (9.9 $\pm$ 1.28 and 59.3 $\pm$ 9.6). (2) In contextual fear conditioning the animal is placed in a cage, where a mild foot shock is given. When replaced at the cage, freezing behavior is measured. Males spent more time in freezing behavior (56.14 $\pm$ 17.68s) than females (8.71 $\pm$ 1.7s). (3) Spontaneous alternation is held in a maze with four enclosed arms. The animal is placed in the center of the apparatus and would usually alternate among the arms. No differences were found in percent alternation (44.4 $\pm$ 4.2 – male; 48.47 $\pm$ 6.1 – female) or total number of entries in the arms. (4) Open field habituation is tested in an arena which the rats can explore for 10 minutes. Habituation is evaluated by decreased locomotion in the second exposition. Both genders showed similar open-field habituation, evaluated by a decrease in the distance travelled (1st session: 18.7 $\pm$ 1.67m – male; 21.7 $\pm$ 1.56m – female; 2nd session: 15.1 $\pm$ 1.27m – male; 18.96 $\pm$ 1.25m – female).

Conclusion

The results presented here corroborates with previous data, indicating sex differences in aversive memory, although no differences between genders were shown during tasks without emotional contexts.

Palavras-Chave: memory, emotion, rats, sex differences

Apoio Financeiro: CNPQ, CAPES (PROAP - UFRN)

Consumption of caffeine during adulthood prevents recognition memory decline and changes BDNF and TrkB immunocontent in mice.

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OBJECTIVE: The signaling operated by brain derived neurotrophic factor (BDNF) seems to be essential for learning and memory process. Caffeine usually has positive effects on this process, but there are no studies focusing on the role of neurotrophic factors in its benefits. Besides, effects of a long term administration of caffeine on memory have not been investigated. Thus, this study investigated the effects of caffeine administered from adulthood up to old age on recognition memory and BDNF its receptor TrkB immunocontent in the hippocampus.

METHODS: Adult mice (6 months old) received either drinking water or caffeine (1 mg/mL) during 12 months. Mice (18 months old) were tested in the object recognition task. Recognition index of each mouse was expressed by the ratio $T_N/(T_N+T_F)$ (T_N = time spent exploring the novel object; T_F = time spent exploring the familiar object). BDNF and TrkB immunocontent was analyzed by Western Blot.

RESULTS: Our results showed that aged mice presents lower recognition index than adult ones ($0,58 \pm 0,023$; $0,66 \pm 0,015$, respectively, $P < 0,05$). Caffeine-treated mice showed similar recognition index to adults ($0,69 \pm 0,02$) and an improvement compared to their age-matched control mice. Western blotting analysis revealed that hippocampus from aged-mice presented an increase in the BDNF (from $9,78 \pm 1,19$ to $22,79 \pm 0,45$, $P < 0,05$) and TrkB (from $19,77 \pm 0,89$ to $14,49 \pm 0,68$, $P < 0,05$) immunocontent than adults samples. Hippocampus from caffeine-treated mice presented a partial increase in the BDNF immunocontent ($15,50 \pm 1,22$, $P < 0,05$) compared to age-matched control and adult mice. For TrkB, caffeine counteracted the increase in its immunocontent with aging ($16,17 \pm 0,77$).

CONCLUSIONS: Our results reinforcing positive effects of caffeine on memory, suggesting that its intake during adulthood may prevent recognition memory decline with aging. Besides, its positive effects found here were related to a decrease in the BDNF and TrkB immunocontent with aging. Finally, it is the first time that the effects of caffeine as a cognitive enhancer were related to an important neurotrophic factor involved in learning and memory processes.

Palavras-Chave: caffeine; aging; cognition; memory; neurotrophins; adenosine

Apoio Financeiro: CNPq, FAPERGS, BIC/UFRGS and L'oreal Grants.

F.02.091

Aprendizagem de seqüências em uma tarefa de tempo de reação serial: previsão do desempenho pela entropia acumulada e envolvimento hemisférico

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O aprendizado de seqüências depende da organização dos eventos que a compõem e, por isso, pode ser previsto pela complexidade das relações entre estes. Resultados prévios mostram que este desafio pode ser quantificado pela Entropia Acumulada (EA) destes eventos, mas é possível que a execução de movimentos seguidos dos dedos de mãos diferentes ou da mesma mão e, potencialmente, de hemisférios distintos ou iguais traga desafios além da EA para a realização da seqüência. O presente estudo avaliou (1) a congruência entre o desempenho observado e o desempenho previsto pelo modelo da EA e (2) a influência da execução de pares de eventos que envolva hemisférios distintos (HD) e iguais (HI) no tempo de reação (TR) durante a execução de uma seqüência.

Voluntários (N=48) foram organizados em 6 grupos com 8 voluntários cada, expostos a 1225 tentativas de uma tarefa de tempo de reação serial. Em cada tentativa deveria ser pressionado um botão em resposta ao aparecimento, no monitor de um computador, de um estímulo correspondente. Em cada uma das tentativas foi medido o TR, relativo ao intervalo de tempo entre a apresentação do estímulo e o toque do botão correspondente. Transições HD e HI estavam inseridas em seqüências com complexidades distintas, que foram executadas por grupos distintos.

O desempenho observado nas diferentes seqüências apresentou consistência com o previsto pelo modelo teórico da EA. Quanto maior a complexidade da seqüência, maior os TR e menores as taxas de aprendizagem. Foi observado também faixas de saturação, em que incrementos na complexidade não alteram o desempenho. Quanto à influência da execução HD no desempenho, se observou TR maiores e taxas de aprendizagem menores em transições HD do que em HI. Adicionalmente, a influência da execução HD diferiu dependendo da complexidade da transição: existem faixas de complexidade em que há saturação tal que execução seja HD ou HI não há diferença no desempenho.

Fica claro que (1) o modelo da EA é efetivo para prever o desempenho das seqüências de diferentes complexidades e (2) o poder de predição desse modelo pode ser aumentado considerando o envolvimento da atividade hemisférica.

Palavras-Chave: aprendizagem; seqüências; entropia acumulada; tempo de reação serial; hemisférios cerebrais.

Apoio Financeiro: FAPESP e CNPq

Transcripción sostenida de *Arc* después de la exploración espacial

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Objetivo: La memoria requiere de plasticidad sináptica y esta depende de la expresión de genes inducidos por actividad neuronal asociada a la experiencia conductual; particularmente relevante es la expresión de algunos genes inmediatos relacionados con plasticidad sináptica como el gen inmediato efector *Arc*. En el giro dentado del hipocampo, vimos que el ARNm de *Arc* se observa en el citoplasma 30 minutos después de la exploración de un ambiente novedoso y se mantienen presente por 8 horas. El objetivo de este trabajo es caracterizar la presencia sostenida de *Arc* en el giro dentado después de la exploración espacial y determinar si se trata de un mecanismo de transcripción sostenida.

Metodo: Ratas macho (Wistar) ejecutaron una conducta de exploración (en campo abierto) por 5 min. Las ratas regresaron a sus cajas habitación por diferentes intervalos: 0, 30, 120, 360, 480, 720 y 1440 min. (n=4); inmediatamente después se sacrificaron. Grupo control con exploración (n=4). Los cerebros fueron extraídos y procesados para hibridación *in situ* fluorescente para detectar el intrón del transcrito reciente de *Arc* y el transcrito completo independientemente. El propósito era distinguir sin confusión entre la transcripción reciente (inducida ~5min. antes; *Arc* intron en núcleo) y la temprana (inducida ~30 min. antes; *Arc* completo en citoplasma). En otros animales se administró Actinomicina D después de la exploración (o vehículo) y se sacrificaron 30, 120 o 480 min. después para evaluar si los mecanismos de transcripción subyacen a la presencia sostenida de *Arc* en el giro dentado.

Resultados: 50% de las células con *Arc* en el citoplasma mostraron también la presencia del intron de *Arc* en el núcleo ANOVA (F 7,20 = 12.998 p< 0.0001), esto sugiere que una alta proporción de las células activadas por la conducta sigue transcribiendo. La presencia sostenida de *Arc* regresa a niveles basales a las 12 horas. Resultados preliminares sugieren que la Actinomicina D puede inhibir la transcripción sostenida de *Arc* en el giro dentado.

Conclusión: La presencia sostenida del ARNm de *Arc* en el giro dentado se debe a un mecanismo de transcripción sostenida que puede ser relevante para la memoria espacial.

Palabras-Chave: plasticidad, genes de transcripción inmediata, aprendizaje espacial, hipocampo, células de lugar, consolidación de la memoria

Apoio Financeiro: CONACYT 51028

F.02.093

Assessment of the adaptive value of dreams.

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Objective:

Several lines of evidence indicate that sleep is beneficial for learning, but there is no experimental evidence yet that the content of dreams is adaptive, i.e., that dreams help the dreamer to cope with challenges of the following day. Our aim here is to investigate the role of dreams in the acquisition of a complex cognitive task.

Methods and Results:

We investigated electroencephalographic recordings and dream reports of adult subjects (n=22, 5 females and 17 males) exposed to a computer game task comprising perceptual, motor, spatial, emotional and higher-level cognitive aspects (Doom). Subjects slept two nights in the sleep laboratory, a completely dark room with a comfortable bed and controlled temperature. Electroencephalographic recordings with 28 channels were continuously performed throughout the experiment to identify episodes of rapid-eye-movement (REM) sleep, when dreams are prevalent. Behaviors were continuously recorded in audio and video with an infrared camera. Dream reports were collected upon forced awakening from late REM sleep, and again in the morning after spontaneous awakening. On day one, subjects were habituated to the sleep laboratory, no computer game was played, and negative controls for game-related dream reports were collected. On day two, subjects played the computer game before and after sleep. Each game session lasted 1 hour, and sleep lasted 7-9 hours. Several questionnaires were applied before and after the experiment to assess relevant variables (socio-demographic, pre- and post sleep, Pittsburgh, Fletcher & Luckett, Epworth, Horne). All subjects showed an improvement in game performance from day 1 to day 2. Seventeen out of twenty two subjects reported the intrusion of game elements in their dreams, including potentially adaptive (insightful) strategies.

Conclusions:

The preliminary data collected so far suggest a correlation between game performance and dream content.

Support:

Associação Alberto Santos Dumont para Apoio à Pesquisa (AASDAP)

Pew Latin American Fellows Program in the Biomedical Sciences

FINEP

MCT

Palavras-Chave: Dream , EEG and Learning

Apoio Financeiro: Associação Alberto Santos Dumont para Apoio à Pesquisa (AASDAP); Pew Latin American Fellows Program in the Biomedical Sciences; FINEP; MCT

Sleep-dependent reactivation of the calcium signal transduction pathway in the rat hippocampus

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Objectives: Sleep plays a key role in the consolidation of newly acquired memories. In most mammalian species, two distinct and successive phases of sleep, slow wave (SWS), and rapid eye movement (REM), can be recognized. Both phases have been implicated in the sensorimotor processing of daytime events, but the molecular mechanisms involved remain unknown. Brain expression of the plasticity-associated immediate-early gene (IEG) zif-268 is upregulated during REM sleep in cerebral cortex and hippocampus of animals exposed to rich sensorimotor experience in the preceding waking period (1). ZIF-268 integrates a major calcium signal transduction pathway which includes Ca(2+)/calmodulin-dependent protein kinase II (CaMKII) and mitogen activated protein kinase (MAPK). CaMKII autophosphorylation of T286 is of special importance because it makes the enzyme active in the absence of Ca(2+), providing a biochemical memory that is critical for plasticity (2). Our goal here is to investigate the involvement of CaMKII in neuronal plasticity during sleep in rats exposed to a new rich environment in the preceding waking period.

Methods: Adult Wistar rats (n=3) were exposed to four novel objects for sensorimotor stimulation during 10 minutes. Intracranial local field potentials (LFPs) recorded in the cortex and hippocampus were used to characterize the wake-sleep cycle (3). CaMKII and Zif268 expression were analyzed in immunoblots of brain extracts using specific antibodies.

Results: Our preliminary results show zif268 upregulation in hippocampal neurons two minutes after REM sleep in rats previously exposed to novel objects in the preceding WK period. Additionally, CamKII reactivation was detected during SWS and REM in the hippocampus of the stimulated animals. Interestingly, CamKII reactivation is also observed in cortical areas of these animals after SWS.

Conclusions: Our data suggest that the calcium signal transduction pathway is reactivated in the hippocampus after a few minutes of sleep.

References: (1) Learn Mem. 6: 500, 1999; (2) Curr Opin Neurobiol. 14: 318, 2004; (3) J. Neurosci. 24: 11137, 2004.

Palavras-Chave: sleep kinase zif268 CaMKII

Apoio Financeiro: CNPq

F.02.096

Memória fonológica em crianças com dislexia e bons leitores.

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Objectives: Present study assessed the ability of syllables' phonological discrimination in children with developmental dyslexia and good readers.

Methods: 10 dyslexics and 10 controls matched for age, gender, school grade, intelligence and attention participated in the study. 45 pairs of syllables differentiated by sound, point of articulation and identical syllables were presented in forced choice tests SOUND/SOUND and SOUND/LETTER. The inter stimuli time varied from short, medium to long.

Results: The results showed between-group differences in SOUND/SOUND test with dyslexics doing worse than good readers in sound syllables and point of articulation syllables with long intervals ($p < 0.054$). Analysis of the syllables pairs showed significantly lower score for dyslexics in sound syllables /KA/-/GA/ ($p < 0.035$) and /FA/-/VA/ ($p < 0.046$) and point of articulation syllables /ZA/-/VA/ ($p < 0.052$).

Conclusions: The results showed faster loss of phonological information in working memory in dyslexic children. This loss is significant in inter stimuli time bigger than 10 seconds. This indicates that the sound categories of some syllables are less distinct for dyslexic children than for normal readers and thus carry weaker phonological mark in the working memory reverberation. The cause of this loss shall be further investigated.

Palavras-Chave: dyslexia; phonological discrimination; working memory

Role of neuronal reverberation during slow wave sleep in the consolidation of recently acquired memory traces

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Introduction: Recently, it has been shown that the main sleep states, slow wave (SWS) and rapid eye movement (REM) periods perform two complementary roles for memory consolidation: neuronal reverberation for short-term recall (1) and synaptic plasticity for long-term storage (2). Recent findings on temporal patterns of neuronal ensemble activity in hippocampus (HP) and primary somatosensory cortex (SI) led to the hypothesis that memory traces gradually migrate from HP to SI through consecutive waves of reverberation during SWS (3). Our goal is to test this hypothesis and to assess the importance of reverberatory phenomena in memory consolidation.

Methods: Adult Long Evans rats received an electrode array implant of 32 microwires in SI for single-unit and LFP recordings. Animals also received a bilateral cannula implant in HP for intra-parenchymatous drug micro-injection. Novel tactile stimulation was provided by a 20 minutes exposure to four objects in the middle of an 8 hours recording session. Hippocampal deactivation was achieved by injecting muscimol (0.5 µl; 1 mg/ml; 0.25 mg/min) after a criterion of 30 minutes of SWS was reached in the post-exposure period, as assessed by an online state map algorithm (4). Memory consolidation was tested by re-exposing animals to two familiar objects plus two new objects, and comparing the time rats spent in the exploration of novel and familiar objects.

Results: Firing rates strongly increased during object exploration and remained elevated afterwards during waking. SI single-unit activity during SWS showed even greater Post/Pre differences in firing rates, displaying periodic waves of reactivation which were completely abolished a few minutes after muscimol micro-injection. Importantly, the time spent in exploration of the new objects was not significantly different from the time spent exploring familiar objects.

Conclusions: The results suggest that inhibition of HP neuronal activity impaired SI neuronal reverberation during SWS and decreased memory consolidation for familiar objects.

References: 1-PLoS Bio., 2:0126, 2004; 2-Learn. Mem. 6: 500, 1999; 3-Frontiers Neurosci., 1:43, 2007; 4- J. Neurosci. 24: 11137, 2004.

Palavras-Chave: sleep, memory, neuronal reverberation, synaptic plasticity, single-unit recording

Apoio Financeiro: CNPq, FAPERN, AASDAP

Investigação do papel do sono na aprendizagem escolar.

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Objetivos: Devido a importância do papel do sono na consolidação das memórias em tarefas desenvolvidas em laboratório com humanos e cobaias, e sendo as memórias ligadas diretamente à aprendizagem na construção do conhecimento. O presente trabalho objetiva investigar a influência do sono no ambiente escolar como um fator benéfico no processo ensino-aprendizagem. (1-2)

Métodos: Foram 186 alunos do 6º ano do Ensino Fundamental participantes do trabalho em 6 escolas do Rio Grande do Norte. Eles foram submetidos a uma aula sobre Visão e Memória, tema que supostamente não tenham domínio comprovado por um teste aplicado antes de iniciá-la. Em seguida, de forma aleatória, eles foram separados em dois grupos: 1) sono; 2) aula (controle). O grupo aula voltou às atividades rotineiras da escola, e o grupo sono foi para a sala do sono, contendo colchonetes e máscaras para os olhos, convidados a dormir entre 90 e 120 minutos. No dia seguinte e com cinco dias pós-aula específica foram aplicados questionários de múltipla escolha sobre a aula para avaliar a retenção do conhecimento aprendido de ambos os grupos experimentais.

Resultados: A média de acertos foi estimada pelo número de respostas corretas dividido pela soma de respostas corretas e incorretas. O desempenho de acerto (média $\pm$ SEM) no teste 1 foi de $55,5 \pm 2,4$ e $52,3 \pm 2,2$ para o grupo sono e aula, respectivamente. Já no teste 5 foi de $59,5 \pm 1,9$ e $55,9 \pm 1,6$ para o grupo sono e aula, respectivamente.

Conclusão: Apesar deste trabalho apresentar resultados ainda preliminares, eles sugerem que o sono pode ser importante para aprendizagem dentro do contexto escolar com suas programações e interferências sensoriais características.

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Palavras-Chave: Sono e Aprendizado, Aprendizado e Memória, Sono na Escola

Apoio Financeiro: AASDAP - Associação Alberto Santos Dumont para Apoio à Pesquisa

THE EFFECTS OF DIAZEPAM ON MEMORY RETRIEVAL ARE NOT RELATED TO ANXIOLYSIS

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Introduction

Benzodiazepines such as diazepam (DZP) have been shown to interfere with memory processes. However, most of these studies focused on the evaluation of anterograde amnesia induced by these drugs. Additionally, it has been suggested that this effect can be related to anxiolysis. Our aims are: (1) verify if DZP would have any effect on memory retrieval; (2) assess the effects of DZP on memory retrieval and anxiety concomitantly, verifying a possible relationship between these two variables in the plus maze discriminative avoidance task.

Methods and Results

The task is held in a modified elevated plus-maze able to evaluate anxiety and memory simultaneously. Female Wistar rats (n=14) received DZP (2.0 mg/kg) or saline i.p. thirty minutes before the test session. The experiment consisted of a training and a test sessions. During the training session the animals were placed in the center of the apparatus and aversive stimuli (light and noise) were produced every time the animal entered the aversive arm. Glass walls were used to block the open arms, so the animal would not be able to explore them, avoiding the one-trial-tolerance phenomenon. In the test session the glass walls were removed and the aversive stimulus was not produced. The time spent in each arm and the distance traveled were registered. In the test session the DZP-treated animals presented increased locomotion (mean±SE were 26.0 ± 1.6 m to DZP-treated and 17.4 ± 2.3 m to control) and open-arms exploration ($19.6 \pm 5.8\%$ to DZP-treated and $6.8 \pm 2.5\%$ to control), indicating an anxiolytic effect. An increased aversive-arm exploration ($57.6 \pm 7.4\%$ to DZP-treated and $30.2 \pm 5.6\%$ to control) was also detected, revealing a memory retrieval impairing. Nevertheless, Pearson's correlation test did not show statistical correlation between these variables.

Conclusion

DZP impaired memory retrieval, suggesting that benzodiazepines can induce retrograde amnesia as well. Despite the fact that the DZP-treated animals presented memory impairment and anxiety reduction these variables did not correlate, suggesting that the memory impairing is not a secondary effect of the anxiolysis induced by the drug.

Palavras-Chave: Memory, Anxiety, Diazepam

Apoio Financeiro: CNPQ, CAPES (PROAP - UFRN)

AMYGDALA INACTIVATION IMPAIRS LEARNED (BUT NOT INNATE) FEAR RESPONSE IN RATS

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Introduction: Studies have suggested that the amygdala has a crucial role in acquisition of aversive memories. However, the possibility that amygdala is merely related to any kind of fear sensation or response, indirectly modulating memories based on fearful situations has been not tested. We investigated the effects of bilateral inactivation of the amygdaloid complex in rats tested in the plus maze discriminative avoidance task. This task concomitantly evaluates aversive memory (by discrimination of the two enclosed arms) and innate fear (by open arm exploration).

Materials and Results: Wistar rats (3-4 months-old) were implanted with bilateral guide cannulae into the amygdala. After surgery, all subjects were given one week to recover before behavioral experiments. In experiment 1, 15 minutes prior to training, 0.5 ml of saline (S, n = 8) or muscimol (M, n = 7) (1 mg/ml) was infused in each side via microinjection needles. In the experiment 2 the animals received the same treatments immediately after the training (n = 5 and 7). In both experiments a test session was performed 24h later. The animals were placed individually in the center of the plus maze that had two open arms and two closed arms. In one of the closed arms there was an aversive stimulus (a strong light and noise) every time that the animal entered in the arm during the training, but not the test session. The main results showed that (1) pretraining muscimol induced acquisition (evaluated by aversive arm exploration along the training) and memory (evaluated by aversive arm exploration in the test session) deficits, but did not alter innate fear (evaluated by percent time in the open arms (S = $30.8 \pm 5.0\%$ and M = $54.5 \pm 16.7\%$, mean $\pm$ SE); (2) post-training muscimol apparently impaired consolidation, inducing increased percent in aversive arm exploration in the test session (S = $7.1 \pm 2.0\%$ and M = $56.2 \pm 1.5\%$).

Conclusion: The results suggest that amygdala inactivation specifically modulated the acquisition and consolidation of the aversive task, since innate fear was not affected.

Palavras-Chave: Memory, Learning, Anxiety, Amygdala

Apoio Financeiro: CNPq, CAPES

Teste Pictórico de Memória: Correlações entre Alunos da Segunda e Quarta Séries de Escola Pública da Cidade de Uberlândia.

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A memória de trabalho é didaticamente dividida em 4 subsistemas funcionais: sistema executivo central, laço fonológico, armazenador episódico e memória visuoespacial. O executivo central supervisiona o fluxo de informação vinda do ambiente e da memória de longo prazo. O Laço fonológico é armazenador de informações verbais/auditivas. O armazenador episódico armazena temporariamente informações provenientes dos outros subsistemas e da memória de longo prazo que são aglutinadas numa representação episódica unitária. A memória visuoespacial mantém e manipula a informação referente aos objetos e suas relações espaciais. O presente trabalho visa avaliar a memória visuoespacial de crianças com o teste Pictórico de Memória, que contém uma paisagem com 51 itens. Para correção, atribui 1 ponto a cada item lembrado, e a pontuação total é produto da soma de todos os itens corretos assinalados. O teste foi aplicado em 119 crianças em 4 turmas (2 de 2ª série e 2 de 4ª série do ensino fundamental) de uma escola pública da cidade de Uberlândia. A idade média das crianças foi de 8,76 sendo 66 do sexo masculino (2º série=34; 4º série=32) e 53 do sexo feminino (2º série= 22; 4º série=31). A aplicação do teste ocorreu de forma coletiva em sala de aula, utilizando retro projetor. O teste foi projetado por 2 min para observação e após a retirada do estímulo, teriam 1 min para escrever os itens recordados. Não houve diferenças significativas de pontuação em relação ao sexo dos participantes (sexo feminino M= 8,3, masculino M=7,9; DP= 3,1). A diferença dos resultados por série mostrou um salto significativo de itens recordados das segundas para as quartas séries (M2ªsérie1=7,8 a M4ªsérie2=9,6). A amostra foi significativa em relação à idade entre os 7 e os 10 anos (N=92), havendo um acréscimo de itens recordados de acordo com a idade (7anos=7,1; 8anos=7,3; 9anos=8,6 e 10anos=9,2). Por se tratar de um teste recentemente validado, ainda não existem escalas de comparação de pontuações em relação à população específica. Porém pode-se perceber que, como relatado na literatura pertinente, que foi obtido um acréscimo de itens corretos lembrados de acordo com o aumento da idade.

Palavras-Chave: memória , crianças , testagem

A privação de sono paradoxal prejudica a aquisição de esquiva dos braços abertos em um paradigma de teste-reteste no labirinto em cruz elevado

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Objetivos: O labirinto em cruz elevado (LCE) é um teste de ansiedade composto por dois braços abertos e dois braços fechados. Numa sessão de livre exploração, os braços abertos tendem a ser evitados por ratos. Tipicamente, numa segunda sessão no LCE, ratos exploram menos os braços abertos que na primeira. Tem sido sugerido que tal queda na exploração decorre de uma aprendizagem aversiva, a qual envolve localização espacial. Existem evidências de que, após o treino de uma aprendizagem espacial, a privação de sono paradoxal (PSP) leva a uma redução no desempenho. O presente estudo tem como objetivo avaliar se o paradigma de teste-reteste no LCE é capaz de detectar efeitos da PSP.

Método: Doze ratos foram submetidos a uma sessão de 5 min no LCE. Nas 14 h seguintes, 6 ratos foram submetidos a PSP: postos em plataformas (7,5 cm de diâmetro) de 5 cm de altura em um tanque com água (profundidade: 4 cm). Em tais plataformas, se o animal entra em sono paradoxal, a decorrente perda de tônus muscular faz com que ele caia na água e desperte. Os ratos restantes (grupo controle) foram submetidos ao mesmo procedimento, porém, em plataformas maiores (20 cm de diâmetro) que possibilitam todas as fases do sono. Sete dias depois, todos os animais foram retestados no LCE.

Resultados: De acordo com uma ANOVA de duas vias para medidas repetidas (fatores: PSP e sessão), a porcentagem de entradas nos braços abertos mostrou efeito da sessão ($F [1,10] = 21,7$; $p < 0,05$) e interação entre os fatores ($F [1,10] = 5,67$; $p < 0,05$). Uma comparação post hoc mostrou (Fisher LSD, $p < 0,05$) que o grupo controle apresentou uma redução na porcentagem de entradas nos braços abertos (teste: $39,9 \pm 6,4$; reteste: $21,1 \pm 5,6$). O grupo PSP não mostrou equivalente queda (teste: $35,0 \pm 2,6$; reteste: $28,9 \pm 5,2$).

Conclusões: Os resultados indicam que a aprendizagem de esquiva dos braços abertos envolvida no paradigma de teste-reteste no labirinto é uma tarefa de aprendizagem espacial que é prejudicada pela PSP.

Palavras-Chave: privação de sono paradoxal, labirinto em cruz elevado, teste-reteste, esquiva

Organização temporal do condicionamento clássico aversivo em processos de memória ao contexto e na expressão da proteína Zenk.

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OBJETIVOS: No condicionamento clássico aversivo (CCA), ocorre a associação de um estímulo incondicionado aversivo com um estímulo neutro para a resposta analisada, assim, o estímulo inicialmente neutro adquire a capacidade de eliciar as respostas incondicionadas ao primeiro estímulo. Estudos prévios indicaram que o treino ao CCA induziu a expressão de Zenk no hipocampo de pombos. O presente estudo investigou as variações dia-noite na aquisição do CCA e possíveis variações na expressão da proteína *Zenk* no hipocampo de pombos. **MÉTODOS E RESULTADOS:** Pombos machos adultos, divididos em grupos condicionados (GE), controle (GC) e manipulação (GM), foram mantidos em fotoperíodo esqueleto. As sessões de treino e teste ocorreram pela manhã (ZT02) ou à noite (ZT14). O grupo GE foi treinado com apresentação de três choques elétricos e testado no contexto, o grupo GC foi exposto ao contexto sem apresentação de estímulos e o grupo GM foi somente pesado. A análise comportamental foi feita com auxílio do programa Etholog 2.2. A análise das células Zenk-positivas foi feita por microscopia de campo claro e a contagem com programa ImageJ (NIH-USA). A ocorrência de *freezing* no teste foi maior para GE ZT02 e ZT14 comparados com GC ZT02 e ZT14. ANOVA indicou diferença significativa de *freezing* entre grupos ($p < 0,05$), mas não entre os horários ($p > 0,05$). Comparações entre bloco final do treino e bloco inicial do teste mostraram que GE ZT02 mantém a ocorrência de *freezing* elevada no início do teste e GE ZT14 mostra queda significativa na ocorrência de *freezing* no bloco inicial do teste ($p < 0,05$). A análise imunohistoquímica indicou maior número de células Zenk-positivas no hipocampo do GE ZT02 em relação aos demais grupos. **CONCLUSÕES:** O aumento de *freezing* nos GE ZT02 e GE ZT14 demonstrou a eficácia do condicionamento aversivo. As comparações do *freezing* no final do treino e início do teste sugerem uma variação na memória aversiva dependente do horário. Os dados referentes ao número de células Zenk-positivas sugerem uma variação dependente do condicionamento e do horário na expressão do gene *zenk*.

Palavras-Chave: Condicionamento Clássico Aversivo; Zenk; Freezing

Apoio Financeiro: Fapesp; CNPq

PROCESSOS DE APRENDIZAGEM E MEMÓRIA AVERSIVA EM POMBOS: ANÁLISE DA PROTEÍNA QUINASE C (PKC) NA REGIÃO HIPOCAMPAL

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Introdução: O condicionamento clássico aversivo é utilizado para investigar os mecanismos celulares e moleculares que sustentam a formação da memória em diferentes espécies de animais. Os processos sinápticos ativam mecanismos de sinalização intracelular que envolvem quinases ativadas em momentos específicos. A ativação da PKC é um dos mecanismos moleculares necessários à plasticidade sináptica subjacente à formação de memória.

Objetivo: Investigar o envolvimento da PKC α / β II hipocampal no condicionamento clássico aversivo em pombos.

Métodos: Foram usados 41 pombos adultos, distribuídos em diferentes grupos. No Experimento 1, 11 animais foram submetidos à cirurgia para implante de microcânulas (A9, L1, V3) para injeção i.c.v. do inibidor da PKC, callostina C (GCdCa, n=6; 5ml de solução 60mg/ml, DMSO 2%) ou do veículo (GCdVe, n=5; DMSO 2% em salina). Após 7 dias, houve uma sessão de treino, 20 min, com 3 pareamentos som-choque. O teste realizado 24h após consistiu na re-exposição ao contexto de treino. O Experimento 2 investigou por meio de Western Blot, a variação temporal da ativação da PKC α / β II após o condicionamento. Os animais foram distribuídos em 5 grupos: grupo controle não treinado (GC, n=6) e grupos condicionados que tiveram a sessão de condicionamento e foram sacrificados em diferentes tempos após o treino: GE1min (n=6), GE1h (n=6), GE2h (n=6) e GE24h (n=6). A região hipocampal foi dissecada e o tecido processado para análise da PKC α / β II. As sessões de treino e teste foram gravadas digitalmente para posterior análise.

Resultados: No Experimento 1 houve menor expressão de *freezing* no GCdCa comparado ao GCdVe ($p < 0,05$). No Experimento 2, mudanças nos níveis da PKC α / β II, após o treino, foram expressas como porcentagem dos grupo controle. Não houve diferença significativa na quantidade de PKC α / β II entre os grupos treinados e sacrificados em diferentes pontos temporais ($p > 0,05$).

Conclusão: Os resultados do Experimento 1 mostram que a administração da callostina interferiu na expressão da resposta de *freezing* condicionada ao contexto aversivo. O Experimento 2 indica que outras isoformas da PKC podem estar envolvidas no processo de aprendizagem e memória.

Palavras-Chave: Memória, Hipocampo, PKC

Apoio Financeiro: CNPq e FAPESP

EFEITOS DO MIDAZOLAM SOBRE A RECONSOLIDAÇÃO DE MEMÓRIA EM FILHOTES DE RATOS

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A evocação de uma memória previamente consolidada inicia 2 processos: a reconsolidação ou a extinção. Neste período, a memória torna-se lábil e sensível a tratamentos amnésicos, por isso, a administração de benzodiazepínicos nesta fase tem sido estudada. O trabalho avalia os efeitos de longa duração da injeção de midazolam (MDZ) 30 min após a reativação (RE) no 20º dia pós-natal de filhotes machos e fêmeas sobre a reconsolidação de memória aversiva.

Ratos Wistar foram submetidos ao condicionamento (CON, 15º ao 18º), RE (20º) e testes (21º, 51º e 81º dia, T1; T2 e T3 respectivamente). O CON consistiu de 1 sessão de treino por dia, onde se colocou um animal na caixa de condicionamento na presença de odor cítrico (estímulo condicionado; 0,2ml de Citral-CIS+TRANS, Fluka) onde recebeu 3 choques (estímulo incondicionado, 0,5mA/0,4s) com intervalos de 30s, totalizando 2 min. A RE consistiu no mesmo procedimento do CON e, após 30 min, aplicou-se intraperitonealmente solução salina (0,9%) ou MDZ (1mg/kg), dividindo os animais em 2 grupos: salina (n=27) e midazolam (n= 28). Nos testes, os ratos foram colocados no mesmo ambiente apenas com o odor cítrico por 5 min. A RE e os testes foram filmados e posteriormente analisados quanto à porcentagem de freezing (ANOVA de duas vias, seguido de Newman-Keuls- $p < 0,05$).

Nos machos do grupo midazolam ocorre uma redução da porcentagem (média±EPM/ RE;T1;T2;T3, respectivamente) de freezing (34,36± 5,26; 33,29± 6,28; 26,39± 7,25; 21,68± 9,94) em comparação com o grupo salina (36,12± 4,67; 49,55± 7,07; 42,94± 8,22; 24,45± 7,33). Isso ocorre também nas fêmeas midazolam (36,59±4,75; 31,21±7,39; 14,11±6,14; 8,94±2,13) quando comparadas com as fêmeas do grupo salina (28,92±4,80; 47,32±4,95; 27,33±7,22; 24,20±5,73).

Os resultados mostram que o midazolam aplicado em filhotes afeta a reconsolidação de memórias aversivas mesmo com o passar do tempo, pois há redução da porcentagem de freezing nos testes em ambos os sexos. Os resultados também mostram uma diferença sexual, já que o efeito do midazolam nas fêmeas parece mais pronunciado do que nos machos, e essa diferença sexual também aparece quando se avalia o efeito do midazolam ao longo do tempo. **Palavras-Chave:** Condicionamento, Reconsolidação, Odor, Choque, Benzodiazepínico, Amnésia.

Apoio Financeiro: CNPq, CAPES, FAPESP, FAPERGS.

Efeitos de um episódio de deficiência de tiamina durante a amamentação sobre o desempenho cognitivo espacial e parâmetros gabaérgicos e glutamatérgicos em ratos jovens

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A deficiência de tiamina (DT) induz lesões em diversas regiões cerebrais podendo afetar os circuitos glutamatérgico e gabaérgico, os quais estão relacionados com vários aspectos comportamentais. Diversos trabalhos sobre alterações cognitivas e bioquímicas em ratos adultos submetidos à DT têm sido realizados, entretanto, raros estudos abordam esse tema utilizando animais jovens submetidos à deficiência durante a amamentação. O objetivo deste trabalho foi avaliar os efeitos da DT e da idade sobre o aprendizado e memória espacial de ratos submetidos à deficiência durante a amamentação e a correlação desses parâmetros comportamentais com as concentrações de ácido γ -aminobutírico (GABA) e glutamato (GLU) no tálamo, hipocampo e córtex pré-frontal (CPF). As variáveis comportamentais e bioquímicas foram determinadas usando Labirinto Aquático de Morris e cromatografia líquida de alto desempenho (HPLC), respectivamente. Foram realizados dois experimentos, com o mesmo delineamento experimental, diferindo apenas na idade (30 e 75 dias), na qual os animais foram submetidos aos testes. Os grupos experimentais foram constituídos de ratos provenientes de mães que receberam ração padrão ou deficiente, durante o período de amamentação. A deficiência causou efeito significativo ($p < 0,01$) na velocidade de aquisição da tarefa apenas para os animais de 30 dias. Entretanto, tanto os ratos de 30 quanto os de 75 dias foram capazes de aprendê-la, não apresentando déficit de memória. Não foi observado efeito significativo da deficiência sobre os níveis de GABA e GLU nos animais de 30 dias, enquanto que para os ratos de 75 dias, para o GABA (tálamo) e GLU (todas as regiões) foram verificados efeitos significativos ($p < 0,01$) desse tratamento. Os resultados sugerem que o déficit cognitivo provocado pela DT nos ratos de 30 dias não está diretamente relacionado aos circuitos gabaérgico e glutamatérgico no tálamo, pois, não foi verificada correlação significativa entre o prejuízo no desempenho e os níveis de GABA e GLU. Além disso, a melhora do desempenho nos testes comportamentais e alteração de GABA e GLU nos animais de 75 dias corroboram a hipótese do papel destes sistemas na plasticidade neuronal.

Palavras-Chave: Deficiência Tiamina GABA Glutamato Tarefa Espacial

Apoio Financeiro: Capes

INFLUÊNCIA DO ENRIQUECIMENTO AMBIENTAL NO CONSUMO, PESO FINAL E HABITUAÇÃO EM RATOS SUBMETIDOS AO TESTE DE *OPEN-FIELD*.

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Objetivos: Avaliar o impacto do enriquecimento ambiental (EA) sobre o consumo total, peso final e habituação no teste de *open-field* em ratos num experimento nutricional. **Métodos:** 36 *Rattus Wistar*, machos recém-desmamados, divididos em três grupos dietéticos (n=12): Controle (GC), recebendo ração à base de caseína; Linhaça (GL), recebendo ração à base de linhaça com 5,43% de caseína; Grupo caseína modificada (GCM), recebendo ração à base de caseína com aumento no teor de óleo e fibras para se igualar ao GL, e que foi mantido em *pair feeding*. As rações foram isoprotéicas (10%), seguindo AIN-93G. Submeteu-se 6 animais de cada grupo ao processo do EA passando a ser GCEA, GLEA e GCMEA, mantendo-se os demais em gaiolas padrão (GSEA), durante 56 dias, com temperatura constante e iluminação controlada. Neste período, duas vezes na semana, alternou-se o lugar dos brinquedos e se registrou consumo e peso dos animais. Ao final do experimento, uma vez ao dia, durante 3 dias consecutivos, registrou-se o comportamento dos animais no *open-field*, avaliando as atividades como alta, baixa e inatividade, pré-estabelecendo que quanto menor a movimentação, maior a habituação. Para essa análise juntou-se todos os animais enriquecidos (GEA) e os não enriquecidos (GSEA), desconsiderando as dietas. Uso do ANOVA para análise do consumo e peso final, e *Exact Wilcoxon rank-sum test* para análise do *open-field*, ambos ao nível de $p \leq 0,05$. **Resultados:** Não houve diferença no consumo total entre GLEA (895,7 $\pm$ 27,0) e GLSEA (913,8 $\pm$ 47,5), GCMEA (905,0 $\pm$ 3,3) e GCMSEA (905,6 $\pm$ 0,6), GCEA (1029,1 $\pm$ 31,1) e GCSEA (1062,4 $\pm$ 25,7). O mesmo se verificou em relação ao peso final, no qual o GLEA (307,4 $\pm$ 25,9) não apresentou diferença com o GLSEA (316,3 $\pm$ 53,5), GCMEA (341,5 $\pm$ 20,8) e GCMSEA (342,9 $\pm$ 19,5), GCEA (395,9 $\pm$ 28,9) e GCSEA (388,3 $\pm$ 28,1). Já na habituação a partir do 2º dia notou-se diferença, no qual a inatividade do GEA (15 $\pm$ 1,9) foi superior ao GSEA (7 $\pm$ 1,7), o mesmo foi visto no 3º dia, onde o GEA (19 $\pm$ 7) foi mais inativo que o GSEA (4 $\pm$ 4). **Conclusão:** Por não acarretar alterações no peso e consumo dos animais e por induzir a uma maior habituação destes, o EA pode ser adotado a fim de melhorar a qualidade de vida dos animais cativos.

Palavras-Chave: Enriquecimento ambiental, open-field teste, ratos

Apoio Financeiro: FAPERJ e ARMA ZEN Ltda

Neuronal avalanches across the sleep-wake cycle of rats

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Objectives: Previous studies of neuronal avalanches in brain slices and cultured cells (1) have shown that the distribution of size of avalanches obeys a power law relationship, with an exponent of -1.5. This is compatible with the hypothesis that in vitro neuronal networks are critical, but little is known about the properties of neuronal avalanches in vivo. Here we investigated the statistics of neuronal avalanches recorded across the sleep-wake cycle of freely behaving rats before and after novel object exploration.

Methods: Adult male rats (n=5) were surgically implanted with multielectrode arrays of teflon-coated tungsten microwires (35 μ m, 1.0-1.2 MOhm at 1 KHz). The targeted brain areas comprised the hippocampus, the primary somatosensory cortex and the primary visual cortex. After a 1-week recovery period, extracellular recordings of neuronal action potentials (spikes) were performed across the sleep-wake cycle before and after novel object exploration. The data were divided in temporal bins. An avalanche was defined as a sequence of bins whose activity surmounted a pre-defined threshold. To perform our statistical analysis, we measured the size (number of spikes), duration and interval between avalanches, for a known bin size.

Results: We found that the statistics of neuronal avalanches vary according to wake-sleep state. For any given state, longer and larger avalanches become more frequent following novel experience. This could be trivially caused by increased firing rates after novel experience, a phenomenon previously known to occur after novel object exploration (2). To elucidate this question, control analysis with surrogate random data of same mean rates are currently being performed.

Conclusions: The occurrence of neuronal avalanches is not nearly as evident in freely behaving animals as they are in anesthetized animals, brain slices or cultured cells. Our preliminary results suggest that the statistics of neuronal avalanches are not solely constrained by firing rate changes.

References: 1- J. Neurosci. 23:11167, 2003; 2- FINS 1: 43-55, 2007 **Palavras-Chave:** Neuronal avalanches, sleep, memory, engram, ongoing activity

Apoio Financeiro: CNPq, FACEPE, CAPES, AASDAP, Finep, MCT

Reversão Farmacológica de memórias aversivas em um modelo do Transtorno de Estresse Pós-Traumático (TEPT).

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OBJETIVOS: O TEPT caracteriza-se por intensas reações em resposta a memórias traumáticas. Acreditamos que um fármaco administrado após a reativação de uma memória traumática poderia atenuá-la. O objetivo deste trabalho é estudar se o midazolam (MDZ, 3mg/kg i.p.) é capaz de reverter expressões de medo, comportamentos do tipo ansioso e déficits cognitivos em ratos submetidos a um modelo de TEPT.

MÉTODOS E RESULTADOS: Ratos machos Wistar com 60 dias foram expostos ou não a um evento estressor (modelo de TEPT - choque inescapável, 1mA/20s) e a mais 3 recordatórios situacionais (RS), um a cada 7 dias, onde o animal era colocado em ambiente semelhante àquele onde havia recebido o choque, durante 150s. Havia 5 grupos: (1)= após cada RS recebia salina i.p.; (2)= recebia MDZ após o 1º RS e, nos demais, salina; (3)= recebia MDZ após cada RS; (4)= recebia salina após RS 1 e 2, mas MDZ após RS3; e (5)= não recebeu choque e foi tratado com salina após os RS. Um mês após o RS3, os animais foram expostos a outro RS onde o tempo de imobilidade (TI, medida de medo condicionado) foi avaliado. Uma semana depois foi realizado o labirinto aquático de Morris (LAM) e o labirinto em cruz elevado (LCE). Os resultados estão expressos por média $\pm$ EPM e foram analisados por ANOVA (Post-Hoc de Duncan). Grupos 2, 3 e 4 tiveram seus TI diminuídos em comparação ao 1. No LAM foram analisados os tempos nos quadrantes alvo (tqa) e oposto e os grupos 2, 3 e 4 tiveram o mesmo desempenho que o grupo 5, enquanto o grupo 1 demonstrou prejuízo na memória. No LCE os animais do grupo 1 permaneceram menos tempo nos braços abertos (tba), enquanto que os dos grupos 2, 3 e 4 apresentaram um comportamento similar ao grupo 5.

Grupos	TI (s)	Grupos	LAM - tqa (s)	LCE – tba (s)
1	123,8 $\pm$ 4,9	1	20,5 $\pm$ 3,0	48,4 $\pm$ 11,3
2	67,0 $\pm$ 7,6	2, 3, 4	33,7 $\pm$ 3,4	135,7 $\pm$ 26,3
3	77,1 $\pm$ 11,5	5	26,9 $\pm$ 2,3	136,8 $\pm$ 33,2
4	86,0 $\pm$ 7,3			
5	28,2 $\pm$ 8,6			

CONCLUSÃO: O MDZ foi capaz de atenuar os efeitos causados pelo TEPT, de lembranças aversivas até aspectos comportamentais e esses resultados sugerem que se poderia utilizar a teoria da reconsolidação da memória no sentido de alterar memórias fortes já bem consolidadas em pacientes com esse transtorno.

Palavras-Chave: Transtorno de Estresse Pós-Traumático, Midazolam, Medo condicionado, Ansiedade, Memória

Apoio Financeiro: CAPES, CNPq, FINEP/IBNnet.

F.02.110

Efeito da lesão seletiva do giro denteado hipocampal no desempenho de ratos em um esquema de reforçamento diferencial de baixas taxas em função da privação alimentar

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Objetivo: Avaliar o desempenho de ratos sob efeito da lesão seletiva do giro denteado hipocampal em função de diferentes níveis de privação alimentar durante uma tarefa de reforçamento diferencial de baixas taxas (DRL).

Método: Foram utilizados 10 ratos submetidos à lesão seletiva do giro denteado hipocampal por aplicação múltipla e tópica de colchicina (**grupo lesado**) e 7 ratos controle-operados (**grupo controle**). Sob regime de privação alimentar, a 90% do peso *ad lib*, os ratos foram submetidos a 40 sessões de treino em DRL-20 s, agrupadas em 8 blocos. Neste procedimento, a resposta de pressão à barra é reforçada se, e somente se, ela segue a resposta precedente por um intervalo de tempo de, no mínimo, 20 segundos. Após o treino, empregou-se 20 sessões de DRL-20s com os animais a 80% do peso *ad lib* (*teste 1*) seguidas de mais 20 sessões de DRL-20s com os animais a 100% do peso *ad lib* (*teste 2*).

Resultados: no treino, o índice de eficiência (IE) aumentou de 29,4% $\pm$ 6,2 (grupo lesado) e 30,8% $\pm$ 9,8 (grupo controle) no 1º bloco para 61,4% $\pm$ 9,7 e 55,5% $\pm$ 10,5 no 4º bloco de sessões, respectivamente. A análise de variância (ANOVA) mostrou que (1) houve diferença significativa entre os quatro primeiros blocos e os quatro últimos blocos ($F_{7,105} = 92,9$ $p < 0,01$), indicando à aquisição da tarefa de DRL; (2) não houve diferenças significativas entre os dois grupos ($F_{1,15} = 0,01$ $p > 0,05$). No teste 1 não houve alteração no desempenho, em relação ao treino, de ambos os grupos, com exceção do grupo lesado que apresentou uma queda no IE (56,5% $\pm$ 8,5) no primeiro bloco. No teste 2 houve um aumento médio do IE para 70,6% no grupo lesado e 67,5% no grupo controle.

Conclusão: os resultados obtidos mostraram que: (1) a lesão do giro denteado não prejudicou a aquisição da tarefa de DRL; (2) a alteração do nível de privação de 90% para 80% do peso *ad lib* não interferiu no desempenho final dos animais na tarefa; (3) a alteração do nível de privação de 80% para 100% do peso *ad lib* promoveu uma melhora no desempenho de ambos os grupos, sendo esta mais acentuada nos animais lesados.

Palavras-Chave: DRL, Privação Alimentar, Hipocampo

Apoio Financeiro: CNPq e CAPES

F.02.111

Environmental enrichment influences object recognition task.

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Objective: Environmental enrichment (EE), a paradigm used to induce plasticity in the central nervous system (CNS), involves increasing sensorial complexity by providing novelty with inanimate objects, increasing exercise and adding social interaction. The object recognition task consists in quantifying the natural behavior of rodents of readily approaching and exploring a novel object instead of the known object; thus this task deals with the natural motivation of the animals to explore novelty, an innate instinct that drives animals to learn about their environment. In the present study, our objective was to evaluate the performance of mice after exposure to environment enriched during 8 weeks in the object recognition task.

Methods: 21 Days mice were divided in two groups: control (n=27) and enrichment (n=22). They were maintained 8 weeks in the enrichment housing apparatus, contained running wheels and a variety of objects, which were changed three times per week; controls were maintained in standard condition (SC). The object recognition was performed protocols by (Bevins et al, 2006).

Results: Both groups present same results in discrimination ratio, EE (0.50 ± 0.02 vs 0.60 ± 0.02 $p < 0.001$) and SC (0.51 ± 0.01 vs 0.63 ± 0.01 $p < 0.001$) in training session and test session respectively. However, time of exploration in two objects for EE group decrease compared to SC (30.54 ± 3.61 vs 89.55 ± 4.79 s $p < 0.001$) in training session and (13.46 ± 1.67 s vs 54.52 ± 4.13 s $p < 0.001$) test session respectively.

Conclusion: The increased plasticity at CNS in EE animals is a possible explanation to our findings, once these animals show a decrease in the total time of exploration but present same discrimination ratio. A faster habituation to novelty has been found as a consequence of EE thus suggesting that improved spatial abilities (due to EE) could explain at least some of the findings of EE effects on behavior under novelty conditions.

References: 1- Nature Protocols 1306-11, 2006.

Palavras-Chave: Environmental enrichment , object recognition task, mice

Apoio Financeiro: IBN-Net, FINEP, CAPES, CNPq, BIC/UFRGS

Effects of paradoxical sleep deprivation on phosphorylated CREB expression in the basal and central nuclei of the amygdala: relationship to memory deficits.

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Objective: The aim of the present study was to verify if there is a relationship between performance impairment in contextual fear conditioning (CFC) induced by paradoxical sleep deprivation (PSD) and CREB (*cAMP – response element binding protein*) phosphorylation of in the basal and central nuclei of the amygdala. We also investigated if changes could be prevented when PSD was followed by a sleep recovery period. **Methods:** Male Wistar Rats were trained in CFC after 24h, 48h or 72h of PSD and were tested 24h later. Another group was trained after 72 h of PSD or 72h of PSD followed by 24h of sleep recovery (SR). Brains from a third group of trained rats were examined for CREB and phosphorylated CREB (pCREB) immunoreactivity in the basal and central nuclei of amygdala. **Results:** Seventy-two hours of PSD before training impaired performance in CFC (table 1) and this impairment was prevented by 24 hours of sleep recovery before training (table 2). Immunohistochemical analyses showed that CREB expression did not differ in the basal and central nuclei of amygdala in any group. In contrast, pCREB expression in the basal nucleus was lower in 72h PSD animals when compared to SR. pCREB expression in the central nucleus was lower in 72 h of PSD animals when compared to the control group (C) and with the SR (table 3).

Table 1:

Group	Freezing time in CFC (mean $\pm$ S.E.M)
Control	27.51 $\pm$ 1.98
24h of PSD	16.45 $\pm$ 3.59
48h of PSD	19.98 $\pm$ 4.50
72h of PSD	14.1 $\pm$ 2.45 *

Table 2:

Group	Freezing time in CFC (mean $\pm$ S.E.M)
Control	22.23 $\pm$ 3.96
72 of PSD	7.76 $\pm$ 3.09 #
SR	31.01 $\pm$ 3.70

Table 3:

Group	Number of immunopositive cells in basal and central nuclei of the amygdala(mean $\pm$ S.E.M)			
	CREB		pCREB	
	Basal	Central	Basal	Central

Control	43.72 $\pm$ 2.93	83 $\pm$ 6.7	0.54 $\pm$ 0.13	0.43 $\pm$ 0.09
72h of PSD	45.68 $\pm$ 3.52	87.75 $\pm$ 3.61	0.25 $\pm$ 0.10 **	0.16 $\pm$ 0.06 #
SR	47.72 $\pm$ 2.41	87.75 $\pm$ 3.61	0.69 $\pm$ 0.09	0.48 $\pm$ 0.08

One-way ANOVA with Newman-Keuls post hoc test ($p < 0.05$; *compared to Controls, ** compared to SR, # compared to both of the other groups).

Conclusions: Both sleep deprivation and sleep recovery led to alterations in pCREB levels in the basal and central nuclei of the amygdala. These alterations paralleled the rats' performance in CFC and may be related to the memory deficits observed in CFC after PSD. This study also provided evidence for behavioral benefits of 24h of sleep recovery before a CFC training session.

Palavras-Chave: memory, sleep, CREB, pCREB, contextual fear conditioning

Apoio Financeiro: Fapesp, CNPq, AFIP, FADA

Effects of acute administration of the hydroalcoholic extract of mate tea leaves (*Ilex paraguariensis*) in animal models of learning and memory.

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Objectives: Cognitive deficits can be observed at the early stages of Parkinson's disease (PD), even before the development of its classical motor symptoms, consisting mainly of visuospatial working memory deficits. Recently, we demonstrated an antiparkinsonian profile of the hydroalcoholic extract of *Ilex paraguariensis* (**1**), a plant widely cultivated in South America with CNS stimulant and antioxidant activities. Here we investigated the effects of *Ilex paraguariensis* on the short- and long-term learning and memory of rats. **Methods:** Adult male Wistar rats received a single intraperitoneal (i.p.) administration of the hydroalcoholic extract of *Ilex p.* (62.5-250 mg/kg) and were tested 30 min later in different learning and memory paradigms including the social recognition (**2**), Morris water maze (**3**) and step-down inhibitory avoidance tasks (**4**). **Results:** *Ilex p.* improved the short-term social memory [investigation time (s): CONT: 1st exp: 123.5±11.9; 2nd exp: 108.80±8.5; *Ilex p.*: 1st exp: 108.0±7.80; 2nd exp: 47.6±10.3] as well as facilitated the step-down inhibitory avoidance short-term memory evaluated 1.5 h after training [latency to step-down (s): CONT=40.8; *Ilex p.*: 115.6]. In contrast, pre-training administration of the extract did not alter the step-down inhibitory avoidance long-term memory evaluated 24 h after training [latency to step-down (s): CONT=32.9; *Ilex p.*: 39.0], while the highest dose tested (250 mg/kg, i.p.) disrupted the animals' performance in a cued version of the Morris water maze [escape latency (s): CONT=10.9±1.7; *Ilex p.*: =23.2±3.5]. **Conclusions:** These results indicate that the hydroalcoholic extract of *Ilex p.* differentially modulates short- and long-term learning and memory in rats probably through its antagonist's action on adenosine receptors. Therefore, the evaluation of the potential of *Ilex paraguariensis* extract as cognition-enhancing drug to counteract cognitive deficits in animal models of PD appears to be important.

References: 1-Phytoter Res. 771:21, 2007 2-Behav pharmacol. 209:16, 2005 3- Exp neurol. 391:202, 206. 4-Neurobiol learn mem. 83:113, 2005

Palavras-Chave: *Ilex paraguariensis*; Learning and memory; social recognition; Morris water maze; step-down inhibitory avoidance; Adenosine receptor; Parkinson disease.

Apoio Financeiro: Conselho Nacional de Desenvolvimento Científico e tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Fundação de Apoio a Pesquisa do Estado de Santa Catarina (FAPESC); Universidade Federal de Santa Catarina -UFSC; Universidade do Extremo Sul Catarinense -UNESC; all of Brazil.

Discalculia do desenvolvimento e transtornos de aprendizagem: um estudo com a ZAREKI-R.

Silva, P. A.¹; Santos, F. H.¹ - ¹UNESP - Campus de Assis - Psicologia Experimental e do Trabalho

Objetivo: O presente estudo investigou habilidades matemáticas de crianças com transtorno de aprendizagem que exibiram escores médios abaixo da nota de corte (<19 ; $M=14,1 \pm 4,7$) no Teste de Aritmética/TA do TDE (1) utilizando a Bateria Neuropsicológica de Testes de Processamento Numérico e Cálculo para Crianças (ZAREKI-R; 2) que avalia tanto o processamento numérico quanto o cálculo por meio de 12 subtestes.

Método: Participaram do estudo 30 crianças com transtorno de aprendizagem, de 9 e 10 anos etários, de ambos os sexos, de escolas públicas do Município de Assis-SP. As crianças foram divididas em dois grupos, conforme o seu comprometimento no TA: Prejuízo Leve/PL ($N=25$; $M=15,6 \pm 3,7$), Prejuízo Moderado/PM ($N=05$; $M=6,8 \pm 1,8$); e avaliadas no Laboratório de Neuropsicologia da UNESP/Assis. O grupo PM obteve escores um desvio-padrão abaixo da média da amostra no TA.

Resultados: No escore total da ZAREKI-R, o test t identificou efeito de grupo ($t=5,16$; $p<0,0001$), no qual o escore do grupo PL ($M=122,8 \pm 22,1$) foi maior que o do grupo PM ($M=88,8 \pm 24,1$). A MANOVA evidenciou efeito de grupo entre os subtestes da ZAREKI-R [$R(12,17)=2,33$; $p=0,05$]. Conforme test post-hoc de Tukey, o grupo PM foi prejudicado nos subtestes Ditado de Números ($p=0,01$), Cálculo Mental ($p=0,01$), Leitura de números ($p=0,002$) e Memória de Dígitos ($p=0,019$). Houve correlação (Pearson; $r=0,62$) entre o TA e o ZAREKI-R.

Conclusão: Houve uma associação positiva entre o ZAREKI-R e o TA, entretanto, além de identificar a dificuldade aritmética (cálculo mental), a ZAREKI-R identificou também dificuldades associada ao processamento numérico e à memória operacional fonológica do grupo PM. Portanto, a ZAREKI-R identificou um perfil quantitativo e qualitativo distinto entre os grupos e caracteriza-se como um instrumento mais apropriado para a avaliação das habilidades matemáticas e da discalculia do desenvolvimento.

Referências: 1- Stein, 1993; 2-von Aster & Dellatolas, 2006.

Palavras-Chave: ZAREKI-R; Habilidades Matemáticas; Transtornos de Aprendizagem; Avaliação Neuropsicológica

Apoio Financeiro: FAPESP processo 05/60375-1.

INFLUENCE OF ESTROUS CYCLE ON MEMORY AND ANXIETY IN RATS.

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OBJECTIVES – Several studies show differences between male and female rats when their performances on memory tasks are compared. These differences could be related to female behavioral variations along the estrous cycle, but studies investigating this possibility show controversial results. On the other hand, memory tasks can be modified by emotional states, which in turn are variable among estrous cycle phases. The aim of the current study was to compare memory performance and anxiety-like behavior in female tested in the plus maze discriminative avoidance task indifferent phases of the estrous cycle.

METHODS AND RESULTS – Wistar female rats (3 months old, n=15) were trained in the in the plus-maze discriminative avoidance task. This paradigm is held in a modified elevated plus-maze able to evaluate anxiety and memory simultaneously. The animals were placed individually in the center of the plus-maze, which had two open arms and two closed arms, in one of them an aversive stimulation (light and noise) was produced each time the animal entered the arm. A test session (without aversive stimuli) was performed 24h later. For determination of estrous cycle stage, vaginal smears were obtained from all rats immediately after the behavioral sessions. For data analysis, each rat was allocated to one of the following groups: diestrus/metestrus (DM), proestrus (PE) or oestrus (OE). The main results were: (1) no differences were found in task acquisition (evaluated by aversive arm exploration along the training); (2) rats in PE showed increased percent time in open arms ($21,9 \pm 0,6\%$, mean $\pm$ SE) compared to other groups (DM= $10,6 \pm 2,3\%$, OE= $8,0 \pm 5,0\%$) and (3) rats in DM presented decreased percent time in aversive arm at the beginning of the test session ($19,4 \pm 5,4\%$) compared to other groups (PE= $38,1 \pm 5,2\%$, OE= $46,4 \pm 6,0\%$).

CONCLUSION – Our data suggests that acquisition of the aversive task was not influenced by estrus cycle, while task retrieval seems to be improved in DM compared to other phases. Additionally, this difference in retrieval is probably not related to anxiety levels, since only PE rats showed decreased anxiety. **Palavras-Chave:** Memory, anxiety, estrous cycle

Apoio Financeiro: CNPQ, CAPES (PROAP - UFRN)

Can regular environmental shifts be predicted by hippocampal neurons during sleep?

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OBJECTIVES: The ability to predict future rewards or threats is crucial for survival. Recent studies have addressed future event prediction by the hippocampus. Hippocampal neurons exhibit robust selectivity for spatial location, defining "place fields"(1). This selectivity allows the hippocampus to be involved in the formation of spatial and episodic memories, including the sequential ordering of events (2). A decision-making study reported that CA3 neurons representing the path forward fired when the rat was at the choice point of a T-maze (3). Two fMRI studies examining mental travel time in humans showed that the hippocampus is involved in future event simulation (4, 5). In addition, damage to the hippocampus impair the capacity of imagining new experiences (6). On the other hand, consolidation of newly acquired memories has been shown involve neuronal reverberation during sleep. Such reverberation occurs in different brain regions during two main cyclical sleep stages: slow-wave sleep and rapid-eye-movement sleep. At present, it is unclear whether neuronal ensemble simulations of future events occur during sleep. To address this issue, the present study assesses the ability of hippocampal neurons to reverberate and predict reward location in an ordered spatial task during pre-experience and post-experience sleep.

METHODS: Multielectrode arrays were surgically implanted in the dentate gyrus and CA1 field of the hippocampus of two adult male Wistar rats. Hippocampal neuronal activity was daily recorded for 12 days comprising four cycles of the sleep-maze behavior task. After sleeping in the 'SleepBox' for up to half an hour, mildly food-deprived rats were put in the center of a cross maze and allowed to search for a reward pellet. At each cycle the reward target was repositioned in the next arm, according to a clockwise sequential order.

RESULTS: Memory reverberation related to past locations as well as future environmental prediction was detected using the Euclidian distance of consecutive firing rate patterns and a template matching algorithm based on Pearson correlations.

CONCLUSIONS: Our preliminary results suggest that the hippocampus is involved with both the reverberation of past and future events. Further investigation of this issue is ongoing.

Palavras-Chave: sleep memory future prediction place cells single unit recording

Apoio Financeiro: CAPES AASDAP

RECONSOLIDATION OR EXTINCTION: DIFFERENTIAL ROLE OF NF-kB IN BOTH PROCESSES

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Objectives: Evidence supports that the cellular and molecular mechanisms underlying memory consolidation are different from those underlying reconsolidation and extinction. Differences may rely, not only in the molecules involved, but also in the activation time of these molecules, and even in the brain areas involved. In addition, little is known about the mechanisms of memory extinction. Contrarily wise, increasing evidence supports the participation of the transcription factor NF-kB in memory. It has already been show that in the crab *Chasmagnathus* model of associative contextual memory, NF-kB is activated specifically by retrieval and that this activation is required for memory consolidation and reconsolidation. Besides, the inhibition of NF-kB after memory reactivation impaired retention in inhibitory avoidance in mice. Now, our aim is to study the role of this transcription factor in reconsolidation and extinction in a paradigm of fear conditioning in mice.

Methods: We used a paradigm of fear conditioning in mice (C57BL/6J). In the training session a tone presented in context A, co-terminated with an aversive stimuli, as an electric shock. The response measured in the test session, which could be contextual or cued (context B), was the freezing of the animals (absence of movements except those related with breathing).

Results: We found that hippocampal inhibition of NF-kB after reactivation impairs long-term memory, raising the question if this procedure impedes reconsolidation or facilitates extinction. We also found that the time course of NF-kB activation in hippocampal formation differs from what we have observed in extinction.

Conclusions: These results supports the hypothesis that both reconsolidation and extinction are sustained by different mechanisms.

Palavras-Chave: memory, NF-kB, fear conditioning, reconsolidation, extinction

Discalculia do desenvolvimento e transtorno de aprendizagem: um estudo com a ZAREKI-R.

Silva, P. A.¹; Santos, F. H.¹ - ¹UNESP - Campus de Assis - Departamento de Psicologia Experimental e do Trabalho

Objetivo: O presente estudo investigou habilidades matemáticas de crianças com transtorno de aprendizagem que exibiram escores médios abaixo da nota de corte (<19 ; $M=14,1 \pm 4,7$) no Teste de Aritmética/TA do TDE (1) utilizando a Bateria Neuropsicológica de Testes de Processamento Numérico e Cálculo para Crianças (ZAREKI-R; 2) que avalia tanto o processamento numérico quanto o cálculo por meio de 12 subtestes.

Método: Participaram do estudo 30 crianças com transtorno de aprendizagem, de 9 e 10 anos etários, de ambos os sexos, de salas de apoio de escolas públicas do Município de Assis-SP. As crianças foram divididas em dois grupos, conforme o seu comprometimento no TA: Prejuízo Leve/PL ($N=25$; $M=15,6 \pm 3,7$), Prejuízo Moderado/PM ($N=05$; $M=6,8 \pm 1,8$); e avaliadas no Laboratório de Neuropsicologia da UNESP/Assis. O grupo PM obteve escores um desvio-padrão abaixo da média da amostra no TA.

Resultados: No escore total da ZAREKI-R, o teste t identificou efeito de grupo ($t=5,16$; $p<0,0001$), no qual o escore do grupo PL ($M=122,8 \pm 22,1$) foi maior que o do grupo PM ($M=88,8 \pm 24,1$). A MANOVA evidenciou efeito de grupo entre os subtestes da ZAREKI-R [$F(12,17)=2,33$; $p=0,05$] em que, conforme o teste post-hoc de Tukey, o grupo PM foi prejudicado nos subtestes Ditado de Números ($p=0,01$), Cálculo Mental ($p=0,01$), Leitura de números ($p=0,002$) e Memória de Dígitos ($p=0,019$). Houve correlação moderada entre o TA e o ZAREKI-R (Pearson; $r=0,62$).

Conclusão: Houve uma associação positiva entre o ZAREKI-R e o TA, entretanto, além de identificar a dificuldade aritmética (cálculo mental), a ZAREKI-R identificou também dificuldades associadas ao processamento numérico e à memória operacional fonológica do grupo PM. Portanto, a ZAREKI-R identificou um perfil quantitativo e qualitativo distinto entre os grupos e caracteriza-se como um instrumento mais apropriado para a avaliação das habilidades matemáticas e da discalculia do desenvolvimento.

Referências: 1- Stein, 1993; 2-von Aster & Dellatolas, 2006.

Palavras-Chave: ZAREKI-R; Habilidades Matemáticas; Transtorno de Aprendizagem; Avaliação Neuropsicológica

Apoio Financeiro: FAPESP processo nº 2005/60375-1.

F.03.001

MIDAZOLAM REDUCES FOS LABELING IN THE MEDIAL PREFRONTAL CORTEX AND AMYGDALA OF RATS SUBMITTED TO THE RETEST SESSION IN THE ELEVATED PLUS-MAZE

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The anxiolytic effects of benzodiazepines are reduced after a single exposure of rats to the elevated plus-maze test (EPM). Midazolam showed an anxiolytic profile in animals submitted to one session, but did not change the usual exploratory behavior of rats exposed twice to the EPM. The retest session is characterized by the emergence of a fear factor, more powerful risk assessment and the activation of distinct encephalic areas. OBJECTIVES: The purpose of this study was to investigate the effects of the benzodiazepine midazolam in telencephalic structures activated in rats submitted to retest sessions of the EPM. METHODS: Wistar rats received an i.p. injection of saline or midazolam 0.5 mg/kg and they were submitted once or twice (intertrial interval of 24 h) to the EPM. Two hours after the test, the brains were removed, sliced and processed free-floating for Fos immunohistochemistry analyses. RESULTS: Fos expression was markedly increased in medial prefrontal areas and amygdala during the retest session (PrL: 44.83±6.8; IL: 28.45±6.0; Cg1: 70.28±5.9; Cg2: 37.51±8.8) when compared to the first exposure (PrL: 21.38±5.2; IL: 13.57±3.3; Cg1: 60.81±7.7; Cg2: 17.25±4.1) and midazolam injected before the retest produced a significant reduction of Fos labeling in these areas (PrL: 20.84±3.1; IL: 15.38±2.7; Cg1: 57.28±4.6; Cg2: 18.72±2.3). CONCLUSIONS: These findings present more arguments for the dissociation between the anxiolytic effects of benzodiazepines and the cognitive activity of rats submitted to the retest session in the EPM.

Palavras-Chave: fos expression, retest session, elevated plus-maze, medial prefrontal cortex, amygdala

Apoio Financeiro: CNPq

Categorização semântica e visual de fotografias com conteúdo emocional

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A normatização de materiais tanto verbais (e.g., listas de palavras) quanto não-verbais (e.g., figuras) fornece ao pesquisador uma maneira de selecionar estímulos com características específicas e que lhe permitem evidenciar relações de causa e efeito entre variáveis de interesse. Um material muito utilizado em estudos envolvendo emoção e neuropsicologia em diversos países é o *International Affective Picture System* (IAPS) (Lang, Bradley & Cuthbert, 1999), um conjunto normatizado com mais de mil fotografias capazes de induzir uma gama de estados emocionais que variam em valência e alerta. Embora várias fotos do IAPS estejam relacionadas a um mesmo tema (e.g., bebês), nenhum estudo havia sido realizado com o intuito de agrupar as imagens em categorias consistentes, como já existe com palavras semanticamente associadas (Stein, Feix & Rohenkohl, 2001). Portanto, o objetivo do presente trabalho foi obter categorias associadas emocional, semântica e visualmente para parte das fotos do IAPS. Primeiramente, dois juízes realizaram a seleção das fotos baseados na temática e semelhança das mesmas, de forma que 20 grupos de oito fotos cada foi criado. A amostra de 396 estudantes ($m = 23,5$ anos de idade) e ambos os sexos avaliou a emocionalidade e o nível de associação semântica e visual, nomeou cada um dos 20 grupos. Após, hierarquizaram as fotografias conforme o nível de representatividade dentro do grupo ao qual pertenciam, obtendo, assim, uma espécie de protótipo. Os resultados apontam que mais da metade das categorias selecionadas para avaliação apresentaram significativo grau de associação interna. Esse trabalho pretende oferecer à comunidade científica fotos associadas e padronizadas do IAPS que poderão ser usadas de várias formas em investigações envolvendo emoção e cognição.

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Palavras-Chave: emoção; memória; imagens; categorização; IAPS.

Apoio Financeiro: Cnpq

EFEITO DO ENRIQUECIMENTO AMBIENTAL NO TRAÇO ANSIOSO DE RATOS ADULTOS

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Objetivo: A resposta comportamental de animais de experimentação ao enriquecimento ambiental pode ser de grande valor científico para o entendimento da neurobiologia dos transtornos psiquiátricos, que demonstram um alto nível de complexidade em relação a fatores genéticos, ambientais e suas interações. O papel da estimulação ambiental na redução do traço ansioso de animais mantidos em ambiente enriquecido desde o nascimento já foi demonstrado (Behav. Genet., 29:41, 1999). Sabendo-se que o cérebro adulto é capaz de responder plasticamente a estímulos ambientais (Nature 386:493, 1997), o objetivo deste estudo foi avaliar a possível influência do enriquecimento ambiental no traço ansioso de animais submetidos a esta condição já na fase adulta.

Métodos e Resultados: Quarenta ratos Wistar machos, adultos (10 semanas) foram avaliados no Paradigma da Exploração Livre (PEL) para que fossem categorizados de acordo com seus níveis de ansiedade-traço: alto (< 1º quartil), médio (>1º quartil e <3º quartil) e baixo (>3º quartil), através do parâmetro “porcentagem de tempo no lado novo”. Os resultados apresentaram-se assim: mediana = 82,3; 1º quartil = 75,3; 3º quartil = 84,8. Após esta avaliação, os animais foram alocados em dois grupos, sendo que um passou as três semanas seguintes em um ambiente enriquecido (AE) e outro, em ambiente padrão (AP). A seguir, cada indivíduo foi novamente submetido ao PEL. Os resultados revelaram que o AE diminuiu significativamente os níveis de ansiedade dos indivíduos com alto traço ansioso (média ± dp: AE= 84,3 ± 6,8; AP= 56,0 ± 39,4; p< 0,01), mas não dos animais com média (média ± dp: AE= 84,3 ± 5,5; AP= 84,1 ± 6,8; p> 0,05) ou baixa ansiedade-traço (média ± dp: AE= 88,7 ± 4,7; AP= 80,7 ± 10,6; p> 0,05).

Conclusão: Os resultados obtidos indicam que a plasticidade do cérebro adulto permite que um indivíduo responda às alterações do ambiente modificando até características de personalidade, como o traço ansioso. O efeito significativo foi observado nos indivíduos com alta ansiedade-traço, característica dos pacientes com transtornos de ansiedade, sugerindo que terapias ocupacionais possam ser desenvolvidas para o tratamento destes quadros.

Palavras-Chave: ansiedade-traço, enriquecimento ambiental, ratos.

Apoio Financeiro: PIBIC / CNPq

F.03.004

INFLUENCIA DO NÍVEL EDUCACIONAL E GÊNERO NA AVALIAÇÃO DA VALÊNCIA E DA ATIVAÇÃO DE IMAGENS EMOCIONAIS

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Objetivo:

Pesquisas em neurobiologia da emoção utilizam amplamente fotos afetivas e neutras do *international affective picture system* (IAPS). Uma maneira de acessar a eficiência destes estímulos em ativar os sistemas defensivo e apetivo é através da escala psicométrica, *Self-Assessment Manikin* (SAM). O objetivo deste trabalho é investigar, utilizando o SAM, se nível educacional e gênero influenciam a avaliação da valência hedônica e da ativação emocional associada à visualização de várias imagens do IAPS.

Métodos:

217 voluntários (97 homens, idade entre 18 a 24 anos), de 2 níveis educacionais (ensino fundamental e superior) avaliaram 65 fotografias utilizando o SAM. As imagens foram de 3 categorias: Negativa, Neutra e Positiva.

Resultados:

Realizamos ANOVAs com medidas repetidas para o fator categoria (negativa, neutra e positiva), tendo como variáveis independentes o gênero (masculino e feminino) e o nível educacional (fundamental e superior). Não houve interação entre valência e nível educacional. Observamos uma interação tripla entre valência, gênero e nível educacional ($F_{(2,426)} = 6,1382$, $p=0,023$). Mulheres com alto nível educacional relataram mais negatividade para os estímulos aversivos ($p=0,002$) e mais positividade para os estímulos agradáveis ($p=0,015$) quando comparado aos homens. Verificamos uma interação entre ativação e escolaridade ($F_{(2,422)} = 9,3056$; $p=0,0001$) em que a categoria positiva foi avaliada como menos ativante pelos indivíduos de baixo nível educacional comparado a voluntários de alto nível educacional ($p=0,0005$).

Conclusão:

Esses resultados mostram que as mulheres com alto nível educacional são mais reativas aos estímulos emocionais utilizados do que os homens. A avaliação de valência mostrou que o nível educacional não foi um fator que influenciou nos relatos, demonstrando que a escala foi entendida por todos os voluntários e que os estímulos têm impacto emocional similar independente da escolaridade. Para ativação houve diferença entre os níveis educacionais, indicando uma menor ativação pelos estímulos agradáveis nos indivíduos de baixa escolaridade.

Palavras-Chave: IAPS, Relato Avaliativo, Emoção

Apoio Financeiro: PRONEX/FAPERJ, CNPq, CAPES, IBN-net/FINEP, MCT/CNPq Projetos Institutos do Milênio

CONDITIONED FREEZING RESPONSE INCREASED SEROTONERGIC ACTIVITY IN THE AMYGDALA BASOLATERAL NUCLEUS.

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Amygdala and the dorsal periaqueductal gray (dPAG) are brain areas involved in the regulation of unconditioned and conditioned reactions to threatening stimuli. It has been proposed a dual role of serotonin (5-HT) in the mediation of these defensive responses: 5-HT inhibits the expression of unconditioned responses in the dPAG and facilitates conditioned defensive reactions on structures such as the amygdala. **OBJECTIVES:** To investigate the activity of serotonergic neurons of the amygdala basolateral nucleus (BLA) on the unconditioned response induced by aversive chemical stimulation of the dPAG with semicarbazide (SEM, 5 µg/0.2 µl), an inhibitor of the GABA synthesizing enzyme. We also examined the activity of the BLA serotonergic neurons on the contextual conditioned freezing using foot shocks as unconditioned stimulus (US). **METHODS:** Animals were submitted to microdialysis procedure and received intra-dPAG injections of SEM or saline (SAL) to verify the content of 5-HT and its metabolite 5-hydroxyindole acetic acid (5-HIAA) in the BLA. Next, the microdialysis procedure was also used in animals submitted to the contextual fear-conditioning test. In both studies, samples were collected every 30 min and analyzed for the content of 5-HT and 5-HIAA using high-performance liquid chromatography. **RESULTS:** The unconditioned freezing behavior induced by aversive chemical stimulation of the dPAG did not alter neither intra-BLA 5-HT content [% 5-HT extracellular (mean±S.E.M): SAL: 91.3±9.8; SEM: 113.9±11.38] nor 5-HIAA content [SAL: 86.9±5.5; SEM: 91.9±12.97]. However, animals displaying an increased conditioned freezing response to contextual cues [mean±S.E.M, in seconds; control group (CON): 48.0±6.6; conditioned group (CDT): 200.9±19.2] showed an increase of 5-HT level [% 5-HT extracellular - CON: 113.30±13.17; CDT: 290.45±43.16], but not of the 5-HIAA level (CON: 76.0±16.5; CDT: 65.9±10.37) in the BLA. **CONCLUSIONS:** Our results indicate that the facilitation of the conditioned fear response, but not of the unconditioned fear response, is related to an increase of the serotonergic neurotransmission in the BLA. **Palavras-Chave:** microdialysis procedure, high-performance liquid chromatography, conditioned fear, unconditioned fear, basolateral nucleus of the amygdala.

Apoio Financeiro: FAPESP

F.03.006

Psicofisiologia da Emoção e Novas Advertências para Maços de Cigarro

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Objetivos: Estudos de emoção revelam que imagens altamente aversivas ativam áreas cerebrais envolvidas no controle de atitudes e comportamentos de esquiva. Neste trabalho, empregamos dados de psicofisiologia da emoção para a elaboração e seleção de um novo grupo de imagens para advertências sanitárias de produtos tabagistas.

Métodos: Jovens (N=338; 18-24 anos; 3 níveis educacionais; 43% de fumantes; 50% mulheres) avaliaram os protótipos de imagens para advertência sanitárias utilizando uma escala psicométrica (Self-Assessment Manikin), utilizada em diversos estudos de neurobiologia da emoção. Esta avalia 2 dimensões afetivas: valência hedônica e ativação emocional. Utilizou-se como controle imagens agradáveis, neutras e desagradáveis retiradas de um catálogo padronizado (International Affective Picture System). Os voluntários não tinham conhecimento de que as imagens seriam utilizadas em maços de cigarro.

Resultados: A escolha de imagens chocantes foi baseada em estudos de reações fisiológicas relacionadas ao comportamento de afastamento. Comparados com as imagens já utilizadas nas embalagens, os protótipos foram avaliados como mais aversivos (menor valência: MWW Test $Z[212,119]=7,253$, $p<0,001$; maior ativação: MWW Test $Z[212,119]=-3,038$, $p=0,002$). Mulheres (valência: MWW Test $Z[168,170]=2,058$, $p=0,039$) e participantes de nível educacional mais baixo, (valência: Kruskal-Wallis $H(2,337)=6,354$; $p=0,041$) consideraram os protótipos estatisticamente mais desagradáveis, comparadas aos homens e jovens de maior nível educacional, respectivamente. Fumantes e não fumantes não se diferenciaram na avaliação dos protótipos. (Valência: MWW Test $Z[144,193]=1,096$; $p=0,27$; Ativação: MWW Test $Z[144,193]=1,372$; $p=0,17$).

Conclusões: Os resultados embasam a seleção e implementação das novas imagens de advertência sanitária para embalagens de produtos tabagistas no Brasil, contribuindo para os objetivos de prevenção e cessação de fumar. Os dados também sugerem que os efeitos serão ainda mais significativos para jovens do sexo feminino e aqueles com baixa renda, estratos mais atingidos pelo marketing da indústria e alvo especial das ações de saúde para o controle do tabagismo.

Palavras-Chave: Emoção, Psicofisiologia, Tabagismo, Advertências Sanitárias

Apoio Financeiro: CNPq, INCA, FINEP, FAPERJ

F.03.007

Aspectos comportamentais e hipocampais de ratos selecionados para o medo condicionado

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A seleção de ratos pelo paradigma do medo condicionado possibilita o estudo de aspectos comportamentais e neurofisiológicos característicos dos traços de medo. Este trabalho objetivou investigar os comportamentos de duas linhagens de ratos, com alta (Cariocas com Alto Congelamento, CAC) e baixa (Cariocas com Baixo Congelamento, CBC) resposta de congelamento a estímulos contextuais previamente associados a choques elétricos e o número de células do hipocampo, já que esse participa da neurobiologia do aprendizado contextual. Foram utilizados os testes de ansiedade do labirinto em cruz elevado (LCE; n=16 por grupo) e de interação social (n=6 pares por grupo) e o teste de depressão do nado forçado (n=9 por grupo). Os resultados mostram que os CAC apresentam número menor de entradas nos braços abertos ($30\% \pm 3,6$ CBC; $16\% \pm 3,8$ CAC; $p < 0,05$), sem diferenças no número de entradas nos braços fechados. O tempo nos braços abertos (15 ± 3 s CBC; $6 \pm 2,5$ s CAC; $p < 0,05$) foi menor do que os CBC. No teste de interação social os CAC gastaram menos tempo do que os CBC em interação (169 s CBC; 70 s CAC; $p < 0,05$), sem diferenças na ambulação. Por fim, não houve diferença no teste do nado forçado. Para análise do número de células hipocampais, os animais foram perfundidos, os cérebros fixados e seccionados ($40\mu\text{m}$) em criostato. Os cortes eram tratados com o marcador nuclear fluorescente 4',6-diamidino-2-fenilindol (DAPI) e visualizados em microscópio de fluorescência. A análise não revelou diferenças no número de células hipocampais (giro denteado e áreas CA1 e CA3) entre os grupos. Esses resultados, embora preliminares, mostram que as linhagens obtidas representam modelo consistente para o estudo do medo, já que as diferenças entre elas se mantêm em outros testes de ansiedade; não se devem a diferenças locomotoras, como mostram os dados de entrada nos braços fechados do LCE e os de ambulação, e não se estendem a sistemas emocionais diversos, como a depressão. Já as diferenças neurofisiológicas entre os grupos possivelmente se devem a aspectos mais específicos que o número de células no hipocampo. Alterações serotoninérgicas e/ou GABAérgicas nessa estrutura serão futuramente avaliadas.

Palavras-Chave: medo condicionado; comportamento; hipocampo

Apoio Financeiro: FAPERJ, CNPQ e PRONEX

F.03.008

Efeitos da omissão de reforço em ratos submetidos a esquemas de tempo fixo com sinalização exteroceptiva

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Objetivos: Examinar os efeitos da omissão do reforço (OR) sobre o repertório comportamental de ratos submetidos a esquemas em tempo fixo (TF) com sinalização exteroceptiva (Si) do reforço.

Método: 24 ratos foram submetidos a 90 sessões de treino com liberação de reforço (R) em 100% das práticas. Cada prática consistia na apresentação do Si no final do TF, sendo R liberado no último segundo do Si. Os ratos foram divididos em 6 grupos: TF30Si10, TF60Si20, TF120Si40; TF30Si20; TF60Si40; TF120Si80 (durações dos eventos em segundos). Nos 3 primeiros grupos o Si ocupou 1/3 do TF e nos outros, 2/3. Em seguida, foram realizadas 4 sessões com 50% das práticas não-reforçadas (N). Registrou-se 9 categorias do repertório comportamental do animal.

Resultados: As frequências dos comportamentos, distribuídas em médias (M) mostram que nas sessões de OR há um decréscimo na frequência de lambe o bebedouro após N quando comparada às frequências após R (MR: 59,3; MN: 25,9). Por outro lado, as taxas de farejar o bebedouro e farejar na região do bebedouro foram maiores após N que após R (MR: 13,9; MN: 24,9; MR: 46,6 e MN: 61,8; respectivamente, para os grupos Si 1/3 e Si 2/3. Além disso, a frequência de lambe o bebedouro após N é mais baixa no TF longo do que no TF curto (MN: 21,6; MN: 14,1; 30 e 120s, respectivamente). Nos TFs de curta duração, a frequência de farejar distante do bebedouro foi maior após N que após R (MR: 3,3; MN: 16,1), entretanto apenas para os grupos 1/3 Si.

Conclusão: A OR afeta diferentemente a organização do repertório comportamental de ratos, dependendo da relação temporal entre sinais exteroceptivos e a liberação do reforço. O efeito de OR no decorrer do TF é modulado pela duração do TF e pela proporção que o sinal exteroceptivo ocupa no intervalo entre reforços.

Palavras-Chave: Omissão do Reforço, Sinalização, Repertório Comportamental

Apoio Financeiro: CNPq e CAPES

F.03.009

Efeito do aroma de laranja doce sobre níveis de ansiedade em ratas wistar.

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Objetivos: A aromaterapia é o uso de óleos essenciais como terapia complementar empregada com a finalidade de proporcionar conforto e diminuir estresse, apesar da insuficiente comprovação científica. Fragrâncias cítricas têm sido particularmente usadas por aromaterapeutas para o tratamento dos sintomas da ansiedade. De fato, o aroma de laranja doce já demonstrou, em modelos animais de ansiedade, efeito ansiolítico em ratos machos. A fim de verificar se este efeito também estaria presente no sexo feminino, o objetivo do presente estudo foi investigar o potencial efeito ansiolítico do óleo essencial de *Citrus sinensis* (laranja doce) em ratas.

Métodos e Resultados: 95 ratas Wistar foram primeiramente monitoradas em relação às fases do ciclo estral (metaestro/diestro, proestro e estro). A seguir, representantes de cada fase do ciclo (n= 6-9) foram colocadas individualmente numa câmara de acrílico, onde foram expostas ao aroma de laranja (0, 100, 200 ou 400 ml = Cs0, Cs100, Cs200 ou Cs400) por cinco minutos. Imediatamente após este período, foram submetidas ao teste do Labirinto em Cruz Elevado, onde foram avaliadas Porcentagem de Tempo nos Braços Abertos (%TBA), Porcentagem de Entradas nos Braços Abertos (%EBA) e Distância Total percorrida no aparato (DT). Considerando DT como covariada, os resultados mostraram uma interação entre o tratamento e a fase do ciclo estral, sendo que Cs100 foi ansiolítico no estro ($\xi \pm dp$: %TBA: Cs0= 14,1 $\pm$ 10,3; Cs100= 26,4 $\pm$ 19,5; %EBA: Cs0= 28,1 $\pm$ 11,5; Cs100= 39,1 $\pm$ 5,4), ansiogênico no metaestro/diestro ($\xi \pm dp$: %TBA: Cs0= 28,0 $\pm$ 20,9; Cs100= 14,7 $\pm$ 5,9; %EBA: Cs0= 37,2 $\pm$ 11,6; Cs100= 23,2 $\pm$ 6,3) e sem efeito no proestro ($\xi \pm dp$: %TBA: Cs0= 21,0 $\pm$ 13,8; Cs100= 10,0 $\pm$ 4,4; %EBA: Cs0= 28,6 $\pm$ 14,3; Cs100= 27,3 $\pm$ 11,6), enquanto Cs200 não apresentou efeito em nenhuma fase e Cs400 foi ansiogênico no metaestro ($\xi \pm dp$: %TBA: Cs0= 28,0 $\pm$ 20,9; Cs400= 14,7 $\pm$ 10,1; %EBA: Cs0= 37,2 $\pm$ 11,6; Cs400= 20,5 $\pm$ 11,5).

Conclusões: Os resultados apresentados mostraram que o efeito do aroma de laranja doce pode variar de ansiolítico a ansiogênico de acordo com o ciclo ovariano, sugerindo que a aromaterapia deva ser administrada a mulheres com cautela.

Palavras-Chave: Ansiedade; aromaterapia; citrus sinensis; ratas; ciclo estral; labirinto em cruz elevado

F.03.010

Behavioral evaluations in mice treated chronically with nevirapine and efavirenz.

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Objectives: Evaluate the effects of chronic administration of NVO and EFV on behaviors linked to anxiety and depression in mice. **Methods:** In the present study, 3 groups of 20 mice CF-1 male adult HIV non-infected were treated orally, once a day for 36 days, with EFV 10 mg/kg, NVO 3.3 mg/kg or vehicle in a volume of 1 mL/kg. Behavioral tests were conducted one hour after drug injection. On day 28, 32 and 36 of drug treatment, mice were subjected to the elevated plus-maze test, open-field test, and forced swimming test, respectively. Results were analysed by Graphpad INSTATO version 3.0 software. The data from the behavioral tests are reported as means $\pm$ SEM, and were analyzed by one-way ANOVA followed by Dunnett's test. Differences were considered statistically significant when $p < 0.05$. **Results :** Animals treated with EFV, but not NVO, showed significant reduction on the frequency of entries and time spent in open arms of the elevated plus-maze test compared to control group. On the forced swimming test, treatment with EFV and NVO did not cause changes on the immobility time of the animals when compared to control group. Finally, administration of NVO and EFV did not change in a significant way the animals locomotor activity. **Conclusions:** The results obtained suggests that in mice the chronic treatment with EFV, on doses equivalent to those employed to human therapy, shows an effect like anxiogenic, but no behavioral change was observed after chronic treatment with NVO. **Palavras-Chave:** behavior, antiretroviral drugs; efavirenz; nevirapine;

Apoio Financeiro: UNESC, FAPESC, CNPq

F.03.011

APOIO SOCIAL E EMOÇÃO POSITIVA ATENUAM O REFLEXO DE SOBRESSALTO

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O reflexo de sobressalto é uma resposta defensiva a um estímulo sensorial intenso e abrupto. Este reflexo é influenciado por fatores externos e internos e é avaliado experimentalmente como um índice da modulação do sistema defensivo. Os vínculos sociais são essenciais à sobrevivência e importantes recursos de regulação emocional. No presente estudo investigamos se o grau de apoio social e a pré-ativação com estímulos emocionais podem influenciar na habituação do reflexo de sobressalto. Os participantes (N=81, 60 mulheres) visualizaram fotos agradáveis de bebês e famílias ou fotos desagradáveis de corpos mutilados. Em seguida todos realizaram uma tarefa de estresse psicológico e foram expostos a dois ruídos abruptos e intensos com intervalo de 12 min. Uma escala padronizada estimou o grau de apoio social e o escore médio foi utilizado para subdividir a amostra nos grupos de alta e baixa pontuação. O sobressalto era mensurado pela magnitude do reflexo de piscar através da eletromiografia do músculo orbicular do olho. A análise de variância (ANOVA) mostrou interação entre apoio social e pré-ativação emocional [$F_{(1,77)} = 3,81$; $p=0.05$]. Independente da pré-ativação emocional, os indivíduos com baixo apoio social não habituarão o reflexo de piscar ao segundo ruído (média ruído 1= 48.7 μ s; média ruído 2= 48.9 μ s; $p=0.97$). Os indivíduos com alto apoio social e que viram fotos agradáveis apresentaram uma diminuição significativa na magnitude do reflexo de piscar ao segundo ruído (média ruído 1= 74.9 μ s; média ruído 2= 58.7 μ s; $p=0.02$). Concluimos que a associação de fatores externos e internos de vinculação social contribui significativamente para uma maior capacidade de regulação emocional.

Palavras-Chave: reflexo de sobressalto, regulação emocional, apoio social, IAPS

Apoio Financeiro: PRONEX-FAPERJ#E-26/171541/2006; CNPq # 420122/2005-2, FINEP Rede Instituto Brasileiro de Neurociência# 01.06.0842-00 e CAPES/DGU #086-05

F.03.012

Substance P injections into the dorsal hippocampus decrease the contextual conditioned fear.

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Substance P (SP) is found in brain regions associated with fear/ anxiety such as the amygdala, hypothalamus, periaqueductal gray and hippocampus. The hippocampus has been related with cognitive and emotional processes. Recent study showed that the SP injection into the dorsal (DH), but do not into the ventral (VH), hippocampus caused an “anxiolytic-like” effect as measured in the elevated plus maze and open field tests without changing the general motor activity. OBJECTIVE: Considering that hippocampal SP mechanisms are included in the unconditioned fear, the aim of the present study was to investigate whether SP injections into the DH and VH affect the conditioned contextual fear. RESULTS: SP injections into the DH significantly decreased the conditioned freezing behavior [Control = 249.55 ± 42.11 ; SP 100 ng/ 0.5 μ L = 123.90 ± 14.72 ; SP 1000 ng/ 0.5 μ L = 167.60 ± 38.44], differently of SP injections into the VH [Control = 296.87 ± 33.85 ; SP 100 ng/ 0.5 μ L = 260.90 ± 23.57 ; SP 1000 ng/ 0.5 μ L = 250.6 ± 48.19]. CONCLUSIONS: The data show a differential involvement of the VH and DH in mechanisms associated with anxiety/defense. Moreover, the present results suggest that SP mechanisms of the DH are implicated in the expression of the conditioned contextual fear.

Palavras-Chave: dorsal hippocampus, ventral hippocampus, contextual fear, defensive reactions, anxiety

Apoio Financeiro: CAPES

Efeitos do envenenamento com chumbo sobre a preferência claro-escuro no Tambaqui (*Colossoma macropomum*)

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OBJETIVO

Verificar os efeitos do chumbo sobre o comportamento da espécie *Colossoma macropomum* em um teste de preferência claro-escuro.

MÉTODOS

Sessenta peixes da espécie *C. macropomum* (EMBRAPA, AM) foram submetidos a três sessões de exploração em aquários de vidro, providos de uma área central de 5 cm (compartimento inicial), delimitada por portas removíveis. Após 3 minutos de habituação, as portas foram suavemente removidas, dando acesso a ambos os compartimentos, permitindo ao animal explorar livremente o ambiente durante um período de 15 minutos em uma de três configurações: aquário todo branco, todo preto, ou meio preto/meio branco. Os animais foram divididos em grupos conforme a dose de chumbo intraperitoneal (acetato de chumbo, nas doses de 0, 30, 45, 60, 75 e 90 mg/Kg). Cada animal foi submetido a todas as condições. O tempo total, porcentagem de tempo e número de alternâncias (cruzamentos da linha central) foram registrados.

RESULTADOS

A análise estatística dos dados da porcentagem de tempo (ANOVA) indicam que o animal tem preferência pelo lado escuro [$F(1,324)=8,518$, $p<0,001$], sem influência do envenenamento sobre a preferência, porém com efeitos sobre a motilidade evidenciada pela alteração nas alternâncias [$F(10,323)=3,120$ $p<0,001$]. Os resultados indicam ainda uma interação entre a presença do estímulo aversivo como escolha (caixa branco/preto) sobre a taxa de exploração entre os grupos. O pós-teste (Tukey) indicou diferenças entre o controle e todas as doses nos parâmetros descritos.

CONCLUSÃO

O animal tem preferência pelo escuro e mesmo quando submetido ao chumbo, essa preferência não é alterada. O metal altera a motricidade do animal de forma dose-dependente e esta é inibida pela presença do estímulo aversivo ao claro em uma situação de contraste. **Palavras-Chave:** comportamento, preferência, tambaqui, chumbo

Apoio Financeiro: FAPEAM

**INFLUÊNCIA DA ANSIEDADE SOBRE AS RESPOSTAS COMPORTAMENTAIS
NOCICEPTIVAS EM UM MODELO DE DOR OROFACIAL.**

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Objetivo: Verificar a influência da ansiedade-traço sobre a dor orofacial e desta sobre a ansiedade-estado.

Métodos e Resultados: Quarenta e quatro ratos Wistar adultos foram inicialmente avaliados através do Paradigma de Exploração Livre (PEL) para verificação do perfil de ansiedade. Foram considerados com alto traço de ansiedade os animais com valores de porcentagem no ambiente não-familiar inferiores ao 1º quartil (47,2%), e com baixo traço ansioso os animais com valores superiores ao 3º quartil (81,1%). Numa segunda etapa, após o PEL, foi administrada aos animais, formalina-1,5% na região do lábio superior. A resposta comportamental nociceptiva caracterizada pelo aumento da frequência de coçar a região orofacial (CO) foi quantificada por 30 minutos da seguinte forma: Frequência total de CO durante os 30 minutos, Frequência de CO no intervalo de 0-6 minutos (1ª fase–Dor neurogênica), Frequência de CO no intervalo de 12-30 minutos (2ª fase–Dor inflamatória). Imediatamente após o teste de dor orofacial, mas ainda sob o efeito da formalina, foi verificado o estado de ansiedade através do teste do Labirinto em Cruz Elevado (LCE). Os resultados mostraram que, considerando-se o período total de avaliação da dor ou apenas a 2ª fase do teste, os animais com alto perfil de ansiedade apresentaram um aumento da frequência de CO (média ± dp: Total = 326,1% ± 179,4%; 2ª Fase: 427,8% ± 243,9%) estatisticamente superior ao do grupo com baixo perfil de ansiedade (média ± dp= 189,2% ± 163,1%; 2ª Fase: 254,1% ± 222,3%), o que não aconteceu durante a 1ª fase do teste (média ± dp: Alta ansiedade = 288,3% ± 278,4%; Baixa Ansiedade: 214,8% ± 189,2%). Além disso, não foi verificada correlação entre dor, avaliada pelo aumento da frequência de CO nos 30 minutos (média ± dp: 257,6 ± 181,4), e estado de ansiedade, avaliado pela porcentagem de tempo nos braços abertos (11,3 ± 10,8).

Conclusão: Os animais com alto perfil de ansiedade foram mais susceptíveis à dor orofacial, entretanto, a dor não influenciou a ansiedade-estado.

Palavras-Chave: ansiedade, dor orofacial, ratos.

F.03.015

O Efeito Ansiolítico e a Atoxicidade Aguda da *Erythrina velutina*: Uma Planta Endêmica do Nordeste Brasileiro.

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Objetivos

O presente estudo teve por objetivos avaliar, em animais de laboratório, os possíveis efeitos ansiolíticos e tóxicos da administração aguda do extrato aquoso (EA) de folhas de *Erythrina velutina*, uma planta utilizada popularmente no Brasil como tranqüilizante.

Métodos e Resultados

Para a avaliação do efeito ansiolítico foram utilizados ratos Wistar, adultos, machos, submetidos ao teste do Labirinto em Cruz Elevado, onde foram avaliados os seguintes parâmetros: Porcentagem de Tempo nos Braços Abertos (%TBA), Porcentagem de Entradas nos Braços Abertos (%EBA) e Distância Total percorrida no aparato (DT). Foram testadas agudamente, por via intraperitoneal, as doses de 0, 10, 20, 40, 80, 160 e 320 mg/kg do EA (n= 11-15/grupo), sendo que somente as três últimas apresentaram efeito ansiolítico/sedativo ($x \pm ep$: %TBA: EA0= $5,77 \pm 1,57$; EA80= $10,70 \pm 2,68$; EA160= $11,20 \pm 3,05$; EA320= $7,74 \pm 2,08$ / %EBA: EA0= $17,13 \pm 4,44$; EA80= $27,01 \pm 4,91$; EA160= $33,29 \pm 6,59$; EA320= $33,94 \pm 6,94$ / DT= covariada). Para a avaliação toxicológica, ratos Wistar, adultos, machos e fêmeas foram tratados agudamente por via oral com a dose limite de 5 g/kg do EA (n=12) ou com água (n=12) e observados por 14 dias consecutivos. Nenhum animal veio a óbito e nenhum sinal de toxicidade foi detectado nas observações comportamentais ou nas autópsias.

Conclusões

Estes resultados demonstram que o extrato aquoso de *Erythrina velutina* possui atividade ansiolítica aguda e uma razoável atoxicidade, trazendo algum apoio científico para o uso desta planta como calmante natural pela medicina popular.

Palavras-Chave: *Erythrina velutina*, ansiedade, toxicidade aguda, ratos.

F.03.016

Does neonatal handling influence aversive emotions triggered by surprising reward loss?

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OBJECTIVES: The purpose of the present work was to explore whether neonatal handling could influence the state of frustration induced by two successive negative contrast (SNC) situations, in which the magnitude of an expected reward was suddenly reduced. **METHODS.** In Experiment 1, handled (H) and non-handled (NH) rats were first tested in the elevated plus maze to have a measure of the characteristic effects of that treatment on fearfulness. In Experiment 2, food-deprived rats were exposed during five minutes to different sucrose concentrations: 32% in pre-shift phase (ten days) and 4% in post-shift phase (six days); the amount of solution consumed by these two groups was compared to controls, who received the 4% solution throughout the training. In Experiment 3, the rats were exposed to a straight alley, recording the latency between leaving the start box and reaching the food available in the goal box at the end of the alley; to induce the SNC effect the amount of reinforcement went from 12 pellets in the pre-shift phase (10 sessions of 6 trials) to 2 pellets in the post-shift phase (8 sessions of 6 trials), whereas control groups received 2 pellets throughout the experiment. **RESULTS.** Though NH rats were more fearful than their H counterparts in the elevated plus maze (time in the open arms: 36 s vs. 69 s, respectively), they did not differ in both consummatory and instrumental SNC experiments, relative to their corresponding controls. **CONCLUSION.** These results suggest that neonatal handling could not attenuate frustrative non-reward (at least) under food-deprivation conditions.

Palavras-Chave: neonatal handling, frustration, reward, elevated plus maze, successive negative contrast

Apoio Financeiro: by MCYT, Ministerio de Ciencia y Tecnología (SEJ2004-03231/PSIC) and by Universidad de Málaga (Proyecto Puente 2006-2007, Modalidad B).

F.03.017

Atualização das Normas Brasileiras para o International Affective Picture System (IAPS): um novo estudo comparativo das avaliações afetivas entre sujeitos brasileiros e norte-americanos.

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Objetivo: Este trabalho apresenta as normas brasileiras de 240 novos estímulos do *International Affective Picture System* (IAPS), um instrumento de imagens afetivas largamente utilizado em pesquisas, e estabelece comparações com os valores da normatização americana. **Métodos:** seguindo os mesmos padrões do estudo original, 448 estudantes universitários brasileiros (269 mulheres e 179 homens) avaliaram as fotografias do IAPS através das dimensões prazer, alerta e dominância. Os dados foram comparados entre as populações por correlações lineares de Pearson e testes t de Student. **Resultados:** As correlações foram altamente significativas para todas as dimensões, porém as médias de alerta dos brasileiros foram mais elevadas que as dos norte-americanos. **Conclusões:** Os resultados mostram estabilidade com a primeira parte da normatização brasileira e boa consistência com os valores da normatização americana, a despeito de pequenas diferenças concernentes à interpretação da dimensão alerta. Fica demonstrado que o IAPS é um instrumento confiável para estudos experimentais na população brasileira. **Palavras-Chave:** Emoções manifestas, reconhecimento visual, percepção visual, comparação transcultural, características sexuais, técnicas projetivas/normas

Apoio Financeiro: FAPESP e AFIP

MODULAÇÃO DAS RESPOSTAS SACÁDICAS: EFEITOS DA VISUALIZAÇÃO PRÉVIA DE FIGURAS EMOCIONAIS

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Objetivo: Trabalhos anteriores mostraram que o tempo de reação manual e sacádico (TRS) são influenciados pela visualização de figuras negativas em relação às figuras neutras, mesmo quando estas não são importantes na realização da tarefa. Neste estudo avaliaremos se o conteúdo emocional da figura apresentada no ensaio anterior à coleta da resposta ocular também é capaz de interferir na latência das respostas sacádicas.

Métodos: 32 alunas da Universidade de Granada (Espanha) participaram do experimento consistindo de 3 sessões de 106 testes. Elas deveriam manter o olhar num ponto de fixação localizado no centro de uma tela e mover os olhos, o mais rapidamente possível, para um alvo visual que poderia ocorrer à direita ou à esquerda. O alvo era precedido pelo aparecimento de uma figura negativa (corpos mutilados) ou neutra (pessoas) no centro da tela. Esta poderia permanecer acesa por 800ms até o acender do alvo (condição “overlap” – “ovr”) ou permanecer por 600ms e se apagar 200ms antes do alvo (condição “gap”). Era medido o tempo para o início do movimento sacádico a partir do acender do alvo – tempo de reação sacádico (TRS). Nesta análise, além de considerarmos as valências (neu/neg) e condições (“ovr”/“gap”), avaliamos o impacto da figura apresentada no ensaio anterior ao da coleta do TRS (ensaio “n-1”). Assim, TRS foram submetidas a testes-t pareados onde, para cada combinação condição/valência, comparamos a valência (neutra ou negativa) da figura apresentada no primeiro (n-1) ou nos dois ensaios precedentes (n-1 e n-2).

Resultados: Os testes-t revelaram que os TRS da condição gap/neg são significativamente maiores quando no ensaio n-1 é apresentada uma figura negativa em relação à apresentação de uma figura neutra – 280 x 271, $p < 0,001$. A análise dos efeitos da visualização de duas figuras de mesma valência nos dois ensaios precedentes mostrou que as diferenças acima se tornam maiores e passam a ser significativas também nas condições ovr/neu e gap/neu.

Conclusões: As figuras apresentadas nos ensaios anteriores (n-1 e n-2) interagem com as figuras visualizadas no ensaio n, onde os TRS são coletados, podendo ampliar as interferências geradas pelas figuras utilizadas. **Palavras-Chave:** emoção, sacada, atenção

Apoio Financeiro: Capes, PRONEX/FAPERJ, CNPq

F.03.019

Efeito do óleo essencial de Laranja e Rosas em ratos submetidos aos testes de Labirinto em T Elevado e Open-field.

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Objetivos: Investigar se os óleos essenciais (OE) de Laranja ou Rosas, possuem propriedades ansiolíticas quando administrados por via oral ou inalatória. Foi avaliado ainda um possível ação desses compostos sobre a atividade locomotora.

Métodos: 175 ratos Wistar machos (240 e 280g), foram tratados com OE de Laranja (0,5g/Kg), Rosas ou salina por via oral (1,0 e 2,0 g/kg) ou inalatória. Após 30 minutos foram submetidos ao teste do Labirinto em T elevado (LTE), e registrou-se as latências de esquivas inibitória e fuga (3 tentativas cada) do braço aberto. Imediatamente após, os animais foram submetidos ao teste do Open-field (OF).

Resultados: O tratamento com OE de Laranja v.o. (0.5g/Kg) reduziu a latência de esquivas inibitória [$F_{(2,43)} = 3.14$; $p=0.05$] e não interferiu na latência de fuga do braço aberto [$F_{(2,43)} = 0,04$; $p=0,96$]. Porém, quando inalado não afetou essas latências [esquiva $F_{(1,31)} = 1.18$; $p=0.76$ e fuga [$F_{(1,31)} = 0,08$; $p=0,92$]. Também não houve diferença entre os grupos quando foram considerados os parâmetros: cruzamentos ($p=0.54$), auto-limpeza ($p=0.41$), levantamentos ($p=0.26$) observados no OF. O tratamento com OE de Rosa v.o. não interferiu nas latências de esquivas inibitória [$F_{(2,43)} = 2,79$; $p>0,05$] ou fuga do braço aberto [$F_{(2,43)} = 0,29$; $p>0,05$]. Não houve diferença entre os grupos quando utilizou-se a via inalatória [esquiva: $F_{(1,15)} = 3,59$; $p>0,05$ e fuga $F_{(1,15)} = 4,02$; $p=0,06$] ou quando foram considerados os parâmetros: cruzamentos ($p=0.54$), auto-limpeza ($p=0.41$), levantamentos ($p=0.26$) observados no OF.

Conclusão: A avaliação dos comportamentos observados nos teste do OF demonstrou que o tratamento com OE de Laranja ou Rosas não interferiu na atividade geral dos animais. Entretanto, apenas o tratamento com OE de laranja quando administrado v.o. promoveu efeito ansiolítico observado no teste do LTE.

Palavras-Chave: Ansiedade; Labirinto em T Elevado, Óleo Essencial de Laranja, Rosas

Apoio Financeiro: Fapesp

OPEN FIELD CAN MEASURE HARM AVOIDANCE AND NOVELTY SEEKING SEPARATELY BUT IS UNRELIABLY MODULATED BY PSYCHOTROPIC DRUGS

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OBJECTIVES: In order to understand the variables involved in Open Field Test (OFT) as fear/harm avoidance (F/H.A) and curiosity/novelty seeking (C/N.S), we analyzed the central area (c.a) exploration during five minutes, with each minute analyzed separately(exp.1) and we also treated animals chronically with mood stabilizers drugs that may modify emotionality to investigate the effects of these drugs across minutes in the OFT(exp.2).

METHODS:The OFTs were performed with CF1 male adult mice. Exp.1- we test-retest (OF1 and OF2) the same animals to their exploratory behavior and time spent in the c.a of the OF. In the exp.2 - 6 groups of animals were first submitted to the OFT without treatment and then treated with lithium, olanzapine, valproate and saline and submitted again to the OFT.

RESULTS: In the OF1 the correlation of min1 with the other 4 minutes decreased progressively every minute, ending with no correlation in min 5 ($r = 0.45; 0.34, 0.24$ and 0.20 for minutes 2,3,4 and 5, respectively). Comparing OF1 and OF2, there was an increase in time in the c.a only in the 1st minute.

Treatment with lithium was associated with increased exploration of the OF c.a in the first minute (before $7,83 \pm 1,58$ and after treatment $12,42 \pm 2,51$ $P < 0.05$). Locomotor activity decreased in olanzapine in min 2 to 5 ($P < 0.05$) and also in the valproate group in min 2 and 5 ($P < 0.05$).

CONCLUSION: We reported in this study that time in the c.a between every minute of the two 5-min OFTs decrease progressively suggesting that more than one factor is involved in regulation of time in the c.a and that pre-exposition in the OF decreased the initial anxiety present when the animal is submitted to a new environment. OFT with object produced a strong correlation with the last minutes of both OF1 and OF2, suggesting that the last minutes are more associated with novelty seeking. Drugs that have different therapeutic efficacy in mood disorders were not effective to modulate the exploratory behavior of the central arena, but were somewhat effective on locomotion. This OF is effective to analyze exploratory behavior but is not reliably sensible to analyze pharmacological modulation of F/HA and C/NS.

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Palavras-Chave: Open Field Test, mood stabilizers, exploratory behavior, harm avoidance, novelty seeking.

Apoio Financeiro: CAPES

F.03.021

O IMPACTO DA VIOLÊNCIA URBANA NA SAÚDE MENTAL: AMOSTRA UNIVERSITÁRIA.

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Objetivo: A prevalência do transtorno do estresse pós-traumático (TEPT) varia entre 15 e 24% em pessoas expostas a eventos traumáticos. Uma questão importante é a investigação de fatores de vulnerabilidade para o desenvolvimento desta patologia. O presente trabalho investigou o perfil de uma amostra universitária, vítima de violência urbana, identificando características individuais potencialmente associadas com a vulnerabilidade ao TEPT.

Métodos: Participaram do experimento 58 universitários (21,2 anos $\pm$ 3, 81) que passaram por estresse intenso devido à violência urbana. Todos os participantes responderam escalas que avaliam (1) traços de afeto positivo ou negativo (PANAS) e (2) a história de traumas vivenciados (THQ). A gravidade dos sintomas de TEPT foi avaliada pela escala PCL-C. Em etapa posterior, serão avaliadas as respostas comportamentais e psicofisiológicas desta amostra.

Resultados: Foi observada uma média de 3,31 (DP=2,35) eventos traumáticos por participante. Dos 58 sujeitos, 32 (55,2%) apresentavam sintomas de TEPT subclínico, com um ou dois clusters preenchidos do PCL. Dez sujeitos (17,2%) preencheram os três clusters do PCL apresentando uma pontuação compatível com o diagnóstico de TEPT. A análise de regressão mostrou uma associação significativa entre o traço de afeto negativo (NA) e a pontuação na escala PCL ($r = 0,46$; $p < 0,001$). Especificamente, foi visto que há uma correlação positiva entre o traço de NA e o cluster dos sintomas de Evitação ($r = 0,35$; $p < 0,01$), de Hiperexcitabilidade ($r = 0,46$; $p < 0,001$) e uma tendência para Revivescência ($r = 0,46$; $p = 0,059$). Além disto, foi observada uma associação positiva entre a quantidade de traumas vividos pelos sujeitos (escala THQ) e a gravidade dos sintomas de TEPT ($r = 0,45$; $p < 0,001$).

Conclusão: Estes resultados sugerem que o traço de afeto negativo e o número de traumas vivenciados pelos voluntários representam fatores de vulnerabilidade associados com a gravidade dos sintomas de TEPT. Além disto, o percentual de universitários com pontuação na escala de PCL compatível com diagnóstico de TEPT foi similar ao descrito para outros países em populações expostas a eventos traumáticos.

Palavras-Chave: Estresse Pós Traumático; Afeto Negativo; Emoção

Apoio Financeiro: PRONEX/FAPERJ, CNPq, CAPES, IBN-net/FINEP, MCT/CNPq Projetos Institutos do Milênio.

CAPTURA DA ATENÇÃO PELAS NOVAS IMAGENS IMPRESSAS NOS MAÇOS DE CIGARRO.

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Objetivo: Trabalhos prévios mostraram que imagens negativas apresentadas como distrativas interferem no desempenho de voluntários durante a execução de uma tarefa concorrente de atenção. Nosso objetivo foi investigar se as novas imagens do Ministério da Saúde veiculadas nos maços de cigarro seriam capazes de capturar involuntariamente a atenção com eficiência similar à descrita para outras categorias emocionais.

Métodos: Foram testados 40 voluntários (26 mulheres) com idade média de 20.1 ± 2.0 . Duas barras, uma à direita e outra à esquerda, equidistantes e periféricas ao ponto de fixação eram apresentadas na tela de um monitor por 200ms. Simultaneamente, uma imagem negativa (veiculada nos maços de cigarro) ou neutra era apresentada centralmente. A tarefa do participante era ignorar a figura central e responder com o dedo indicador de uma das mãos caso a orientação das barras fosse igual e com o outro indicador, se diferente. Foi contabilizada a latência do tempo de reação (TR) para a realização da tarefa.

Resultados: O tempo de reação durante a apresentação de distrativos negativos, imagens veiculadas nos maços (576,6 ms), foi significativamente maior (teste t-student, $p=0,002$) do que para distrativos neutros (559,6 ms).

Conclusão: Estes resultados são similares aos já descritos na literatura para outras categorias, tais como imagens de mutilação, mostrando que as novas imagens veiculadas nos maços de cigarro possuem alta capacidade de engajamento involuntário da atenção. Portanto, estas imagens capturam automaticamente recursos de processamento cerebral e têm alto impacto aversivo, levando a uma diminuição da atratividade dos maços e gerando potencialmente um padrão de esquiva comportamental. **Palavras-Chave:** Imagens nos maços de cigarro; Captura da atenção; Tempo de Reação; Emoção

Apoio Financeiro: PRONEX/FAPERJ, CNPq, CAPES, IBN-net/FINEP, MCT/CNPq Projetos Institutos do Milênio

EFEITO DA VISUALIZAÇÃO DE FOTOS DE VIOLÊNCIA SOBRE O COMPORTAMENTO

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Objetivo: Trabalhos prévios na literatura mostraram que a apresentação de estímulos emocionais interferem na execução de uma tarefa atencional. Nosso objetivo foi investigar se fotos de violência também seriam capazes de capturar de forma automática a atenção e se o direcionamento dos estímulos ameaçadores representaria um fator crítico para a modulação do efeito.

Métodos: Foram testados 40 voluntários (26 mulheres). Duas barras, uma à direita e outra à esquerda, equidistantes e periféricas ao ponto de fixação eram apresentadas na tela por 200ms. Simultaneamente, uma foto negativa ou neutra era apresentada centralmente. As fotos emocionais testadas foram cenas de violência “não direcionada” e de violência “direcionada”(armas apontadas para o voluntário). A tarefa do participante era ignorar a figura central e responder com o dedo indicador de uma das mãos caso a orientação das barras fosse igual e com o outro indicador, se diferente. Foi contabilizada o tempo de reação (TR) para a realização da tarefa.

Resultados: Os dados foram submetidos a uma análise de variância com medidas repetidas (ANOVA), tendo como fatores: bloco (violência “direcionada” e “não direcionada”) e valência (negativa e neutra). Houve uma interação significativa entre os dois fatores ($F_{(1,41)}=19,582$; $p<0,05$). Comparações planejadas revelaram um aumento dos TRs quando as fotos de violência “não direcionada” ($580,7\text{ms} \pm 76,5$) eram apresentadas simultaneamente ao alvo, quando comparado à apresentação de fotos neutras ($568,0\text{ms} \pm 68,5$) ($p=0,007$). No entanto, a apresentação de fotos de violência “direcionada” ($573,2\text{ms} \pm 65,3$) promoveu uma aceleração nos TRs quando comparada com as neutras ($583,1\text{ms} \pm 65,3$) ($p=0,004$).

Conclusão: Nossos resultados mostram que fotos de violência não-direcionadas produzem aumento do TR. Este retardo se deve possivelmente a ativação de respostas defensivas de imobilização. Por outro lado, estímulos ameaçadores direcionados promoveram o efeito oposto. Esta aceleração no TR pode ser decorrente de uma ameaça mais iminente promovida por estas imagens, gerando assim uma ativação mais intensa no continuum da cascata defensiva e resultando em uma preparação imediata para ação.

Palavras-Chave: Emoção, Captura da Atenção, Tempo de Reação, Violência.

Apoio Financeiro: PRONEX/FAPERJ, CNPq, CAPES, IBN-net/FINEP, MCT/CNPq Projetos Institutos do Milênio

Entorpecimento Emocional como Preditor da Resposta do Cortisol ao Despertar

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Introdução: O Transtorno do Estresse Pós-Traumático é caracterizado pelos sintomas de revivescência do trauma, hiperestimulação autônoma, esquiva e entorpecimento emocional. Diversos estudos têm relacionado o TEPT com disfunções no eixo Hipotálamo-Pituitária-Adrenal (eixo HPA). Contudo, os resultados na literatura permanecem contraditórios. **Objetivos:** No presente estudo, se investigou a associação dos grupos sintomas de TEPT com a Resposta de Cortisol ao Despertar em vítimas de violência urbana. Participaram da pesquisa 28 indivíduos: 14 diagnosticados com TEPT e 14 sem quaisquer transtorno e pareados por características sócio-demográficas. Todos os participantes coletaram 4 amostras de saliva em domicílio: imediatamente após acordar, +30, +45 e +60 minutos. Escalas psicométricas que avaliam os traços afetivos (Escala de Afeto Positivo e Negativo), Depressão e sintomas de TEPT (PCL-Versão Civil). **Resultados:** Modelos de Regressão Linear mostraram que dentre os grupos de sintomas de TEPT, o entorpecimento emocional foi o preditor mais robusto do aumento da Resposta de Cortisol ao Despertar, controlando os efeitos da presença ou ausência de TEPT, Depressão e Afeto Negativo (beta=0,65 p<0,05). **Conclusão:** Os sintomas de entorpecimento emocional podem estar associados com o esforço de regular, implícita e explicitamente, as emoções relativas ao trauma. É possível que a gravidade deste sintoma esteja relacionada com o aumento da Resposta de Cortisol ao Despertar. Além disso, a intensidade deste sintoma pode variar em diferentes amostras, ocasionando, desta forma, resultados contraditórios em estudos que investigam a atividade do eixo HPA.

Palavras-Chave: Estresse, Emoção, Resposta do Cortisol ao Despertar, Entorpecimento Emocional

Apoio Financeiro: MCT/CNPq Projetos Institutos do Milênio - "Instituto Milênio da Violência e Saúde Mental: o Impacto da Violência na Saúde Mental da População Brasileira"

EMOTIONAL MODULATION OF SELECTIVE ATTENTION IN SPECIFIC PHOBIA

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Objectives. According to the hypervigilance-avoidance hypothesis (Amir, Foa & Coles, 1998), anxious patients show greater alertness towards threatening stimuli in early processing stages, avoiding this information in late stages. The aim of the present study was to explore the attentional biases towards potentially phobic stimuli in persons with intense fear and avoidance to them, regarding: (a) the affective valence and arousal of the stimulus, (b) the stimulus location, (c) the stimulus onset asynchrony (SOA).

Method. The performance on two tasks was compared between a group with specific phobias (n=40) and a control group (n=37). The first task was a modified version of the attention task based on Posner's signal-target paradigm in which 5 different categories of affective pictures were presented (12 phobics, 12 non-phobics, 12 pleasant, 12 unpleasant and 12 neutral) as signals of the target's location. The pictures were presented: (a) with a 100 or 800 ms SOA and (b) on the same visual hemifield (2/3 of valid trial) more frequently than on the contrary visual hemifield (1/3 of invalid trials) in which the target appears. The second task consisted on the subjective assessment of 8 phobic pictures and 8 non-phobic pictures through the Valence, Arousal, Dominance and Attention scales of the Self-Assessment Manikin [SAM] (Lang et al., 1999). **Results.** Phobic participants showed: (a) slower reaction times to their phobic pictures presented in the valid trials and in the trials with short SOA; (b) greater number of mistakes in the valid trials compared with control participants. In addition, the results of the SAM confirm that phobic participants rated their phobic pictures as more unpleasant, arousing, with lower level of dominance and higher external attention than control participants. **Conclusion.** The delay and impairment of phobics' performance when viewing phobic pictures in short presentations showed a preattentive vigilance or engagement towards threatening stimuli. On the contrary, the impairment of phobics' performance in the valid trials confirmed an avoidance or disengagement towards the same stimuli, supporting the hypervigilance-avoidance hypothesis.

Palavras-Chave: attention emotion phobia

MODULATION OF ATTENTIONAL BRADYCARDIA BY DISTRACTIVE AFFECTIVE PICTURES

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Previous studies showed that distractive aversive stimuli interfere with the performance of a relevant task. Objectives: Here we studied the effects of attenuating the affective content of aversive distractive pictures on cardiac reactivity during the performance of an attentional task. Methods: Participants (N=25) judged the orientation of two peripheral bars (0.3° x 3.0°) presented bilaterally with a simultaneous central picture (9° x 12°) for 200ms. They had to ignore the central picture and attend to the peripheral bars, pressing one of two keys as quickly and accurate as possible as to whether the bars were in the same or different orientation. The pictures could display either injured or non-injured people. Participants performed the task in two contexts. In the “Fictitious” one, instructions read were that the injuries resulted from cinema make-up; while in the “Real” one, instructions were that they were taken from real scenes. The electrocardiogram was recorded. Results: Data showed a deceleration of heart rate in response to the relevant attention task. This cardiac deceleration was more pronounced in the “Fictitious” condition (M= -1.21; p=0.03), when the relevance of the aversive pictures was attenuated, in comparison to the “Real” condition (M= -0.91). Conclusions: This result suggests that the deceleration to the attention task was more prone to interference when the pictures were real. In this vein, the aversive real pictures drove resources away from the task, reducing the attention orienting bradycardia. This study highlights the impact of an implicit emotional regulation strategy on attentional engagement, assessed by cardiac reactivity.

Palavras-Chave: emotion attention heart rate

Apoio Financeiro: CAPES

F.03.027

Mecanismos serotoninérgicos do circuito núcleo mediano da rafe-hipocampo dorsal no medo condicionado contextual

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Objetivo: Avaliar a participação de mecanismos serotoninérgicos no circuito núcleo mediano da rafe-hipocampo dorsal (NMR-HD) por meio do estudo do curso temporal da ativação das vias serotoninérgicas NMR-HD na expressão do medo condicionado contextual, bem como a identificação dos tipos de receptores serotoninérgicos envolvidos no processo de transferência de informações contextuais aversivas. **Métodos:** Ratos Wistar machos foram avaliados no teste de sobressalto potencializado pelo medo. No primeiro dia de experimento, os animais foram habituados à caixa de sobressalto e no segundo foi registrada a linha de base da resposta. No dia seguinte, os animais foram submetidos à cirurgia estereotáxica para implantação da cânula no NMR ou HD. Uma semana após, os animais foram submetidos à sessão treino, que consiste em associar o contexto da caixa de sobressalto ao choque (0,3 mA/1s). Seis horas após o treino, os animais receberam injeções do agonista de receptores 5-HT_{1A}, o 8-OH-DPAT no NMR ou HD e foram submetidos à sessão teste. **Resultados:** O tempo de congelamento dos animais que receberam 8-OH-DPAT no NMR foi reduzido ($132,93 \pm 41,46$) quando comparado com os animais que receberam salina ($263,18 \pm 42,81$). No HD também houve redução desta resposta (8-OH-DPAT: $117,00 \pm 17,54$; salina: $254,93 \pm 22,67$). Enquanto a injeção no NMR de 8-OH-DPAT não alterou a potencialização do sobressalto induzida pelo medo contextual, essa resposta foi atenuada por essas injeções no HD ($318,71 \pm 34,71$; $295,25 \pm 41,34$, na linha de base e teste, respectivamente). **Conclusão:** Em vista da dissociação farmacológica entre a resposta de congelamento e a resposta de sobressalto promovida pelas injeções locais de 8-OH-DPAT sugerimos que o congelamento e a resposta de sobressalto potencializado pelo medo ativam o circuito NMR-HD, porém a resposta de sobressalto não necessita do NMR para expressão.

Palavras-Chave: Núcleo mediano da rafe. hipocampo dorsal, sobressalto potencializado pelo medo

Apoio Financeiro: Capes

F.03.028

Aplicação de um algoritmo de análise do comportamento de limpeza para avaliação da ansiedade em ratos testados no labirinto em cruz elevado

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Objetivos: Tem sido sugerido que a aplicação de um algoritmo de análise do comportamento de limpeza pode ser um bom método de investigação da ansiedade em roedores (J. Neurosci. Methods 143:169, 2005). No trabalho citado, é indicado que as porcentagens de transições incorretas (diferentes da sequência cefalocaudal típica da limpeza de roedores) e de episódios de limpeza interrompidos (pausas menores que 5 s) seriam bons índices de avaliação da ansiedade. No presente estudo, a aplicação de tal algoritmo foi testada em ratos submetidos ao labirinto em cruz elevado (LCE) após a exposição a contextos diferentemente aversivos.

Método: Ratos machos adultos foram individualmente mantidos em uma gaiola na sala de testes por 5 min em 3 dias consecutivos. No quarto dia, os mesmos foram confinados por 5 min em um de três ambientes: braço aberto do LCE (n = 13), braço fechado do LCE (n = 15) ou a mesma gaiola familiar dos dias anteriores (n = 14). Imediatamente após este período, os animais foram postos por um min na mesma gaiola familiar dos dias anteriores e então testados no LCE por 10 min.

Resultados: A aplicação de uma ANOVA de uma via mostrou que não houve efeito dos diferentes confinamentos sobre o tempo nos braços abertos e a porcentagem de entradas nos mesmos. Também não houve efeito sobre as porcentagens de transições incorretas e de episódios de limpeza interrompidos. O tempo total gasto em limpeza mostrou efeito do tipo de confinamento ($F[2,39] = 9,16; p < 0,05$). O grupo previamente confinado aos braços abertos executou limpeza por mais tempo (s) que os outros dois (braço aberto: $116,5 \pm 22,6$; braço fechado: $44,7 \pm 7,9$; gaiola familiar: $44,9 \pm 5,4$ [Newman-Keuls, $p < 0,05$]).

Conclusão: Os resultados não confirmam a sensibilidade das porcentagens de transições incorretas e de episódios de limpeza interrompidos na avaliação da ansiedade. De todas as medidas avaliadas, a que se mostrou mais sensível foi o tempo total despendido em limpeza.

Palavras-Chave: comportamento de limpeza, algoritmo de análise, ansiedade, labirinto em cruz elevado

Apoio Financeiro: CNPq (processo 981165/2007-0)

F.04.001

Structural Limits of Information Processing: ¿What is possible and not possible to learn?

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Objectives:

When two tasks are presented within a short interval, a delay in the execution of the second task has been systematically observed. Psychological theorizing has argued that while sensory and motor operations can proceed in parallel, the coordination between these modules establishes a processing bottleneck. An intriguing open question is which aspects of this interference are subject to changes with learning and experience and to subject to subject variability. Here we set up an experiment to explore these issues.

Methods:

In this experiment we asked subjects to perform multiple sessions. In each session participants performed two simple decision tasks: a Tone Discrimination and a Number Comparison Task. Participants performed these tasks either in isolation or superposed in time. This allowed us to address specific aspects of learning related to single task performance or to the coordination of both tasks.

Results

First, we found that most independent variables were subject to changes with learning: RTs to both tasks, as well as other aspects which involve the coordination of both tasks improved with training. In striking contrast, the main interference effect remained completely unchanged.

Subject by subject analysis reveals remarkable differences which were very stable across different sessions. We observed a clustered pattern of dependencies with very tight correlation in the different observed variables. A group of subjects was extremely fast in single task performance, showed consistent learning in the dual-task performance, moderate effects of interference and very reliable across session performance. Another group showed a consistently distinct behavior in all these variables.

Conclusions

Our work has shed light on which robust observations in psychology may be variable across individuals or, within each individual, to changes related to practice. While most variables are subject to change with practice, the dual-task interference, which implies a sequential organization of certain cognitive operations seems to be unchanged by learning. This argues in favor of a purely structural limit probably related to architectonic properties of the human brain. In addition the observed pattern with very strong correlations in different presumably independent measurements argues in favor of common processes which underlie different aspects of performance in a broad range of cognitive tasks. Palavras-Chave: information processing, dual-task, PRP, decision making

PREFRONTAL CORTEX AND DECISION MAKING: ON A NEUROBIOLOGY OF AUTONOMY

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PURPOSE: to present an updated debate about the neurobiology of processes involved in decision making, discussing the difficulties of conceiving autonomy as free arbitrium in the light of the current neuroscience concepts.

METHODS: Review of Literature – using the databases BVS (*Biblioteca Virtual em Saúde*), SCIELO (*Scientific Electronic Library Online*) and PUBMED (*U.S. National Library of Medicine*) as well as books and textbooks in neuroscience — and critical reflection on the concerning texts.

RESULTS and DISCUSSION: Autonomy has been put in question by neuroscience — in the light of the debate free arbitrium/ determinism — whenever one accepts that autonomy is the result of decision making processes determined by subjacent neurobiological processes, primarily with the participation of prefrontal cortex (PFC). The ventromedial prefrontal cortex (VMPFC) is highly correlated with decision making, and by consequence with aspects implied in autonomy and morality (1). In fact, the integrity of this region seems crucial to processing, evaluating and filtering emotion and social information, which are essential for the decision making process. One event historically marks this debate the famous “Phineas Gage case” — that took place in the XIX century —, when a lesion in VMPFC turned a most capable foreman into a capricious and vacillating man of great difficulty to plan his actions, aspects as such with severe implications in decision making. From then on several similar situations were published in the international medical literature, the issue in question being how much of human reason and emotion is determined by neurobiology.

CONCLUSION: the brief data presented seem to show feasible de hypothesis that says brain functioning is decisive for the different cognitive moral elements related to judgment and behavior, putting in question how much the neurobiological aspects may influence — if not determine — man’s thinking and acting . The consequence is devastating: the destitution, in the human experience of being, of one of the most genuine prerogatives of man — free arbitrium (2). Would it be the end of autonomy?

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Palavras-Chave: PREFRONTAL CORTEX, Behavior

Apoio Financeiro: UNIFESO

ALTERAÇÕES EXECUTIVAS EM PACIENTES COM Distrofia Muscular de DUCHENNE (DMD)

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Distrofia muscular de Duchenne (DMD) é uma doença genética que causa degeneração progressiva dos músculos esqueléticos e cardíaco, acompanhada de eletrorretinopatia e retardo mental em parte dos afetados. Pouco se conhece do desempenho de pacientes com DMD em funções executivas, as quais compõem os comportamentos humanos mais complexos, intrínsecos ao raciocínio abstrato, ao planejamento de ações e às habilidades de responder adaptativamente ao ambiente. **Objetivo:** examinar o desempenho executivo de pacientes com DMD. **Método:** 35 pacientes com DMD, sexo masculino, 16 ± 5 anos de idade, 6 ± 4 anos de estudo formal e inteligência normal (indicada por $QI > 80$ conforme a escala de Wechsler de Inteligência) foram submetidos ao Teste Wisconsin de Classificação de Cartas. Foram computados números de: erros, respostas perseverativas, erros perseverativos, respostas de nível conceitual, categorias completas e perdas da meta. Os escores brutos foram transformados em escala de percentis, com percentil 10 como limite mínimo de normalidade. **Resultados:** dentre os 35 pacientes testados, apresentaram percentil $< 10\%$: 22/35 (63%) em erros, 20 (57%) em erros perseverativos e 18 (35%) em respostas perseverativas, indicando dificuldade de flexibilização do comportamento para o recrutamento de estratégias diversificadas na resolução da questão problema. Onze pacientes (31%) obtiveram percentil < 10 no número de respostas de nível conceitual e 2 (6%), em capacidade de manter a meta. **Conclusão:** os resultados sugerem que pacientes com DMD podem apresentar déficits em funções executivas, principalmente da flexibilidade mental necessária para adaptar-se às mudanças nas demandas do ambiente, o que funcionalmente pode traduzir-se em uma dificuldade na resolução de problemas complexos e lidar situações novas no cotidiano.

Palavras-Chave: distrofia muscular de Duchenne, funções executivas, teste de Wisconsin, distrofina

Apoio Financeiro: FAPESP

Circadian modulation of interval timing in mice

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Timing and time perception are fundamental to survival and goal reaching in humans and other animals. To deal with timing, organisms have developed multiple systems that are active over a range of 10 orders of magnitude, the most important being circadian timing, interval timing and millisecond timing [1]. The circadian pacemaker is located in the suprachiasmatic nuclei (SCN) of the hypothalamus, and is driven by a self-sustaining oscillator with a period near to 24 h. The estimation of time in the second-to-minutes range – known as interval timing – involves the interaction of the basal ganglia and the prefrontal cortex. There are several evidences indicating a possible link between circadian and interval timing [2-7].

In this work we tested the hypothesis that interval timing is sensitive to circadian modulations. The ability of mice to estimate time intervals of short duration was examined. Animals were trained following the protocol of Drew et al [8], slightly modified. Briefly, mice were trained in three consecutive phases - pre-training, fixed interval training and peak interval training - during approximately 60 days. Two independent experiments were conducted, in which mice were trained in the middle of the diurnal phase (ZT 5-7, group 1) or in the middle of the nocturnal phase (ZT 17-19, group 2). Our preliminary results show significant differences in the estimation of 24-second intervals at different times of day.

Currently we are studying the estimation of time throughout the circadian cycle, as well as interval timing in animals subjected to circadian desynchronizations. We also aim to develop theoretical models which tell about the mechanisms involved in interval timing

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Palavras-Chave: circadian rhythms, cortico-striatal circuits, interval timing, suprachiasmatic nuclei

Apoio Financeiro: ANPCyT, CONICET and Universidad Nacional de Quilmes.

Discriminação condicional e bi-seção temporal em ratos com lesão hipocampal.

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OBJETIVO: O efeito da lesão seletiva do giro denteado hipocampal foi avaliado durante tarefa operante de discriminação condicional e bi-seção temporal com duas barras. **MÉTODO:** Foram utilizados ratos lesados no giro denteado por microinjeções de colchicina e ratos controle-operados. Sob privação de água, os ratos foram submetidos a treino cujas práticas apresentaram luz de 4 ou 16 s como estímulos condicionais. Independentemente da duração, a luz foi sempre seguida de intervalo vazio de 5 s e, depois, de tom puro de 5 s. Pressões à barra A foram reforçadas com água somente se emitidas durante o tom precedido pela luz de 4 s e pressões à barra B foram reforçadas somente durante o tom precedido pela luz de 16 s. No mesmo treino, apresentaram-se práticas com o tom não precedido por luz, durante as quais pressões em qualquer barra não causaram efeito algum. O teste empregou práticas sem liberação de reforçador, iniciadas com luz de 4,0 s, 5,0 s, 6,4 s, 8,0 s, 10,0 s, 12,8 s e 16,0 para a obtenção de uma curva psicofísica de bi-seção temporal. **RESULTADOS:** Escolhendo a barra A ou B, os ratos dos dois grupos adquiriram a discriminação entre as duas durações apresentadas no treino, pois aprenderam a pressionar a barra A durante tons precedidos por luz de 4 s (efeito de sessões: $F_{11,187}=21,70$; $P<0,05$) e a pressionar a barra B durante tons precedidos por luz de 16 s ($F_{11,187}=12,31$; $P<0,05$), segundo a ANOVA de dois fatores com medidas repetidas. Independentemente das barras, os ratos aprenderam a responder durante tons precedidos por luz de qualquer duração (efeito de sessões: $F_{11,187}=4,93$; $P<0,05$) e a não responder durante tons sozinhos ($F_{11,187}=17,31$; $P<0,05$). Além disto, o decurso das respostas operantes ao longo das práticas mostrou que a atividade operante aumentava durante o intervalo vazio entre luz e tom, indicando que a escolha entre as duas barras não foi mediada apenas por mero controle temporal, mas por uma relação condicional entre o sinal temporal (luz) e o estímulo condicionado (tom). **CONCLUSÕES:** A lesão do giro denteado não prejudicou nem a aquisição da discriminação condicional de durações nem o desempenho no teste de bi-seção temporal. **Palavras-Chave:** Discriminação condicional, bi-seção temporal, comportamento operante, giro denteado do hipocampo, lesão seletiva, colchicina, privação de água.

Apoio Financeiro: CAPES - PROEX

F.05.003

Tonal Modulation and Subjective Time

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Objectives: We examined the effect of tonal modulation on time estimation with experiment and model. The cognitive processing of such high order pitch structures of music relative to subjective timing has not been studied. For low/middle orders, there are over-, under-, or quasi-accurate time estimations for melody-last-pitch/sequence-last-chord ending later or earlier than expected, or “in-time”, respectively (1;2).

Method: One stimulus was retrospectively reproduced by one in four subject groups (n=66). The stimuli (20s) were: no-modulating chord sequence (CC); sudden-close modulating one (CF); sudden-distant modulating one (CGb); gradual-distant modulating one (CEbGb).

Results: ANOVA showed time estimations affected by tonal modulations (CC: $m=21.242s/sd=6.290s$; CF: $m=17.689s/sd=3.655s$; CGb: $m=13.474s/sd=4.166s$; CEbGb: $m=16.916s/sd=4.829s$; $F_{(3,62)}=6.49$; $p<0.05$). *Post hoc* Duncan test showed time underestimations as an inverse function of interkey distances, with a major impact for sudden modulations.

Conclusions: As modulations did not elicit overestimations, models based on cognitive storing such as the storage-size (3), attentional demand (4), and contextual change (5) do not match the results. Subjects recovered the stimulus image to make the retrospective reproduction implying implicit working memory: stimuli-responses were non-verbal, and such image might be kept only during the test. Tonality and time are environmental patterns, incidentally internalized by passive long-term exposition (6;7), featuring semantic memory. Our expected development fraction model claims: When interkey distance is traversed, expectation for the target key occurs (semantic memory duration). This seems longer than the perceived duration (implicit working memory). Depending on the modulation, sudden or gradual, the final moment will appear more or less anticipated, leading to time underestimation to a greater or lesser degree, respectively.

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Palavras-Chave: Tonality (Music); Subjective Time; Model

Apoio Financeiro: CAPES

Associações implícitas e o comportamento alimentar no esporte

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O Teste de Associações Implícitas (TAI) é capaz de acessar informações sobre redes de associações implícitas entre determinados conceitos, através da medida do tempo de reação em tarefas de discriminação combinada. Os tempos de reação manual (TRM) mais rápidos ao emparelhamento de 2 conceitos em uma mesma tecla de resposta mostram que a associação entre eles é mais fortalecida do que a dos emparelhamentos mais lentos. Nesta pesquisa buscamos estudar as associações entre conceitos próprios (EU/Não-EU) e conceitos sobre a indicação alimentar esportiva: alimentos indicados (AI) e não-indicados (ANI). Dezoito atletas nascidos entre 1988 e 1992, responderam inicialmente ao questionário de frequência de consumo alimentar, o qual teve uma pontuação variando de 0 (nenhuma frequência) a 5 (frequência diária). A seguir, foram submetidos ao TAI e orientados a responder prontamente às seguintes configurações: **1** - Alimentos indicados (14 palavras sobre frutas, verduras, legumes e cereais) associados com EU (com as palavras auto, eu, meu, sou e mim) e Alimentos não indicados (14 palavras de alimentos ricos em gordura e/ou com pouco valor nutricional) com Não-Eu (com as palavras deles, eles, seu, vocês, outros); **2**- Alimentos Indicados com Não-Eu e Alimentos não indicados com EU. Na análise utilizamos o coeficiente de Pearson (r) para identificar possíveis correlações das duas configurações com a pontuação obtida no questionário. A análise identificou uma correlação negativa moderada ($r = -0,37$) entre o TRM da configuração 1 com a pontuação obtida no questionário de alimentos indicados. Ou seja, quanto mais rápido o TRM na configuração 1, mais habitual é o consumo dos alimentos indicados e quanto mais lento, menor a ingestão desses alimentos. Também identificamos uma correlação negativa moderada na análise do TRM da configuração 2 com a pontuação obtida em alimentos não indicados ($r = -0,36$). Indicando que os TRMs mais rápidos se relacionam com o consumo mais habitual dos alimentos não indicados e os mais lentos com um menor consumo. Nossos resultados oferecem indícios de que o comportamento alimentar pode ser estudado e avaliado através de medidas implícitas de associações.

Palavras-Chave: Medidas implícitas; TRM; comportamento alimentar

F.06.001

CORRELAÇÃO ENTRE PRECISÃO, VELOCIDADE, PADRÃO E COMPREENSÃO DE LEITURA EM ESCOLARES DO SEGUNDO ANO DO ENSINO FUNDAMENTAL

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OBJETIVOS

Determinar as médias esperadas para as habilidades de leitura no início do segundo ano do ensino fundamental, a saber, precisão, velocidade, padrão e compreensão, a fim de que sirvam como parâmetros para identificação precoce de alterações no desenvolvimento deste processo.

Correlacionar estas habilidades entre si, a fim de estabelecer a importância de cada uma delas para as demais.

MÉTODO

A amostra foi formada por 45 crianças com idade, em meses, de 92.29 (DV=3,40), estudantes do segundo ano do ensino fundamental do CAP-UFRJ, submetidos à mesma metodologia de alfabetização.

Foram aplicadas as seguintes avaliações: leitura de palavras isoladas (Pinheiro, 1994 – reduzido por Capovilla e Capovilla, 2000) e a leitura de um texto narrativo compatível com a escolaridade, com 196 palavras e 939 caracteres, excluindo os espaços. O padrão foi classificado em silabado, pausado e fluente. A compreensão foi verificada a partir de 4 questões eliciadoras, envolvendo o conteúdo do texto. Foi realizada uma estatística descritiva, seguida da correlação (Pearson).

RESULTADOS

As médias encontradas nas tarefas foram: precisão de leitura (palavras lidas corretamente) T 48 M 43,22 DV 7,997; velocidade de leitura (em segundos) M 314,67 DV 219,483; padrão de leitura (1-silabado, 2-pausado e 3-fluente) M 1,93 DV ,863; compreensão de leitura (de 0 a 5 acertos) M 2,96 DV 1,147.

Em relação às correlações, todas as interações possíveis mostraram alta significância estatística: precisão-velocidade $p < ,000$; precisão-padrão $p < ,003$; precisão-compreensão $p < ,000$; velocidade-padrão $p < ,000$; velocidade-compreensão $p < ,000$; padrão-compreensão $p < ,000$.

CONCLUSÕES

A maior parte dos alunos do segundo ano do ensino fundamental possui um padrão de leitura silabado, a compreensão de textos oscila entre total e parcial por uma única falha, a leitura de palavras isoladas revela ótima precisão 43,22/48 e a velocidade é de 314,67 S, para 196 palavras (939 caracteres).

As habilidades envolvidas no desenvolvimento favorável da leitura se retroalimentam, mostrando igualmente a interdependência entre fluência e compreensão.

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Palavras-Chave: linguagem leitura fluência compreensão

F.06.002

Lexical and visuospatial processing in brazilian children with cerebral palsy: a pilot study

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Objectives

Use of cognitive-neuropsychological models of assessment in developmental populations is currently being the focus of interest and debate. Our research efforts aim at the use of information processing models to assess lexical and visuospatial processing in children with early focal brain lesions.

Methods

Sample was composed of 189 children, 137 of whom were typically developing, and 31 of whom had cerebral palsy [9 with left hemispheric damage (LH), 7 with right hemispheric lesions (RH), and 15 with bilateral lesions (DIPLEGICS)]. Mean age was 6.75 years (sd= 1.14 years), 43.8 % were female. Age and gender were approximately balanced across groups. Lexical processing was assessed by tasks covering a serial information-processing model comprising phonological (syllable and phoneme discrimination), input lexical (lexical decision task), semantic (word-figure categorization), and output lexical (word and nonword repetition) levels. The visuospatial assessment model covered both dorsal (input, storage/imaging, execution) and ventral (graphic formulae or “constructional lexicon”) visual processing streams.

Results

Intelligence was in the normal range (Raven's Matrices). Lexical-processing tasks discriminated between groups, with LH exhibiting lower performances ($d's > 0.8$). Visuospatial tasks distinguished typically developing from brain-damaged children, but not between LH and RH. LH children showed a clearcut profile of semantic-lexical impairment. RH and DIPLEGIC children did not present a unique pattern of impairment on the tasks used. CONTROL children performed at ceiling. Internal consistency of the instruments was high (Cronbach's $\alpha > 0.8$).

Conclusions

The tasks used were able to evaluate the investigated issues. It was also possible to identify a development and 'kind of school' effect for the children of the control group. These instruments were able to identify questions concerning the cognitive development of the different groups that were investigated: the LH showed a lower and consistent pattern, characterized by difficulties in the lexical processing's abilities. These instruments can also be used in the neuropsychological clinic, specially concerning the diagnosis and rehabilitation. Due to these questions, it is necessary to further study these issues and to explore them in single-case's studies.

Palavras-Chave: perfil neuropsicológico, paralisia cerebral, processamento lexical e visoespacial

Apoio Financeiro: conselho nacional de desenvolvimento científico e tecnológico, fundação de amparo à pesquisa do estado de minas gerais

Abstraindo estrutura fonológica a partir das formas fonéticas do input

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Objetivos: Aquisição de padrões fonológicos a partir da hipótese de que a variação sociofonética modela a aquisição de padrões fonológicos, uma vez que as variantes são formas fonéticas diferentes da mesma palavra e podem também refletir competição de padrões fonológicos. Segundo Pierrehumbert (2003), a representação da forma sonora das palavras no léxico mental inclui informação fonética detalhada e a representação fonológica, que envolve os padrões fonológicos correntes no léxico, é emergente da representação da forma das palavras no léxico. **Método:** Dados de produção espontânea de 34 entrevistas de crianças com idade entre 2;0 e 5;0 anos. **Resultados:** A acuracidade na realização do padrão CCV, tanto em itens lexicais com a lateral como alvo (ex. blusa) ou o tepe (ex. prato), reflete a natureza da variação sociofonética observada no input recebido. As poucas realizações de CCV com lateral alvo nas faixas etárias iniciais ocorreram com o tepe. As distribuições de frequência de realização de CCV em função da líquida alvo por idade revelam diferenças desenvolvimentais que são estatisticamente relevantes ($X^2=5.946$, $df=1$, $p\text{-value}=0.014$). Os percentuais mais altos de realização de CCV correspondem às palavras com a lateral como alvo entre 3;0 e 4;6 anos, se comparadas às que apresentam o tepe como alvo. As crianças só chegam ao mesmo percentual de ocorrência de CCV para os dois grupos de palavras aos 5;0 anos. Dado que as representações lingüísticas mudam no curso da aquisição em função da exposição da criança ao input e a constante reorganização do léxico em expansão, os resultados apontam para uma explicação das diferenças desenvolvimentais observadas em função da ocorrência das formas no input ao invés de restrições universais, que seriam gerais para a estrutura CCV. **Conclusão:** A acuracidade progressiva na realização da estrutura silábica em questão está relacionada às variantes disponíveis no *input*, que envolvem competição entre realizações fonéticas diferentes da mesma palavra e competição entre tipos estruturais diferentes, como no caso da alternância CCV – CV das palavras que têm o tepe como alvo.

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Palavras-Chave: aquisição de padrões fonológicos; léxico mental

Apoio Financeiro: CNPq

Análise do efeito da escolaridade no desempenho de idosos em tarefas de Fluência Verbal

Semântica e Fonêmica.

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Objetivo: Avaliar o efeito da escolaridade nas tarefas de fluência verbal (FV), semântica (FS) e fonêmica (FF) em idosos sem diagnóstico de demência. **Método:** Para o diagnóstico foi realizada uma avaliação médica, funcional e neuropsicológica abrangente. A amostra foi constituída de 176 idosos sem demência, que faziam parte do atendimento ambulatorial em centros de referência universitários das cidades do Rio de Janeiro e São Paulo. Estes foram subdivididos em duas faixas etárias: inferior a 75 anos e superior/igual a 75 anos, para isolar o efeito escolaridade. No teste de fluência verbal (FV), o sujeito é solicitado a evocar, em um tempo limite de um minuto, palavras iniciadas com as letras F, A, S (FF), animais (semântica – FSA) e itens de supermercado (semântica – FSS). **Resultado:** Observou-se correlação significativa entre anos de escolaridade e desempenho nas tarefas de FF ($r=0,479$; $p<0,05$) para idades inferior a 75 e também para superior/igual 75 anos ($r=0,466$; $p<0,05$). Em relação a FS, correlações significativas foram observadas para idade inferior a 75 anos no FSA ($r=0,409$; $p<0,05$) e para idosos com idade igual/superior a 75 anos no FSS ($r=0,317$; $p<0,05$). Ao se comparar as diferentes faixas de escolaridade através da análise de variância (ANOVA), encontra-se diferenças significativas para níveis de escolaridade entre 1-4 anos, 5-8 anos e 9 anos ou mais no FF ($F=12,36$; $p=0,000$) e em FSA ($F=5,40$; $p=0,006$) para o grupo com idade inferior a 75 anos. Para o grupo com idade igual/superior a 75 anos, observou-se diferenças significativas ($F=4,28$; $p=0,019$) apenas para FF entre os níveis 1-4 anos e 9 anos ou mais. **Conclusão:** Há efeito de escolaridade nas tarefas de FV no envelhecimento, que é mais consistente para a tarefa de FF. Na FS há uma variabilidade desse efeito em relação às tarefas e a idade, sugerindo que a educação e seu impacto no desenvolvimento das funções executivas podem estar mais relacionadas com o desempenho de tarefas de FF.

Palavras-Chave: Fluência verbal; idosos; escolaridade.

F.06.005

Neurofisiologia comparada do acesso lexical: um estudo de ERPs (potenciais relacionado a evento) em bilíngües de português-alemão, bilíngües tardios e monolíngües de ambas as línguas

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Objetivos: Este é um projeto de estudo de priming que pretende comparar o acesso lexical de quatro grupos distintos de falantes: monolíngües de alemão, monolíngües de português, bilíngües e bilíngües tardios (que são fluentes em ambas, mas que têm uma única língua materna). Partindo-se do pressuposto de que há diferenças neurofisiológicas estabelecidas pelo contato precoce ou não com dados primários das línguas estudadas, este estudo pretende caracterizar as representações e esmiuçar os processos derivacionais e heurísticas utilizadas por cada grupo de indivíduos estudados

Método: O método consiste na aplicação de um experimento constituído por dois cenários, que serão rodados seguindo um esquema *between subject*. Cada um dos cenários é composto por 60 pares, formados por uma figura e uma palavra ou não palavra. Assim, tem-se 15 pares de figuras combinadas com palavras corretas em alemão, 15, de figuras combinadas com palavras corretas em português, 10 de figuras combinadas com palavras erradas, 5 com palavras erradas em português e 5 com palavras erradas em alemão; e 20 combinações de figuras com não palavras, sendo 10 delas não palavras em português e 10 não palavras em alemão (não palavras são seqüência de letras em uma dada combinação que poderiam ser palavras e respeitam as regras fonológicas da língua, mas não o são).

Resultados: O experimento ainda não foi executado, mas espera-se encontrar diferentes tempos de resposta para cada grupo de sujeitos, uma vez que se parte do pressuposto de que há diferenças neurofisiológicas entre indivíduos monolíngües e bilíngües.

Conclusões: Espera-se encontrar um tempo de resposta menor entre monolíngües de qualquer uma das línguas do que entre bilíngües tardios. Em relação à diferença entre bilíngües tardios e aqueles que aprenderam duas línguas ainda na infância, acredita-se que haverá uma maior quantidade de erros no grupo de bilíngües tardios, entretanto, talvez, o tempo de resposta do mesmo seja relativamente menor (pelo menos na língua materna) do que em qualquer uma das línguas no outro grupo de bilíngües. Acredita-se que não somente o tempo de resposta do grupo de bilíngües “verdadeiros” seja maior do que o de monolíngües, como o grupo de bilíngües tardios tenha um tempo de resposta menor do que o dos bilíngües verdadeiro em uma das duas línguas. Se, de fato, esta predição for confirmada, poder-se-á concluir que o conhecimento de uma língua L2, tanto por bilíngües verdadeiros como tardios interfere no conhecimento da língua L1 do sujeito.

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Palavras-Chave: Acesso lexical, bilingüismo, neurofisiologia

Apoio Financeiro: CAPES

EL EFECTO PARADÓJICO DE LA FACILITACIÓN MORFOLÓGICA SUPERFICIAL

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Los resultados de la investigación sobre el reconocimiento de las palabras morfológicamente complejas sugieren una relación estrecha del procesamiento de las propiedades morfológicas con las propiedades semánticas de las palabras. El objetivo de este experimento es comparar los efectos de los pares de palabras relacionadas por transparencia semántica, seudo-derivación y solapamiento ortográfico en el idioma español. El estudio se desarrolló midiendo los tiempos de reacción en una tarea de decisión léxica mediante la técnica de inducción visual enmascarada. Se realizaron pruebas de diferencias de medias para comparar los efectos. Los resultados muestran que los pares seudo-derivados tienen un efecto de facilitación similar a los pares de transparencia semántica. La diferencia entre el tipo de relación para los pares transparentes es significativa ($F=3,53, p=0,06$). La diferencia entre el tipo de relación para los pares seudo-derivados no resulta significativa ($F=0,66, p=0,42$). Las diferencias entre el tipo de relación para los pares de solapamiento ortográfico no resulta significativa ($F=0,08, p=0,76$). La diferencia de los promedios de los tipos de par en presencia de la relación de priming fue significativa ($F=3,73, p=0,03$). La facilitación observada en los pares seudo-derivados es contradictoria a las explicaciones de las propuestas teóricas tradicionales del procesamiento morfológico subléxico y supraléxico, puesto estos modelos no logran dar cuenta este efecto de facilitación morfológica sin relación semántica producido por los pares seudo-derivados. Los hallazgos de esta investigación aportan evidencia que apoya parcialmente las propuestas de procesamiento morfológico preléxico y a su vez dan lugar a otros cuestionamientos. Dado que las propiedades formales del estímulo son las que primero activan representaciones morfémicas, se demuestra que la morfología superficial produce un efecto de facilitación. En consecuencia se puede afirmar que el procesamiento morfológico está más relacionado con las propiedades formales que con las propiedades semánticas, durante los momentos iniciales del procesamiento de las palabras morfológicamente complejas.

Palavras-Chave: Procesamiento morfológico, reconocimiento visual de palabras, palabras morfológicamente complejas

Apoio Financeiro: Universidad Católica de Colombia, Facultad de Psicología

MODELOS DE PROCESAMIENTO LÉXICO EN EL RECONOCIMIENTO DE PALABRAS MORFOLÓGICAMENTE COMPLEJAS

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Las palabras morfológicamente complejas (PMC) se componen de dos o más unidades morfélicas que pueden ser prefijos, sufijos e infijos. El objetivo de los investigadores contemporáneos en psicolingüística es identificar las condiciones que acompañan el procesamiento cerebral para reconocer las palabras. El propósito de este documento es presentar una revisión del desarrollo que ha tenido la investigación psicolingüística en este campo y el papel que juega la morfología de las PMC en su procesamiento cognoscitivo, además de abordar los supuestos teóricos que sustentan los procedimientos experimentales y las hipótesis que subyacen a estos. La revisión contiene tres grandes apartados: en el primero se hace una introducción a la psicolingüística y algunos de sus conceptos fundamentales. En el segundo se exponen los paradigmas dominantes desde los cuales se postulan los modelos hipotéticos que dan cuenta del procesamiento morfológico. Y, en el tercer apartado, se describe el procedimiento experimental de *priming*; la mayor fuente de evidencia empírica en el campo. Finalmente, se exponen las nuevas perspectivas de investigación que buscan incrementar la evidencia empírica frente a la hipótesis sobre el cómo procesan los humanos las palabras compuestas por morfemas. **Palavras-Chave:** psicolingüística, reconocimiento de palabras, morfología, priming

Apoio Financeiro: Universidad Católica de Colombia, Facultad de Psicología

F.06.008

Percepção de fala: análise das vogais do Português Brasileiro em tempo comprimido.

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Objetivo: avaliar a percepção das vogais do Português Brasileiro (PB) em diferentes taxas de elocução e investigar se a estimação de magnitude é um procedimento adequado para avaliação da percepção de fala em tempo comprimido.

Métodos: a amostra foi composta por 32 participantes, ambos os sexos, distribuídos igualmente em dois estudos para realizarem julgamento de estimação de magnitude das frases. Foram apresentadas 28 frases aleatorizadas quanto às sete vogais do PB em uma frase-veículo com duas taxas de locução. Estas frases foram gravadas por um locutor falante nativo do PB com taxas de elocução considerada “normal” ou confortável para o locutor, rápida (aproximadamente 18% mais rápido que a “normal”) e mais rápida (aproximadamente 30% mais rápido que a “normal”). No estudo I foram apresentadas as taxas de locução normal e rápida, e no estudo II normal e mais rápida. Para avaliação das durações dos estímulos acústicos nas diferentes taxas de elocução foi utilizada a análise acústica por meio do *software PRAAT 4.0.2*.

Resultados: O teste estatístico utilizado para análise dos resultados foi ANOVA. *Análise acústica:* houve diferença significativa na duração das frases em relação às taxas de elocução (normal, rápida e mais rápida), porém não difere quanto às diferentes vogais alvo das frases. Contudo, quando analisada as vogais isoladamente há diferença estatística entre as taxas de elocução e entre as vogais do PB. *Análise psicofísica:* o julgamento da percepção das frases mostrou que os sujeitos não perceberam qualquer modificação da taxa de elocução normal e rápida (ANOVA). Entretanto, quando apresentados com taxa normal e mais rápida os sujeitos perceberam as frases diferentemente.

Conclusão: taxa de elocução pode interferir na inteligibilidade de fala, porém não as vogais do PB. Conclui-se, também, a validade da Psicofísica para o julgamento de percepção de fala.

Palavras-Chave: Inteligibilidade de fala; velocidade de fala; psicofísica e análise acústica.

Apoio Financeiro: CAPES e CNPq

Neurofisiologia do Processamento de Expressões Idiomáticas em Aspergers

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Objetivos: À luz da Morfologia Distribuída (Hale, Marantz, 1993), este estudo, voltado para Aspergers, é um design experimental, com visos a investigar falhas no processamento integrativo entre a Interface conceptual, que faz o pareamento forma e sentido, no âmbito da Faculdade da Linguagem, e a ativação no córtex cingulado anterior responsável pela detecção da resolução do conflito de respostas.

Métodos: Trata-se de um experimento que, a partir da Eletroencefalografia (EEG), se propõe a aplicar a técnica de extração de potenciais relacionados a eventos lingüísticos (ERPs). Participarão do experimento 16 indivíduos aspergers e 16 normais, no corte *between subject*, design quadrado latino. O *software Presentation* será utilizado para apresentação auditiva de 240 sentenças, 120 distratoras e 120 idiomáticas, seguidas de um *target*, relacionado denotativa ou idiomáticamente ao *prime*. Após o término dos estímulos, o informante deverá responder se o *target* relaciona-se semanticamente à sentença. As respostas serão captadas por sinais de voz e posteriormente armazenadas. As variáveis do experimento são índice de erro/acerto e tempo de resposta.

Resultados: Com a aplicação do experimento espera-se analisar as reações do córtex cerebral diante de estímulos idiomáticos da língua, independentemente das variáveis, uma vez que anterior às respostas, a reação elétrica ao estímulo já terá sido captada e registrada pelo EEG.

Conclusões: Muito se tem discutido sobre o papel das disfunções neurológicas na origem da Síndrome de Asperger. Segundo descrição da CID-10, não há déficits lingüísticos no quadro. Contudo, evidências de diferentes teorias têm apontado para problemas dessa ordem, sobretudo na compreensão de idiomáticas. Desse modo, através da aplicação desse experimento, os sinais captados pelo EEG, comparativamente a aspergers e normais, demonstrarão possíveis falhas de processamento da linguagem, elucidando a controvérsia existente na literatura.

Referências: 1- Trends Cogn Sci, 6, 215, 2000; 2- Rev Neurosci, 1, 49, 1999; MIT Press, 111, 1993.

Palavras-Chave: EEG. Síndrome de Asperger. Linguagem. Experimento.

F.07.001

Divergencia de umbrales de respuestas auditivas mesencefálicas en anuros primitivos.

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Estudios de respuestas conductuales de hembras y machos de anuros a señales acústicas indican una importante correspondencia con los cantos de advertencia coespecíficos. Sin embargo, la producción y procesamiento de las señales dependen también de la carga filogenética. Para entender la contribución de los mecanismos neuronales que subyacen a las respuestas conductuales en anuros primitivos, se midieron umbrales auditivos multiunitarios en el mesencéfalo de *Alytes cisternasii*, *A. obstetricans*, *A. dickhilleni*, y *Discoglossus pictus* para tonos puros y cantos de 9 especies de discoglósidos de los géneros *Alytes*, *Bombina* y *Discoglossus*. La frecuencia central de los audiogramas en el rango de frecuencias de la papila basilar fue 1483, 1566, 1831 y 1577 Hz para *A. cisternasii*, *A. obstetricans*, *A. dickhilleni* y *D. pictus*, respectivamente. Los umbrales mínimos para estas especies fueron en promedio 48.9, 42.8, 45.6 y 53.0 dB SPL, respectivamente. Los umbrales para los cantos fueron de 45 a 60 dB SPL, siendo tanto las especies de *Alytes* como *Discoglossus* más sensibles a los cantos de *Alytes*. Además, en *Alytes* no hay una relación óptima entre los umbrales y los cantos coespecíficos, ya que *A. dickhilleni* y *A. obstetricans* son más sensibles a los cantos de *A. muletensis* y *A. cisternasii*, respectivamente. Los resultados muestran que la sintonización entre el receptor y las señales no ha determinado de manera exclusiva la divergencia de los sistemas de comunicación sonora en estas especies y destaca la importancia de eventos filogenéticos en estos procesos.

Palavras-Chave: Filogenia, Umbral Auditivo, Anuros, Discoglossidae.

Apoio Financeiro: Beca doctoral CONICYT, FONDECYT 1040830 y BOS 2003-01413.

F.07.002

Duration and echo-delay tuning in the inferior colliculus of the long CF bat *Pteronotus parnellii*.

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Neurons measuring time in the auditory system of bats can respond selectively to specific delays between call and echo or to certain sound durations, and were described at and above the level of the inferior colliculus. Delay-tuned neurons have been studied in species of FM bats (bats with frequency modulated echolocation calls) and CF bats (bats with constant frequency echolocation calls), and recognize target range. Duration-selective neurons, however, have been studied only in certain species of FM bats and recognize species-specific calls. For both types of neurons the mechanism underlying the measurement of time has been modeled using inhibitory and excitatory neuronal inputs with specific temporal relations. In addition, studies on duration- and delay-tuning have been conducted independently on each of two species of FM bats, in which similar operational time ranges (1 - 20 ms) have been found. It is thus still unclear whether the same neuron could selectively respond to certain durations and delays and whether same mechanisms are involved. The long CF-bat *Pteronotus parnellii* is a unique biological model to clarify these issues. Three main types of duration selectivity, short-, band-, and long-pass, as previously described in others species, are present in the inferior colliculus of *P. parnellii*. Duration-sensitive neurons in the central nucleus were found with a tuning preference to 12-21 ms which correspond to the duration of the echolocation calls. A subpopulation of the neurons was also sensitive to echo-delays in the same time range. We also found a large population of delay-tuned neurons that responded to FM-FM combinations, as they have not been described for the IC previously. **Palavras-Chave:** duration selectivity, delay tuning, coincidence mechanism, inferior colliculus

F.07.003

Análise etológica do comportamento de camundongos no teste de nado forçado: efeitos da apomorfina.

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Objetivo: Descrever o catálogo comportamental e o tempo de curso dos comportamentos emitidos por camundongos tratados com apomorfina no teste de nado forçado (TNF) **Métodos:** Camundongos Swiss (30-35g) receberam 1 injeção de salina (SAL, 1 ml/kg, n=12) ou apomorfina (APO, 10mg/kg, n=10) 30 minutos (min) antes do TNF (6 min em um recipiente com água, 25°C). Dos testes gravados a latência, frequência e tempo gasto em todos os comportamentos exibidos foram registrados e totalizados em blocos de 1 (min-a-min) ou 6 min (total). O efeito do tratamento foi analisado pelo teste Mann-Whitney (significância p=0,05). Os resultados são expressos como média±EPM. **Resultados:** Os comportamentos exibidos foram classificados como escalar, nadar e imobilidade. Em todos os animais (grupo controle e experimental) o nado é o comportamento mais abundante (SAL: 220,7±15,5, APO:197,1±28,9 min) seguido da imobilidade (SAL: 115,5±14,3, APO:106,5±30,9 min) e do escalar (SAL: 23,75±4,8, APO:56,5±11,8 min). A análise qualitativa do registro min-a-min mostra que a imobilidade aumenta durante o teste enquanto que o escalar diminui em todos os animais. O nado é estável durante o TNF nos animais tratados com SAL, mas diminui nos tratados com APO. A frequência de imobilidade total diminuiu significativamente (p= 0,004) nos animais tratados com APO (7,2±1,7) quando comparados ao grupo SAL (16,4±2,3). A frequência e o tempo de escalar total aumentaram significativamente (p=0,002) nos animais tratados com APO (frequência: 6,5±1,2, tempo: 56,5±11,8) comparado aos animais do grupo SAL (frequência: 2,2±0,3; tempo: 23,7±4,8). As demais variáveis não foram afetadas por APO.

Conclusão: O catálogo comportamental e o tempo de curso dos comportamentos emitidos pelos camundongos no TNF são similares aos observados em ratos. O tratamento agudo com APO alterou as variáveis frequência de imobilidade e escalar, que em ratos são correlatos da atividade motora, sugerindo que estas variáveis podem ser indexes de alteração motora também em camundongos.

Palavras-Chave: Teste de nado forçado; Apomorfina; Camundongo

F.07.004

¿El núcleo que comanda la descarga del órgano eléctrico de *Brachyhypopomus pinnicaudatus* es blanco de hormonas esteroideas?

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Brachyhypopomus pinnicaudatus es un pez eléctrico gymnotiforme, reproductor estacional, que emite señales eléctricas sociales (SES) durante el cortejo. Las SES son modulaciones transitorias del ritmo y la forma de la descarga del órgano eléctrico (DOE). Estas señales son sexualmente dimórficas y exhiben variaciones estacionales por lo que es probable que sean moduladas por hormonas esteroideas. El núcleo electromotor bulbar (NEB) comanda la DOE y recibe aferencias de núcleos superiores. Está constituido por células relé y marcapaso.

Objetivos: describir la citoarquitectura del NEB y explorar la presencia de receptores de andrógenos (RA) que puede subyacer a la estacionalidad y al dimorfismo de las SES. Métodos: las técnicas de histología clásica y la construcción de un modelo 3D se usaron para describir la citoarquitectura del NEB. Para buscar RA realizamos inmunohistoquímica y western blot con el anticuerpo PG21 en el SNC de machos dentro y fuera del periodo reproductivo.

Resultados y conclusiones: las células relé son más grandes y están presentes en todo el NEB en sentido caudo-rostral. Las células marcapaso son más dorsales y están agrupadas en la mitad rostral del NEB. La distribución diferencial de las neuronas en el eje rostro-caudal y dorso-ventral puede estar correlacionada con una probable organización somatotópica del núcleo. Los RA están presentes en el SNC, específicamente en hipófisis, cerebro anterior y posterior; pero no en el NEB. Estos resultados sugieren que la modulación por hormonas esteroideas pueda ocurrir en núcleos superiores al NEB. Actualmente exploramos el uso de tratamientos con testosterona para potenciar la expresión de RA en el SNC.

Palavras-Chave: Pez eléctrico, señales eléctricas sociales, receptores de andrógeno,

Apoio Financeiro: PEDECIBA y PDT 043.

F.07.005

Neuroanatomy of the accessory olfactory bulb of the capybara *Hydrochaeris hydrochaeris*: correlating morphology with pheromonal communication.

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Pheromones are detected by the vomeronasal system. Two population of sensory neurons expressing either Gi or Go proteins, project to the rostral or caudal extent of the accessory olfactory bulb (rAOB and cAOB), and are activated by volatile vs. non-volatile ligands, respectively. Although many mammals possess these two pathways, most species with conspicuous sexual dimorphisms have lost the Go-cAOB pathway.

The capybara (*Hydrochaeris hydrochaeris*), a semi-aquatic, social and polygynic caviomorph, is the largest living rodent weighting up to 50 kg. Dominant males scent-mark their territory by rubbing their anal and snout (morrillo) glands on twigs and bushes, depositing an oily substance. Only males have a prominent morrillo. Thus, it is interesting to study capybara's AOB, as it is a sexually dimorphic rodent that is involved in non-volatile pheromonal communication.

Objectives: 1) To assess whether the accessory olfactory bulb (AOB) of the capybara express both G-proteins in segregated domains. 2) To study the organization of neurons in the AOB. 3) To compare morphoquantifications between rAOB and cAOB.

Methods: We used fixated olfactory bulbs of 5 capybaras. Sagittal sections were stained immunohistochemically against Gi or Go proteins, or processed with Nissl stain. We photographed and took measures of serial sections using the Spot camera and software.

Results: 1) The AOB of the capybara is segregated in rostral and caudal subdomains, each expressing either Gi or Go. 2) The mitral cell layer shows an inflection that coincides with the margin of G-protein expression. 3) The cAOB shows larger proportions than the rAOB, both in the area of the glomerular layer (in mm²: 0.95 ± 0.02 vs 0.76 ± 0.02 at the rAOB), and in the number of glomeruli (91.66 ± 2.15 vs 65.14 ± 1.61 at the rAOB).

Conclusions: This is, to our knowledge, the first report of larger glomerular features in the cAOB as compared with the rAOB. Our findings are coherent with previous evidence in rodents indicating that the cAOB would be involved both in sensing non-volatiles and in male-to-male signaling. We propose that non-volatile scent-marking may be crucial for semi-aquatic mammals.

Palavras-Chave: Vomeronasal System, Semiochemicals, Hystricognathi

Apoio Financeiro: Fondecyt 1080094, 1030522

F.07.006

Bases Neurales de la estacionalidad y dimorfismo de señales comunicativas en un pez eléctrico

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Los peces Gymnotiformes tienen comportamientos que se expresan mediante modulaciones de las descargas de su órgano eléctrico (DOE). Durante el período reproductivo (verano) tanto machos como hembras modulan la frecuencia de DOE produciendo señales eléctricas sociales transitorias (SES) que son claves en el cortejo y el desove. En *Brachyhypopomus pinnicaudatus*, los machos generan bruscos aumentos de frecuencia de DOE llamados « chirps » mientras que las hembras responden silenciando sus descargas. Estas SES son producto de entradas moduladoras al Núcleo Electromotor Bulbar (NEB), compuesto por neuronas marcapasos y de relevo, que provoca una DOE mediante un único pulso comando. El objetivo del estudio es encontrar las bases neurales del dimorfismo y la estacionalidad en la generación de señales sociales.

Se hicieron experimentos in vivo, en adultos fuera y dentro del período reproductivo. Se realizaron inyecciones por presión de Glutamato (10mM) en diferentes áreas del NEB, con registro simultáneo de DOE. Se realizaron hibridizaciones in situ de ARNm de diferentes subunidades del receptor NMDA en el NEB.

Los experimentos *in vivo* muestran que la inyección de glutamato en el NEB provoca diferentes modulaciones de DOE dependiendo del sitio de inyección, sexo y estado reproductivo del animal. Es posible generar chirps en machos maduros estimulando en la zona ventral del NEB rostral, que en hembras y adultos no reproductivos provoca una interrupción de la DOE. Dados los antecedentes que las interrupciones y los chirps son mediados por diferentes tipos de receptores glutamatérgicos, se está analizando por hibridización in situ la presencia de ARNm de NMDAR1, NMDAR2A y NMDAR2B en el NEB. Los tres están presentes en las neuronas de relevo del NEB, y las neuronas marcapaso muestran marca de mayor intensidad de NMDAR1.

En conjunto, los resultados del estudio sugieren que hay cambios en un pequeño subgrupo de neuronas del NEB, probablemente relacionados a la presencia o distribución de los receptores de glutamato, que subyacen la estacionalidad y las diferencias sexuales de un comportamiento clave en la vida del animal. **Palavras-Chave:** estacionalidad dimorfismo comunicación

Apoio Financeiro: PEDECIBA, PDT 043, PDT 55/29 (Uruguay), IBRO

F.07.007

Bases neurales de la modulación central de un sistema sensorial por un comando motor. Estudio *in vitro* del control sináptico de un núcleo marcapaso.

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En el pez eléctrico *Gymnotus omari*, la activación de la célula de Mauthner (CM), neurona comando para la respuesta de escape, provoca un aumento abrupto y duradero de la frecuencia de la descarga de su órgano eléctrico (DOE). Esta modulación revela un control eferente ejercido por un comando motor sobre su principal modalidad sensorial, la electrorrecepción activa. El análisis *in vivo* mostró que esta modulación implica aferentes glutamatérgicas que inervan las células marcapaso (cMP) del núcleo electromotor bulbar (NEB), origen del comando rítmico para la DOE, y la activación de receptores NMDA y metabotrópicos, pese a que las cMP expresan además receptores de tipo AMPA. Esto sugiere un patrón de inervación que difiere del descrito en estructuras centrales en mamíferos.

El control sináptico de la actividad de las células del NEB en esta especie se analizó en rodajas horizontales del tronco encefálico mediante registros intracelulares. Estas presentaron descarga espontánea y rítmica con frecuencia similar a las observada *in vivo* (20.9 ± 5.2 Hz). La activación de fibras aferentes al NEB (1 tren de 3 estímulos, 250 Hz) provocó aumentos duraderos (860 ± 310 ms) de la frecuencia de descarga, similares a los observados *in vivo* por estimulación de la CM: amplitud de 13.2 ± 8.5 Hz, tiempo al pico de 45 ± 22 ms y una fase lenta de recuperación de la frecuencia basal.

El antagonista para NMDA ($50 \mu\text{M}$) redujo la amplitud de la respuesta en un $57 \pm 25\%$. Contrariamente a lo observado *in vivo*, el antagonista para AMPA ($20 \mu\text{M}$) también redujo su amplitud ($46 \pm 19\%$). Llamativamente, el bloqueo de receptores AMPA modificó el curso temporal de la recuperación de la frecuencia, incrementando la duración de la respuesta, resultado similar al observado bajo antagonistas de receptores GABA_A (picrotoxina, 50 mM). En bajo calcio, persistió una respuesta (1.4 ± 1.6 Hz) restringida al intervalo de aplicación del estímulo.

Se concluye que la respuesta obtenida por activación de aferentes al NEB *in vitro* implica la activación de receptores NMDA y AMPA en las cMP así como un componente presumiblemente electrotónico. El curso temporal de la respuesta parece depender además de la activación de interneuronas GABAérgicas.

F.07.008

Efeitos da Intoxicação Progressiva e Aguda sobre a Preferência Claro-Escuro no *Betta splendens* (*Anabantidae*, *Teleostei*)

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OBJETIVO: Verificar se o *Betta splendens* apresenta preferência claro-escuro; bem como o efeito da re-exposição ao teste de preferência claro-escuro; e identificar efeitos de acetato de chumbo sobre a preferência claro-escuro.

MÉTODO: (1) Validação do teste de preferência claro-escuro: os sujeitos eram expostos às caixas preto/preto, branco/branco e preto/branco por 900s, com 300s de habituação; (2) Re-exposição ao teste de preferência claro-escuro: os sujeitos eram expostos ao teste durante 3 dias (24h de intervalo); (3) Efeito de doses progressivas na preferência claro-escuro: os sujeitos eram expostos a contaminação hídrica (12mgPb/L, 24h) antes de serem expostos ao teste de preferência claro-escuro, ao longo de 5 dias; (4) Efeito de doses agudas na preferência claro-escuro: os sujeitos eram contaminados agudamente com 60mgPb/L, com o teste de preferência ocorrendo ao longo de 5 dias (intervalo de 24h). Parâmetros observados: primeira latência, tempo total de permanência em cada compartimento, e número de alternância. Foi utilizada ANOVA (*two way*) seguido do Pós-Teste (Turkey).

RESULTADOS: Os sujeitos permaneceram maior tempo no compartimento preto, quando exposto a caixa preto/branco. Branco: $20,9 \pm 0,3s$; Preto: $97,5 \pm 1,0s$, $[F(1,48)=64,15, p<0,001]$. Os sujeitos não apresentaram nenhuma alteração de preferência claro-escuro durante os 3 dias de experimento $[F(2,48)=0,963, p=0,389]$; O chumbo não altera a preferência claro-escuro, mas observa-se uma diminuição no número de alternâncias nos dias 4 e 5 no Grupos Intoxicação Progressiva $[F(5,86)=3,037, p=0,014]$ e no Grupo Intoxicação Aguda $[F(5,80)=6,864, p<0,001]$.

CONCLUSÃO: O *Betta splendens* apresenta comportamento de preferência claro-escuro. A re-exposição ao teste não altera esta preferência. O chumbo em dose progressiva ou aguda não altera a preferência claro-escuro, mas diminui a atividade motora.

Palavras-Chave: Chumbo; *Betta splendens*; Preferência Claro-Escuro; Intoxicação Progressiva; Intoxicação Aguda.

Apoio Financeiro: CAPES

F.07.009

Are whiskers information fundamental to predatory behavior?

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Objective: Insect predation represents a fundamental motivated behavior critical in species maintenance. Lateral superior colliculus (SCI) is known to be related to motor control and sensory guided behavior and has an increase in Fos protein expression during hunting roaches, in rats. Rats with bilateral NMDA lesions placed into SCI handled preys very awkwardly being unable to capture and hold them efficiently. In this study we aimed to evaluate how effectively sensory information from whiskers could mediate predatory hunting in the context of collicular circuitry.

Methods: Rats hunted roaches and afterwards their whiskers were removed. They were exposed to roaches again and their brain immunoreacted for Fos protein. **Predation Test:** Rats were repeatedly handled by the same investigator and individually placed in a closed arena every day for 10 days. They were allowed to hunt by a simultaneous introduction of five mature intact roaches (*Leurolestes circunvagans*) into the cage. **Whiskers Removal:** After predation their whiskers were removed under anesthesia under a microscope with a small tweezer. After a survival time of 48 hours, animals were allowed to predate roaches again.

Results: Rats sniffed vigorously around the cage, located the roaches and rushed towards trying to seize them. Capture was assisted by grasping with the forepaws simultaneously or shortly after a killing bite administered toward the prey's head. Roaches were eaten voraciously. Amazingly rats without whiskers were able to capture and hold preys efficiently; and Fos immunoreactivity was found in the SCI of all animals that predated roaches.

Conclusion: This data suggest sensory information from whiskers are not triggering stimuli to initiate predation and, although sensory information is processed in SCI, Fos immunoreactivity might be more related to motor components of predation and suggest that the reason why animals lesioned at SCI did not predate efficiently (1) is motor loss. Also preliminary data suggest that whiskers might be important in prey recognition and hunting in rats not previously exposed to roaches.

References: (1) Abstract 847.5. Ann Meeting Soc Neurosc 2000, v. 26. p. 2257

Palavras-Chave: predatory behavior; whiskers; superior colliculus; Fos protein

Apoio Financeiro: FAPESP # 06/03655-4

F.07.010

Egr-1 expression induced by spontaneous vocalizations in the ventrolateral prefrontal cortex of the common marmoset (*Callithrix jacchus*)

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Objetivos: Immediate-early gene (IEG) expression has been widely used as a valuable tool to investigate brain areas activated by specific stimuli. Studies of natural vocalizations, specially in songbirds, have largely benefited from this tool. Here we used the expression of IEG *egr-1* (a.k.a. *zif-268*, *krox-24*, NGFI-A and ZENK) to investigate prefrontal areas activated by the utterance of vocalizations in the common marmoset (*Callithrix jacchus*).

Métodos: Eight adult male common marmosets were housed in sound-attenuating cages. Subjects in the control group (C, n=2) remained in silence. The six other animals were stimulated with playbacks of freely recorded natural long distance vocalizations (phee calls; 45 min total duration). The 'hearing and vocalizing' group (HV, n=3) comprised the subjects that spontaneously uttered antiphonal vocalizations, in contrast to the 'hearing only' group (H, n=3). After the stimulation (or no stimulation in the case of the C group), animals were perfused with phosphate-saline buffer and 4% paraformaldehyde. The tissue was frontally sectioned at 20 µm in a cryostat and submitted to immunohistochemistry for the IEG *egr-1*.

Resultados: Marked immunoreactivity was observed in the ventrolateral prefrontal cortex of HV subjects compared to the other groups.

Conclusões: In this *egr-1* expression study, the activation of ventrolateral prefrontal cortex by the utterance of vocalizations in common marmosets was in accordance with previous results for the IEG *c-fos* in *Saguinus fuscicollis* using deep brain electrical stimulation (Eur J Neurosci. 8(1):2, 1996). The ventrolateral prefrontal cortex corresponds to the 12/45 area in the marmoset brain, which encompasses vocal and visual areas (J Comp Neurol. 495(2):149, 2006). The cytoarchitecture of homologous areas in other primates, such as Rhesus macaques and humans (Eur J Neurosci. 16(2):291, 2002), suggests progressive evolution towards refined vocal control. The marked immunoreactivity observed for *egr-1*, an IEG involved in neuronal plasticity (J Biol Chem 269:15294,1994), may account for some degree of plasticity in the vocal communication of a non-human primate species, otherwise considered highly stereotyped.

Palavras-Chave: Vocalizações, genes imediatos, primatas não-humanos

Apoio Financeiro: CNPq, AASDAP, MCT, FINEP

EFEITOS COMPORTAMENTAIS INDUZIDOS EM RATOS POR UM PROTOCOLO DE ENRIQUECIMENTO AMBIENTAL.

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Objetivos: O enriquecimento ambiental é usado com diferentes propósitos como o estudo de mecanismos de memória e a diminuição do estresse. O presente estudo avaliou quais alterações comportamentais foram induzidas em animais submetidos a este protocolo de enriquecimento ambiental.

Métodos e Resultados: Foram utilizados ratos Wistar machos (2 meses) mantidos em caixas com 5 animais e divididos em dois grupos (n=10): ambiente enriquecido (AE) e controle (CONT, mantidos em caixas simples durante os 15 dias de experimento). Nos 6 primeiros dias, o grupo AE foi mantido em duas caixas que se comunicavam, uma com ambiente enriquecido (tampa mais alta e um esconderijo) e outra simples (tampa baixa). Durante este período foi avaliada a preferência dos animais pelo ambiente enriquecido, que chegou a 77,5 %. Nos 9 dias subsequentes, os animais foram mantidos em caixas com ambiente enriquecido (porém não comunicada) sendo então testados por 5 min. nos testes do campo aberto (CA) e do labirinto em cruz elevado (LCE). Os resultados foram expressos como média $\pm$ EPM. No LCE foi observado um aumento significativo do número de entradas nos braços fechados (AE=12,2 $\pm$ 0,9 x CONT=7,0 $\pm$ 1,3), no tempo de permanência na plataforma central (AE=46,3 $\pm$ 11 s x CONT=18,5 $\pm$ 5,6s) e uma diminuição do tempo de auto-limpeza (AE=15,0 $\pm$ 4,3s x CONT=41,4 $\pm$ 11,7s) realizados pelo grupo AE. No CA, houve um aumento significativo do número de quadrados intermediários percorridos (AE=21,0 $\pm$ 1,7 x CONT=14,4 $\pm$ 2,2) uma redução do tempo de inatividade (AE=10,9 $\pm$ 2,2s x CONT=21,5 $\pm$ 4,6s) e do número de auto-limpezas (AE=4,1 $\pm$ 0,6 x CONT=7,9 $\pm$ 0,9) realizados pelo grupo AE em relação ao grupo CONT.

Conclusões: Os resultados mostram que o protocolo de enriquecimento utilizado é capaz de alterar a atividade locomotora horizontal dos animais, além de provocar uma possível alteração na tomada de decisões (*decision-making*), esta última refletida pelo aumento do tempo de permanência na plataforma central do LCE e no número de quadrados intermediários percorridos no CA. Estudos subsequentes são necessários para elucidar os mecanismos envolvidos nas alterações descritas acima assim como avaliar a possível influência deste protocolo em outros modelos comportamentais.

Palavras-Chave: Enriquecimento Ambiental Alterações Comportamentais Ratos

Apoio Financeiro: FAPERJ

F.07.012

A influência do isolamento social sobre as diferenças comportamentais exibidas por ratos Lewis e SHR, um modelo genético de ansiedade

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O isolamento de um animal do seu grupo social para análises experimentais é uma situação frequentemente utilizada. A falta de contato físico gera uma série de reações comportamentais e fisiológicas que poderão afetar consideravelmente a reatividade emocional de ratos adultos. Neste estudo, nós avaliamos o efeito do isolamento social sobre o comportamento das linhagens de ratos, Lewis (LEW) e SHR, validadas como modelo genético para o estudo da ansiedade. Para isso, um grupo foi mantido em condições normais de agrupamento (4 animais/caixa) e outro foi submetido ao isolamento social (1 animal/caixa) a partir de 8 semanas de idade (N=8/linhagem/sexo/tratamento). Os animais foram submetidos aos testes de busca pela novidade (BN), campo aberto (CA) e labirinto em cruz elevado (LCE). Na BN, as linhagens diferiram no tempo gasto no compartimento novo somente nos animais agrupados (SHR 373,4±24,4; LEW 256,1±25,9; p<0,05). No CA, ocorreram diferenças entre as linhagens para tempo (SHR 26,5±2,2; LEW 11,7±2,0; p<0,001) e locomoção no centro (SHR 17,9±1,8; LEW 6,6±0,8; p<0,001), sendo que o isolamento teve efeito apenas na linhagem SHR (agrupados 31,9±3,1 e 23,0±2,2, isolados 21,1±2,7 e 12,9±2,4; p<0,05). O isolamento diminuiu em ambas as linhagens as medidas % de locomoção central (isolados 8,2±1,1; agrupados 12,5±1,2; p<0,001) e locomoção periférica (isolados 91,0±3,9; agrupados 104,8±2,6; p<0,001). No LCE, a ANOVA revelou diferenças de linhagem no número de entradas nos braços fechados (LEW 8,9±0,5; SHR 7,4±0,5; p<0,05) e tempo nos braços fechados (LEW 164,6±4,7; SHR 129,7±3,9; p<0,001). O isolamento causou diferenças significativas no número (LEW 3,4±0,7; SHR 1,3±0,4; p<0,05) e na % de entradas nos braços abertos (LEW 26,1±3,9; SHR 14,3±3,4; p<0,05). O isolamento social parece causar um efeito ansiogênico, sendo que os ratos SHR parecem ser mais suscetíveis a estes efeitos do que os ratos LEW. Isto sugere que as relações sociais durante o desenvolvimento dos ratos destas linhagens são essenciais para o comportamento normalmente exibido durante a idade adulta.

Palavras-Chave: rato, linhagens, isolamento social, ansiedade

Apoio Financeiro: CNPq

AVALIAÇÃO ECOLÓGICA DE METACOGNIÇÃO EM CRIANÇAS COM TRANSTORNO DE APRENDIZAGEM NA CIDADE DO RIO DE JANEIRO.

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Objetivo: O presente estudo pretende mostrar o desempenho de metacognição em crianças com transtornos de aprendizagem e grupo controle. Pais e professores de crianças com transtornos de aprendizagem (TA) e do grupo controle responderam a versão traduzida do Behavior Rating Inventory of Executive Function (Brief) que avalia o funcionamento executivo no dia a dia da criança.

Amostra: 10 crianças, que não distinguiram em sexo ($p < ,575$) e QI ($p < ,333$), meio sócio cultural variado. O grupo controle foi constituído por crianças que não apresentam problemas no comportamento e/ou no aprendizado, QI dentro do esperado, nunca repetiram ano escolar, nunca fizeram uso de medicação neurológica e/ou psiquiátrica. O grupo TA foi encaminhado pela fonoaudióloga com o diagnóstico previamente estabelecido.

Procedimento: Após os pais terem assinado o Termo de Consentimento Livre e Esclarecido, foi realizado o Wisc III reduzido nas crianças para verificar o QI. Os pais e os professores responderam ao BRIEF (questionário com 86 questões).

Os dados foram tratados pelo pacote estatístico SPSS. As provas e as sub-escalas considerados estatisticamente sem significância na diferença entre os grupos foram: pais - iniciativa ($p < ,295$); pais - monitoramento ($p < ,181$); professor – iniciativa ($p < ,106$); professor – memória operacional ($p < ,124$); professor, organização dos materiais ($p < ,293$); professor – monitoramento ($p < ,294$). Significativas foram as sub-escalas organização dos materiais ($p < 0,43$); professor – planejamento/organização. Altamente relevantes na distinção entre os grupos TA e controle foram pais-memória operacional ($p < 0,10$); pais – planejamento/organização ($p < ,006$).

Os resultados encontrados nesta análise exibem dificuldades em metacognição, especialmente na memória operacional, planejamento e organização quando comparados com o grupo controle. As diferenças encontradas no presente estudo são consistentes em toda literatura. Conclusão: O Brief é um questionário de fácil aplicação e sua alta confiabilidade sugere ser este um instrumento ecológico complementar importante de avaliação de crianças com Transtornos na Aprendizagem. **Palavras-Chave:** função executiva, transtornos na aprendizagem, cognição, desenvolvimento

Desempenho cognitivo em ratos jovens alimentados com dieta à base de semente de linhaça (*Linum usitatissimum*) desde a fase intrauterina.

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OBJETIVOS: A semente da linhaça (*Linum usitatissimum*) é considerada uma das melhores fontes vegetais de ácidos graxos (AG) n-3 (alfa-linolênico). Este estudo objetivou avaliar o efeito do consumo da linhaça desde o período pré-gestacional, gestação e lactação sobre a cognição de ratos. **MÉTODOS:** Ratas Wistar recém desmamadas formaram três grupos: Grupo Controle (GC), com ração à base de caseína; Grupo Linhaça (GL), com ração à base de caseína e semente de linhaça como fonte parcial de proteínas e total de lipídeos e fibras; e Grupo Controle Modificado (GCM), com ração à base de caseína adicionada de fibra e óleo de soja de forma equivalente ao GL. As fêmeas acasalaram aos 90 dias, seus filhotes foram desmamados aos 21 dias passando a receber as mesmas dietas das mães. Foram monitorados o consumo de ração e o peso dos filhotes para cálculo do Coeficiente de Eficácia Alimentar (CEA) e *Net Protein Retention* (NPR). A partir do 30º dia os animais foram testados no Labirinto Aquático de Morris (LAM). Os resultados foram tratados com o software SPSS10 através dos testes de Mann Whitney e de Kruskal Wallis. Foi considerado significativo $p < 0,05$. **RESULTADOS:** O GC apresentou maior CEA ($0,35 \pm 0,01$ $p < 0,05$), seguido do GCM ($0,31 \pm 0,02$ $p < 0,05$) ficando o GL com o menor valor ($0,30 \pm 0,02$ $p < 0,05$). O NPR do GL também foi inferior ($3,39 \pm 0,25$ $p < 0,05$) enquanto o GC ($4,29 \pm 0,34$) e GCM ($4,29 \pm 0,39$) foram semelhantes. No dia 1 do LAM o GL apresentou latências inferiores ao GC ($p < 0,05$) e similares ao GCM, também similar ao GC (GL= $35,14 \pm 8,81$; GC= $44,90 \pm 12,50$ e GCM= $45,69 \pm 8,81$ s). O padrão se repetiu nos dias 2 e 3 (GL= $22,38 \pm 5,36$ e $17,01 \pm 4,91$; GC= $29,65 \pm 5,28$ e $22,95 \pm 5,68$ e GCM= $29,55 \pm 13,06$ e $23,11 \pm 17,36$ s). No dia 4 as latências dos três grupos foram semelhantes e no dia 5 o GL apresentou menor valor ($p < 0,05$; $8,70 \pm 5,50$ s) do que o GC ($12,69 \pm 5,65$ s). O GCM ($14,62 \pm 5,76$ s) manteve semelhança com o GL e com o GC. **CONCLUSÃO:** O resultado do LAM sugere maior incorporação de n3 no cérebro favorecendo o aprendizado, no entanto a adição de linhaça à dieta materna interferiu no crescimento do GL provavelmente em função da relação entre AG n-6 e n-3 da semente.

REFERÊNCIAS BIBLIOGRÁFICAS: Prog. Lipid. Res. 40: 1, 2001;

J. Nutr. 127: 184, 1997.

Palavras-Chave: DHA, neurodesenvolvimento, cognição, ratos

Apoio Financeiro: Capes

F.08.003

Capacidad de memoria de trabajo y desarrollo de la teoría de la mente: Evidencia de no asociación en el nivel preescolar

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Objetivos: Se busca determinar si existe una asociación entre la memoria de trabajo y el desarrollo de la teoría de la mente. Aunque esto ha sido previamente sugerido existe evidencia inconsistente (1). Estas inconsistencias podrían derivar del uso de tareas de memoria de trabajo poco precisas (2), cuya resolución involucra el ejercicio simultáneo de otros procesos cognitivos (como el control inhibitorio o habilidad verbal). Aquí, utilizando tareas de memoria de trabajo que no involucran demandas significativas de otros procesos secundarios, analizamos la relación entre estos dos procesos por medio de tareas más específicas.

Métodos: El estudio incluye 60 participantes costarricenses (de 5 a 7 años), quienes fueron evaluados por medio de una batería de cinco pruebas de teoría de la mente y dos tareas de capacidad de memoria de trabajo. Además, tres subpruebas de habilidad verbal y una tarea de control inhibitorio fueron utilizadas como variables de control.

Resultados: Las tareas de capacidad de memoria de trabajo correlacionaron positivamente entre sí, y no mostraron asociaciones con las medidas de habilidad verbal, ni con la medida de control inhibitorio. Las tareas de teoría de la mente cumplieron con los criterios para agregar sus puntajes en un solo marcador. No hubo correlaciones entre las tareas de memoria de trabajo y el puntaje agregado de teoría de la mente en la muestra en general ni tampoco por sexo y edad. Además, se encontró que las diferencias del desempeño de los participantes en la batería de teoría de la mente según la edad, fueron independientes de los puntajes de memoria de trabajo.

Conclusiones: Utilizando tareas más específicas de memoria de trabajo, nuestros resultados descartan la asociación entre este proceso y la teoría de la mente durante el nivel preescolar. El uso de tareas inespecíficas podría involucrar otros procesos cognitivos que sí se asocian con la teoría de la mente, explicando en parte las correlaciones comúnmente reportadas entre ambos procesos. Destacamos la relevancia de analizar a profundidad los componentes de las tareas incorporadas en los diseños.

Referencias: 1- *Infant Child Dev* 11:73, 2002; 2- *Child Dev* 73:752, 2002.

Palavras-Chave: memoria de trabajo, teoría de la mente, desarrollo cognitivo infantil

Apoio Financeiro: Vicerrectoría de Investigación, Universidad de Costa Rica

PERCEPÇÃO DE FACES: ESPECIFICIDADE INATA?

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Objetivos e Método: Reunir, através de pesquisa bibliográfica, os principais estudos recentes envolvendo a questão da preferência inata por faces e sua especificidade de processamento.

Resultados: Desde que Fantz (1961) concluiu que os bebês despendiam mais tempo olhando para desenhos de rostos do que para objetos, algumas pesquisas vêm se aprofundando na investigação da existência de um mecanismo inato de preferência visual por faces **(1)**.

Na década de 1990, o debate se concentrou em torno de quais características da face seriam as responsáveis pela sua atratividade para o bebê: um suposto padrão de três pontos (correspondentes aos olhos e boca) ou sua frequência espacial e seu contraste, mais adequados ao nível de sensibilidade do bebê que a outros estímulos.

Valenza et al. (1996) demonstraram que uma imagem com o padrão de três pontos provocou maior preferência visual que outra com propriedades físicas mais adequadas ao sistema visual do bebê **(2)**. Já De Heering et al. (2008) sugerem a frequência espacial como preponderante no reconhecimento de faces **(3)**.

Através de utilização de ERP's (*event-related potentials*), Halit et al. (2004), sugeriram haver em bebês de 3 meses algum grau de especificidade cortical para o processamento de faces **(4)**.

Pascalis et al. (2002), trabalhando com a percepção de faces humanas e de chimpanzés em bebês de seis e de nove meses de idade, observaram que os bebês mais novos eram capazes de reconhecer diferentes faces humanas e de chimpanzés. Os bebês mais velhos, assim como os adultos demonstraram reconhecer apenas faces humanas, sugerindo um estreitamento dessa habilidade ao longo do desenvolvimento, tal como ocorre na discriminação de fonemas de diferentes línguas **(5)**.

Conclusão: Diante de tantos indícios, são necessárias mais pesquisas que esclareçam o início do desenvolvimento do processamento cerebral das faces humanas.

Referências: 1 – Scientific American 204:66, 1961; 2 – J. Exp. Psychology: Human Perception and Performance 22:892, 1996; 3 – Cognition 106:444, 2008; 5 – Science 296:1321, 2002.

Palavras-Chave: percepção, faces, desenvolvimento perceptivo

Apoio Financeiro: CNPq

ORIGENS DO PROCESSAMENTO CORTICAL DE FACES EM BEBÊS

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Objetivos e Método: Reunir, através de pesquisa bibliográfica, os principais estudos recentes sobre as origens do processamento cortical de faces em bebês.

Resultados: Morton e Johnson (1991) propuseram a existência de um mecanismo subcortical (CONSPEC) cujo objetivo seria direcionar a atenção do recém-nascido para faces humanas. Posteriormente, surgiria um mecanismo cortical (CONLERN) que seria voltado para o aprendizado das faces, substituindo o CONSPEC **(1)**.

Valenza et al. (1996) discordam de que o CONSPEC seria subcortical. Apesar de o córtex visual não estar tão desenvolvido quanto o colículo superior na ocasião do nascimento, os autores apontam indícios de que o hemisfério direito exerceria o processamento de padrões semelhantes à face **(2)**.

Slater et al. (1988) demonstraram que o recém-nascido possui algum grau de funcionamento do córtex visual por haver discriminação de orientação espacial, a qual exige uma atividade exclusivamente cortical **(3)**.

Através de utilização de ERP's (*Event-Related Potentials*), Halit et al. (2004), sugeriram haver, já em bebês de três meses, algum grau de especificidade cortical para o processamento de faces. O cérebro dos bebês demonstrou, durante o processamento visual de faces, uma ativação de amplitude semelhante à encontrada em adultos, mas em uma área diferente, a qual seria precursora da localização adulta **(4)**.

Johnson (2005) reafirma sua teoria de haver, no recém-nascido, uma via subcortical de processamento de faces, a qual orientaria o bebê para as faces e forneceria estímulos para as vias corticais. As projeções corticais da via subcortical provocariam a ativação de áreas específicas, incluindo o córtex fusiforme, durante o processamento das faces. Algumas partes do córtex fusiforme se tornariam, então, sensíveis a faces **(5)**.

Conclusão: Novas tecnologias não invasivas para o estudo do cérebro, como a magnetoencefalografia, podem ser utilizadas em estudos futuros para elucidar essas questões em recém-nascidos.

Referências: 1 – Psychological Review 98:164, 1991; 2 – J. Exp. Psychology: Human Perception and Performance 22:892, 1996; 3 – Perception 17:597, 1988; 5 – Nature Reviews Neuroscience, 6:766, 2005.

Palavras-Chave: percepção, faces, desenvolvimento perceptivo

Apoio Financeiro: CNPq

METACOGNIÇÃO NA FORMAÇÃO DOCENTE

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Objetivo: É grande o interesse mundial em desenvolver a aprendizagem reflexiva, sendo importantes alternativas educacionais que desenvolvam a reflexão e a auto-orientação do aluno frente a sua própria aprendizagem (CLAXTON, 2005). Atividades metacognitivas permitem ao aluno a realização de aprendizagens eficazes, possibilitando a elaboração de conhecimentos sobre como aprende, levando-o à condução gradual de seus processos de conhecer, aprendendo a pensar sobre seu próprio pensamento (GANGREAT, 1999). “O monitoramento e a auto-regulação cognitiva envolvem atividades guiadas pelo conhecimento metacognitivo que proporcionam informações sobre o progresso em alguma iniciativa cognitiva” (FLAVELL, MILLER e MILLER, 1999, p. 143). Para Monereo (apud MATEOS, 2001, p. 120), dificilmente a metacognição encontrará campo fértil para seu desenvolvimento enquanto os professores não transformarem suas práticas docentes, cabendo ao educador desenvolver conhecimentos teórico-práticos nessa área. Nesse contexto, o estudo objetivou investigar a abordagem da metacognição na formação de professores.

A investigação: *corpus* da pesquisa: 100 alunos formandos dos cursos de Licenciatura em Biologia, Física, Química, História, Geografia, Matemática, Letras e Pedagogia da Fundação Universidade Federal do Rio Grande. Foi aplicado um questionário com a questão *O que entendes por metacognição?*

Resultado: 88% desconheciam o termo; 12 % ofereceu algum tipo de resposta que demonstrasse seu entendimento do assunto. Entretanto, as respostas apresentavam lacunas e/ou equívocos, sendo que os alunos usavam como subsídio a análise etimológica da palavra ou suas teorias implícitas.

Conclusão: O desconhecimento acerca da temática demonstra que não é possível esperar práticas educativas inovadoras baseadas na metacognição por parte desses futuros professores a curto prazo. Há necessidade de abordar o tema metacognição nos cursos de formação de professores, contribuindo para uma visão aprimorada do aprender a aprender; uma revisão curricular que promova o entendimento da metacognição, bem como o seu exercício prático; a articulação entre neurociência cognitiva, metacognição e pedagogia. Em consequência desse entendimento, poderiam surgir alterações conceituais de aprendizagem, ensino e aluno, o que poderia se converter na adaptação de processos didáticos em busca da otimização do ensino e da aprendizagem.

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Palavras-Chave: Metacognição, formação de professores, transformação de práticas docentes

Apoio Financeiro: FURG

Redes Neurais: um instrumento no rastreio (screening) de pessoas com risco de transtorno específico de leitura

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Objetivo - Este trabalho apresenta a implementação de uma rede neuronal do tipo Multi Layer Perceptron (MLP) para rastrear pessoas com risco de transtorno específico de leitura (dislexia). **Método** – O delineamento do estudo se fez através de classificação probabilística, usando a técnica computacional de Redes Neurais. Foram utilizados dados de pessoas com e sem diagnóstico do transtorno de dislexia, baseados nas escalas diagnósticas padronizadas. A RNA utilizada neste trabalho é do tipo feedforward com duas camadas de neurônios, com múltiplas entradas $z_1, \dots, z_E$ e uma saída $\hat{y}$. Os dados foram coletados a partir dos questionários fornecidos pela Associação Nacional de Dislexia (AND) sediada no Rio de Janeiro. A amostra é composta de 50 casos, sendo 25 de pacientes disléxicos e 25 de não-disléxicos. A partir do questionário foi construída uma tabela com 50 amostras. **Resultados** – Neste estudo foram realizados dois experimentos. No primeiro experimento verificou-se que a rede neuronal sem a utilização da técnica de Análise de Componentes Principais (PCA) fez uma boa classificação dos dois grupos (Disléxicos e Não-Disléxicos). No segundo experimento, observou-se que, com a utilização da técnica do PCA, a rede apresentou uma classificação melhor para os dois grupos (Disléxicos e Não-Disléxicos) e houve a convergência em um número menor de épocas. Comparando os dois resultados, a rede neuronal sem a utilização do PCA poderá apresentar melhor resultado quando utilizar o pré-processamento nos dados brutos. **Conclusão** - Com base nos resultados obtidos considera-se a pertinência da tecnologia computacional no rastreamento de pessoas com risco de transtorno de leitura, a fim de auxiliar essas mesmas pessoas e profissionais das áreas de saúde e educação. Tendo em vista o tamanho da amostra, não podemos garantir a generalização, mesmo assim consideramos que o conjunto de técnicas computacionais foram ferramentas úteis no rastreamento de pessoas com dislexia neste experimento. O trabalho requer aprimoramento no sentido da criação de uma base de dados representativa para o treinamento da rede. A partir deste trabalho, serão realizados outros experimentos com o público infantil. **Palavras-Chave:** Rede Neuronal, Dislexia, Transtorno de Leitura, Screening

On the relationship between connection pruning and dimensionality reduction in simple recurrent networks

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Objectives: Simple recurrent Networks (SRN) are one of the mostly used artificial neural networks in connectionist language modeling [1]. Hoffman and coworkers [3] trained a SRN and showed that after synaptic pruning the network erroneously perceived words when only noise was present in the input, a behavior they assimilate to verbal hallucinations in schizophrenia. We have recently reproduced these results with a different version of recurrent network, the SPELT model, which is based on multiplicative units and only two layers [2]. Nevertheless, Hoffman and co-workers found that intermediate levels of pruning produced an increase in the performance of the network, something that we did not find in our model. In this work we analyze the reasons that make pruning enhance the SRN network capabilities.

Methods: We trained 20 SRNs using the protocol in [2]. Each network was trained with randomly-selected coding of the input words. The output words used a fixed sparse coding with features indicating part of speech and semantic category. We induced several levels of pruning by eliminating a fraction of the lowest synaptic weights between the working memory and the hidden layer. We evaluated the recognizing capabilities of the network with a set of sentences different from the training set. We performed principal component analysis of the hidden layer's activation vectors [1].

Results: The intermediately pruned networks showed a better clustering of words according to their syntactic/semantic category than both the full trained network and the deteriorated network. The full network is more sensitive to idiosyncratic details in the training set, whereas the deteriorated network groups together unrelated words and noise. This behavior is explained by the distribution of weights in the hidden layer.

Conclusions: The training procedure results in overfitting of the weights to the training set. Pruning partially solves this problem, in a phenomenon akin to dimensionality reduction [4].

References: 1-*Cognitive Science*, 14, 179-211, 1990; 2-*J Cogn Neurosci*, 7: 479-497, 1995; 3-*Neural Networks*, 18:863-877, 2005; 4-*ESANN' 2003 proceedings, ISBN 2-930307-03-X* pp. 287-294, 2003. **Palavras-Chave:** SRN, synaptic pruning, dimensionality reduction

Apoio Financeiro: PDT/FCE S/C/IF754/002

G.01.003

Um modelo computacional neural bio-realista para o estudo da formação de memória em ratos.

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Objective To construct a large-scale computational model of four different rat forebrain regions (primary somatosensory "barrel" cortex, ventral posteromedial thalamic nucleus, hippocampus, and dorsal putamen) in order to study reverberation, storage and propagation of memories.

Methods The model will incorporate known data about neuronal and synaptic densities as well as intrinsic and extrinsic wiring of these areas. Neurons will be modeled by Izhikevich's equations to replicate spiking properties of the major neuronal electrophysiological classes found in these areas (1). Activity-dependent synaptic plasticity rules will be implemented to simulate both short-term and long-term learning paradigms. The model will be submitted to stimuli simulating whisker input during exploratory behavior intercalated with periods without stimuli to simulate sleep stages and the responses will be analyzed by the neuronal ensemble correlation method used by Ribeiro and Nicolelis (2).

Results Successfully assessing the spatiotemporal evolution of ongoing neuronal activity of cell populations of these four rat forebrain areas, we expect to describe *in silico* patterns of memory acquisition and consolidation very similar to those found *in vivo*: for example, the recurrence for several hours of firing patterns generated during the exploration of novel objects after a novel sensory stimulation experience and the long-lasting neuronal reverberation phenomena in the rat cerebral cortex, hippocampus, putamen and thalamus.

Conclusions: The development of this large-scale, anatomically detailed model of these four rat forebrain regions involved in memory processing is crucial to deepen our understanding of how synaptic and neuronal processes interact to produce, to distribute and to maintain memories in the mammalian brain; also, this computer model will allow very specific manipulations that are impossible to carry out with animals, as the global effect of a single spike in the whole neural network.

References: 1 - PNAS 105: 3593-3598, 2008; 2 - Learn. Mem. 11: 686-696, 2004.

Palavras-Chave: neurônio, memória, modelagem computacional

G.02.001

Emergência de mecanismos de seleção sensorial em simulações de vida artificial

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Objetivo

Este trabalho busca esclarecer as vantagens da atenção seletiva através de uma abordagem evolutiva.

Métodos e Resultados

Foram desenvolvidas simulações de vida artificial em computador, nas quais animais artificiais possuem redes neurais com 2 nós de entrada, um número variável de nós internos e 2 nós de saída. Os estímulos fornecidos às redes indicam se há “comida” ou “não-comida” à esquerda e à direita e a saída da rede indica se o animal come ou não o que está em cada uma das posições. Os pesos das redes neurais evoluem por meio de um algoritmo genético. O valor adaptativo do indivíduo é determinado pela quantidade total de comidas (ganho) e não-comidas (perda) ingeridas. Seis simulações foram realizadas, nas quais as redes neurais tinham 2 ou 8 nós internos e os estímulos podiam ter as mesmas relevâncias—a ingestão de comida à esquerda ou à direita resultava no mesmo ganho de 6—ou relevâncias diferentes—a ingestão de comida à esquerda resultava em um ganho maior do que a ingestão de comida à direita (9 para a esquerda e 3 para a direita em duas simulações, 11 para a esquerda e 1 para a direita em duas simulações). Após 40000 gerações, os animais foram testados com 10000 estímulos e foi determinada a detectabilidade (d') em relação aos estímulos à esquerda e à direita. Nas simulações em que os estímulos tinham a mesma relevância, foi observado que os animais com 8 nós internos eram capazes de detectar comida à esquerda e à direita e, quando os animais tinham 2 nós internos, metade deles só detectava comida à esquerda e a outra metade, só comida à direita. Os animais das simulações em que os estímulos à esquerda eram mais relevantes do que os estímulos à direita tinham maior capacidade para detectar comida à esquerda do que à direita.

Conclusões

O surgimento de mecanismos de seleção de estímulos está relacionado não somente ao tamanho da rede neural, mas também à diferença entre as relevâncias dos estímulos. A atenção pode ser capturada por estímulos de maior relevância mesmo nos casos em que o animal tem um sistema nervoso capaz de processar todos os estímulos. **Palavras-Chave:** atenção, vida artificial, rede neural, algoritmo genético

Apoio Financeiro: FAPESP

G.02.002

Search Strategies in Homogenous Environment

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Objectives: The search for food is fundamental for the survival of many animals' species. There are studies conducted to describe animal's strategies of search and also to determine strategy that gives the animals the best chance of finding food [1,2]. Our objective is to explore which of the 3 strategies, Lévy walk (LW), Brownian motion (BM) and straight-line movement (SL), gives consumers the best chance of surviving in a virtual environment.

Methods: In order to investigate movement, we used individual based model approach to simulate consumers in a virtual environment. We tested the 3 movement strategies against 3 different food quantities (10, 20 and 40 units) randomly distributed. In our model, consumers detect food by vision of radius r , expend energy during the search and replace it by food intake. Once consumed, food disappears and reappears in a different location randomly asserted. The time T50 when 50% of consumers died of starvation in each simulation was stored.

Results: It was analyzed 900 simulations. T50 for SL for each food quantity was superior ($1048 \pm 66,5$; $2144 \pm 264,7$; 6286 ± 1281) compared with LW ($727 \pm 55,6$; $922 \pm 83,6$; $1471 \pm 232,1$) and BM ($668 \pm 48,5$; $832 \pm 70,7$; $1184 \pm 138,2$).

Conclusions: Our simulation model shows that SL gives the best chance of survival in an environment where food is scarce. It is due to the fact that consumer's movement describes greater displacement, exploring different areas, not spending time in non-profitable areas. BM is the opposite, its displacement is short and consumer spends too much time exploring the same area. LW is a kind of mixture of both movements, consumers spend part of their time with small displacements and part with bigger ones. Although random walks are widely found as a food search pattern, we hypothesize that straight-line like movements would have an important role in animal survival and might be used as an alternative strategy when food is scarce. There are empirical studies [e.g. 3] that show straight-line movements during foraging reinforcing our theoretical results.

References: 1- Nature 449:1044, 2007; 2- Europhy. Lett. 75:349, 2006; 3- Polar Biosci. 15:75, 2002.

Palavras-Chave: simulation IBM search strategy Lévy walks Brownian

Apoio Financeiro: FAPESP 06/02171-3

THE UTILIZATION OF FACTOR ANALYSIS IN THE STUDY OF ELEVATED PLUS-MAZE AND ARENA EXPLORATORY BEHAVIORS OF WISTAR, WILD AND DERIVED RATS STRAINS.

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Objectives: Several studies have been demonstrated that domestication alters many features and behaviors of Wistar rat, like aggression, exploratory, reproduction and stress response. The present work aimed the comparison of exploratory behaviors of the wild brown rat (*Rattus norvegicus sp*) and Wistar derived strains and the analysis of hybridization effects of Wistar and Wild rats on exploratory behaviors.

Methods: The groups of rats were organized as follows - Wistar derived strains (WT), Wild derived strains (WD), Wistar rats (WIS), Spontaneously hypertensive rats (SHR), Wistar-Kyoto rats (WKY), Wild rats (WLD), Hybrids WIS x WLD (HBR), Backcross hybrids HBR x WLD (HWD). The exploratory behavior was analysed in the special arena and the peritaxis (PX) and centrotaxis (CX) were registered. After, all the rats were submitted to plus-maze test with the number of entries in open and closed arms and the time spend in these arms. Variables were subjected to a two-factor fixed factor analysis. Factors were extracted by PROMAX (Procrustean) rotation of orthogonal factors obtained by VARIMAX prerotation of original factors. PROMAX oblique rotation was selected to get a more parsimonious description of data. Statistics was carried out by the 'Factor Procedure' of SAS[®] software (Statistical Analysis Systems, Cary, North Carolina, USA). **Results:** The rat exploratory behavior is basically characterized by OT, ET and PX variables. Strain exploratory behaviors loaded differently on anxiety and activity factors. Whereas the CX of WIS strain is controlled by anxiety and activity, CX of SHR loaded on activity only (Fig.3). Rats from the WLD strain were the more anxious, whereas those of the WKY strain were the least ones. WKY exploratory pattern differed markedly from both the WIS and SHR strains. Virtually, anxiety and activity account for the entire variance of WLD male rats (91.7%).

Conclusion: Sex had a powerful influence on EPM and OF exploratory behaviors. Activity is the main factor controlling the exploratory behavior of female but not male rats from WIS derived strains. Anxiety is the main factor controlling the exploratory behavior of rats both male and female of WLD derived strains.

Palavras-Chave: Data analysis, strains, wild rat, open field, hibred rat

Apoio Financeiro: CNPq

G.03.002

Fractal motor patterns in the rat motor activity are regulated by the suprachiasmatic nucleus.

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OBJECTIVES. In mammals, the hypothalamic suprachiasmatic nucleus (SCN) drives the circadian motor rhythms, which had in the rat a period >24h when observed under constant darkness (DD). Prolonged exposure to constant bright light (LL) increase circadian period until the rhythm is abolished, while lesion of the SCN tissue (SCNx) produces an abrupt loss of rhythmicity under DD. However, a description in both time and frequency domains of these arrhythmic phenotypes, is not available up to date. Thus, time-series of rats whose motor rhythms were impaired either by LL, or by SCNx, were analysed. Also, data sets collected in a previous study, from animals that showed unexpected lack of a circadian period in spite of being maintained under DD, were included (N=5). **METHODS.** Male rats were submitted to LL (N=13), and to SCNx under DD (N=12), to generate arrhythmicity. The Fourier harmonics, as well as fractal coefficients obtained using Power Spectral Density (*b* slope) and Autocorrelation analyses (*Ac* slope), were calculated for the individual time-series, in 10-day data section with undetectable circadian rhythm. **RESULTS.** Fourier spectrum detected high power content (PC) for ultradian harmonics in both LL and DD groups, (LL: 8-4h; DD: 6-4h), and no dominant harmonic in the SCNx group (mean±SEM of PC: LL: 8h: 5.9±0.8, 6h: 8.6±0.9, 4.8h: 8.7±1.1, 4h: 6.5±0.8; DD: 6h: 4.3±1.3, 4.8h: 4.9±1.5, 4h: 5.9±0.8). Fractal coefficients showed that, while scale-invariant motor patterns were absent in the SCNx group, they were present with a different extent in the LL and DD groups (*b* slope: LL: -0.73±0.02; DD: -0.81±0.07; SCNx: -0.61±0.02; *Ac* slope: LL: -1.04±0.04; SCNx: -2.26±0.09; t-test, p<0.0001). **CONCLUSIONS.** These results give heuristic hypotheses about the SCN function: 1) arrhythmic patterns of SCN-intact rats under LL and DD have a temporal organization, both at ultradian and high frequency ranges, which may depends on the SCN activity beyond the 24-h regulation; 2) since both ultradian and scale-invariant patterns seems to be modulated by lighting conditions, interactions among controlling factors in the SCN (i.e. oscillator elements) could be involved in its generation. **Palavras-Chave:** circadian, suprachiasmatic, rat, Fourier analysis, fractal analysis

Apoio Financeiro: Consejo Nacional de Investigaciones Científicas y Tecnológicas, Argentina.

G.03.003

Análise de sincronização de fase em um modelo para atividades epileptiformes por mecanismos não sinápticos no giro denteado do hipocampo de rato.

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Objetivos: Identificar, usando simulações e análises estatísticas, os mecanismos biofísicos responsáveis por sincronização de fase, em determinadas faixas de frequência, em um modelo de atividade epileptiforme sustentada por mecanismos não-sinápticos (AENS), no giro denteado (GD) do hipocampo de rato.

Metodologia: O GD do hipocampo de rato foi simulado por uma rede de compartimentos celulares justaposta a uma rede de compartimentos extracelulares. Estudaram-se os efeitos dos acoplamentos por eletrodifusão extracelular, *gap-junctions* e efeito de campo elétrico populacional sobre a sincronização dos potenciais transmembrânicos e suas consequências sobre os potenciais extracelulares. Condições iniciais aleatórias foram estabelecidas para as concentrações iônicas e potenciais transmembrânicos neuronais. Em seguida, foram variados aleatoriamente os parâmetros que definem as formas e períodos dos bursts no meio intracelular. A detecção de sincronização de fase seguiu as seguintes etapas: 1) pré-processamento dos sinais usando procedimentos de filtragem; 2) extração das fases usando-se transformada *de wavelet* e transformada de Hilbert; 3) cálculo de índices de sincronização sobre fases e diferenças de fase: coerência de fase média, entropia de Shannon e informação mútua. Os índices clássicos de detecção de correlação temporal também foram usados para comparação.

Resultados: Os índices indicaram tendência à sincronização em fase abaixo de 1 Hz quando da supressão dos acoplamentos por *gap-junctions* e efeito de campo. Isto sugere que a eletrodifusão extracelular deve atuar como mecanismo sustentador desta tendência. Religando esses acoplamentos observaram-se regimes próximos ao de sincronização completa em todas as faixas de frequência. Em outros termos, esses acoplamentos atuaram sobre os potenciais de ação, que oscilam em frequências acima de 10 Hz.

Conclusões: Os resultados sugerem ser o acoplamento por eletrodifusão no meio extracelular o mecanismo essencial para sincronização de *bursts*. As *gap-junctions* e o efeito de campo elétrico populacional demonstram atuar como acopladores rápidos agindo somente sobre sincronização de potenciais de ação.

Palavras-Chave: Sincronização, atividade epileptiforme, hipocampo

Apoio Financeiro: CNPQ

ETÓGRAFO 2: Etografia, Análise de Locomoção e Classificação Automática de Comportamentos através de Recursos Estatísticos

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Introdução: Ferramentas de etografia e análise automática de experimentos auxiliam na classificação e registro dos comportamentos em situação experimental, na extração de dados de movimentação como distância percorrida, velocidade média, permanência em certas áreas da arena, etc, ampliando e facilitando a obtenção de dados. **Objetivo:** Criar uma ferramenta analítica que realizasse etografia e cálculo da atividade motora de animais de laboratório em arenas. **Materiais e Métodos:** Os vídeos digitais de roedores em campo aberto foram adquiridos com uma webcam conectada a um computador Intel-P4 3 GHz, 512MB RAM. O sistema desenvolvido une 2 softwares produzidos pelo laboratório: o Etógrafo®, para registro e análise de comportamentos, e o Espião, que realiza a análise da atividade (tracking). A ferramenta obtida permite, além das funcionalidades anteriores, uma análise etográfica com um relatório de atividade geral e locomoção ao fim do processamento automático. Além disso, o novo aplicativo foi dotado de um classificador automático de comportamentos baseado em recursos estatísticos (média e desvio padrão), aplicados a variáveis de interesse (obtidas automaticamente do vídeo digital): número de pixels do animal e pixels alterados a cada quadro, distância total percorrida, etc. Os padrões de classificação de cada comportamento são obtidos pela extração dos parâmetros estatísticos de trechos de vídeo que representam os comportamentos que o pesquisador deseja identificar, fornecidos pelo mesmo ao criar um catálogo de comportamentos baseado em segmentos. Com o catálogo, o usuário aplica o classificador no vídeo que deseja classificar/analisar, e ao fim, o sistema gera um relatório (latências, durações, frequências) com os comportamentos encontrados e os trechos não identificados. **Conclusão:** Testes em situação experimental real indicam alta precisão temporal dos dados extraídos (até 0.03 s) e alta sensibilidade na detecção, por exemplo, de efeitos de ansiolíticos em campo aberto. Este software permitirá o uso de técnicas de Inteligência Artificial para diferenciar e classificar esses padrões. Versões de teste do software serão apresentadas e distribuídas durante o Congresso.

Palavras-Chave: Etografia, Análise de Atividade, Classificação Comportamental

Apoio Financeiro: CAPES

Development of a Graphical Interface for a MatLab® Application of Neuronal Oscillation Analysis

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Objectives. Oscillatory patterns of extracellular potentials play an important role in organizing the activity of neuronal ensembles across different brain areas. Analysis of such patterns can provide significant information on circuitry activity underlying normal and abnormal brain functions, such as those observed in neurological disorders. Here, we created a graphical interface for a recently developed computational tool to analyze oscillatory activity in extracellular local field potentials recorded from behaving animals [1]. **Material and Methods.** MatLab routines originally running from the command line prompt were implemented in a graphical user interface (GUI) by using the interactive built – in tool from MatLab® 6.5 installed in a PC computer running Microsoft Windows XP Professional. We adopted a modular approach for the development of the GUI, consisting of a main window from which various available tasks could be selected from pull-down menus. **Results.** The interface consists of: (1) Main module: allows plotting of data and access to processing modules; (2) Epoch Save module: allows extraction of epochs from local field potential files; (3) Wavelet module: allows calculation of spectrograms and sustained oscillations from multiple animals through wavelet time-frequency analysis; (4) Cross Subject Analysis module: provides an output descriptive statistical analysis of oscillatory events in multiple subjects; (5) Coherence module: estimates the level of synchronization between pairs of time series. **Conclusions.** The graphical interface will provide a friendlier environment for software use, even for users without knowledge of MatLab commands. It also allows the contribution from other researches to a public access application. **Reference.** [1] Journal of Neuroscience Methods 167:384, 2008. **Palavras-Chave:** Neuronal Oscillation , MatLab® Application , Graphical User Interface, Data Analysis

Apoio Financeiro: FAPESP, CNPq, CAPES

G.03.006

A novel automatic system for analyzing rats' freezing behavior based on video recordings

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Defensive freezing behavior is an immobile and crouching posture that animals present when facing potential threatening or dangerous situations. This response is a widely employed index of innate and conditioned fear. Freezing is commonly measured by trained human observers. The present study presents a novel computational system analysis the rat freezing behavior automatically. The system is based on a supervised learning algorithm. During the training phase, videos of the rat's general activity are first normalized for size, blurred to reduce high-frequency spatial noise and edges are extracted using a Roberts filter. Next, sum-of-differences are calculated independently for each two sequential frames quadrant. The resulting four features are embedded in a dissimilarity space, where each frame is represented by its Cartesian distance to all other frames, and a pseudo-Fisher discriminant is built. The classifier is trained using ground-truth freezing/activity classification as registered by trained human observers. The system was evaluated using 16 videos, totaling 73 minutes, of context conditioning and a leave-one-out procedure. Error rate, calculated comparing automatic and ground-truth freezing/activity classification frame-by-frame or in blocks of 15 seconds, was 6.4% and 4.6 %, respectively. For comparison, the error rates of a second observer were 9.7% and 8.0 %, respectively. These results indicates that the present system can reliably measure freezing behavior in rats. Indeed, error rate of this system is below the variance presented by trained human observers.

Palavras-Chave: Freezing; Automatic Measurements; Moviment Detection

Apoio Financeiro: CNPQ

Characterization of the Effective Connectivity Networks inferred through Partial Directed Coherence during episodes of Mesial Temporal Epileptic Seizure

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Objectives: Modeling studies have pointed that neural networks with a small-world configuration facilitate the onset of synchronized activity. Since epileptic seizures consists of highly synchronized activity we investigated the hypothesis that the topography of functional brain networks changes towards a small-world pattern during medial temporal seizures.

Methods: EEG data of 6 epileptic subjects were divided into three periods: preictal, ictal and postictal. For each subject a 60s segment of each period was selected. We used a sliding window of 10s with 4s overlap and for each of these sub-segments the effective connectivity between all pairs of channels was estimated through Partial Directed Coherence. We divided the frequency domain into six intervals: delta, theta, alpha, beta, gamma and high gamma and derived a significance level for PDC (Takahashi et al., Journal of Applied Stat. 34 10:1259, 2007). Directed connectivity networks between the brain areas were constructed using the significative PDC levels for each sub-segment and band. Then we calculated the cluster index C (a measure of local connectedness) and shortest path length L (a measure of overall network integration) in these networks and adjusted linear regression models to track the trends in these measures over the three periods.

Results: We found significative ($P < 5\%$) increasing C during the pre-ictal period in the beta (mean: 0.73, std. error: 0.30) and gamma (1.8, 0.52) bands and during the ictal period in theta (1.21, 0.30) and alpha (0.68, 0.21). We found significative decreasing C in the post ictal period in theta (-0.71, 0.32), alpha (-0.74, 0.22), beta (-1.76, 0.34) and gamma (-1.45, 0.59) bands. We also found decreasing L in gamma (-3.84, 1.31) during the pre-ictal period and incresing L in alpha (2.43, 1.23) and gamma (4.87, 1.21) bands during the post-ictal period. All quantities are dimensionless and in percentage.

Conclusion: During seizures the neuronal network changes towards a more small-world configuration (characterized by higher C and lower L) in specific frequency domains. The changes in the pre-ictal period may be useful to predict seizures.

Palavras-Chave: epilepsy connectivity data analysis graph complex networks

Apoio Financeiro: CNPQ, ASDAP

G.03.008

Principal Component Analysis of fluorescence spectra of pyrene discloses lipid preferences of the nicotinic acetylcholine receptor

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Useful information from fluorescent spectra is not always straightforward to acquire if only one or a few wavelengths are considered. Here we present an approach which involves analysis of entire spectra by means of Principal Component Analysis (PCA) to investigate the preference of the nicotinic acetylcholine receptor (AChR) protein for different lipids species.

PCA was applied to normalized spectra of the fluorescence probe pyrene-phosphatidylcholine (Py-PC) incorporated into lipid-only and AChR-containing membranes with different combinations of POPA/POPC or DPPC/DOPC, at varying temperature (0 to 57.5°). We exploited the favorable partition of PyPC into fluid phases and its ability to form excimers (E) having spectroscopic properties different from those of monomers (M) when the probe concentration and/or its diffusion rate vary. Spectral differences inform on changes in the physical state and fluidity of bilayers.

By using PCA the multidimensionality of recorded variables (79 wavelengths) was reduced to two principal components (PCs) that sufficed to account for ~99% of the total spectral variance. From the score plots it was possible to group samples according to their lipid composition, changing temperature and the presence or absence of AChR. In AChR-free membranes composed of either POPA/POPC or DPPC/DOPC the score plots of PC1 vs PC2 revealed an abrupt change in the spectral profile of PyPC that matched the transition temperatures of each lipid system. In pure POPC or DOPC bilayers (having transition temperatures <0°) a smooth co-variation between scores was found, reflecting the absence of phase transitions in the assayed interval. Incorporation of AChR protein drastically reduced the phase transition that occurs in similar AChR-free membranes.

The results indicate that the AChR exhibits preference for POPA or DPPC in comparison to POPC or DOPC in its immediate environment, it rigidizes the lipid matrix and impairs the mixing of lipids upon heating. In view of the structure of the phospholipids tested, the protein-lipid interactions appear to depend on both the polar headgroups and fatty acyl chains of phospholipids.

Palavras-Chave: nicotinic acetylcholine receptor - fluorescence - PCA

Apoio Financeiro: ANPCyT - CONICET

CONSISTENCY IN APPROXIMATE ENTROPY GIVEN BY A VOLUMETRIC ESTIMATE

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Objectives: Non-linear methods for estimating variability in time-series are currently of widespread use, both in basic and applied research fields. For instance, variability in ECG and EEG is employed as a predictor of organ disturbances from normality. Two of such methods are approximate entropy (ApEn (1)) and sample approximate entropy (SampEn (2)). The applicability of ApEn and SampEn in analysing data is evident and their use is increasing. However, consistency is a point of concern in these tools, i.e., the classification of the temporal organization of a data set might indicate a relative less ordered series in relation to another when the opposite is true. The objective of this study is to present an ApEn-based method which has consistency.

Methods: ApEn is computed as the positive matches in a given time-series S_0 using windows of length m that are compared to all the other vectors of the same length obtained in S_0 . A positive match is defined within a tolerance error r , also given as a parameter of the analysis. The present tool is developed by using ApEn repeatedly in the entire range of combinations of m and r . The tool is called volumetric ApEn, vApEn. To validate the method we prove some analytical results concerning convergence of the estimator and apply it to nine prototypical time-series artificially generated (sine waves, logistic maps, random noises) and also performed surrogate data analysis.

Results: vApEn correctly classified all the series without lack of consistency: 1-sine = 207; 2-sine = 423; 3-sine = 525; 4-sine = 606; logistic maps = (383, 1016, 952); uniform noise = 1011; white noise = 1141. Surrogate data for each type of sequence were compared to the others by t-tests that confirmed the consistency of the tool.

Conclusions: vApEn was proved as an adequate estimator to address variability in time-series. The tool has consistency and meets the current criteria of validation for non-linear methods.

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(2) Richman, J. S. & Moorman, J. R. (2000). Am. J. Physiol. Heart Circ. Physiol. 278: H2039-H2049. **Palavras-Chave:** Entropia aproximada, séries temporais, métodos não-lineares

Apoio Financeiro: FAPESP (#06/02120-0)

G.03.010

Spatiotemporal pattern recognition analysis of an fMRI mental rotation experiment

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Objectives

We used a spatiotemporal support vector machine (SVM) to discriminate between brain activation patterns during a mental rotation task with different degrees of angular disparity.

Methods

The spatiotemporal SVM (Mourao-Miranda et al. 2007) is a pattern recognition approach that is suitable for investigating dynamic changes in brain networks during a complex mental task. By defining a time window including a cognitive event, one can use spatiotemporal fMRI observations from two cognitive states to train the SVM. During the training, the SVM finds the discriminating pattern between the two states and produces a discriminating weight vector encompassing both voxels and time (i.e. spatiotemporal maps). We evaluated the performance of the classifier using a leave-one-subject-out cross validation test (total number of subjects = 11).

Results

We showed that the spatiotemporal SVM was able to discriminate between brain activation patterns during a mental rotation task with different degrees of angular disparity. The classification accuracy was correlated with amount of difference in angular disparity between the mental rotation task and the control task: 67.0% for 20 vs. 0 degrees, 78.9% for 60 vs. 0 degrees and 89.5% for 100 vs. 0 degrees. The SVM was also able to detect brain regions functionally associated with different aspects of the task and to show how the contributions of these regions change over time.

Conclusions

We have shown that by applying spatiotemporal SVM to a mental rotation event-related experiment we could detect areas involved in different components of the task as they change their behavior over time. In addition we showed that the discrimination accuracy was correlated with the amount of load difference between the tasks.

References:

Mourao-Miranda, J., K. J. Friston, et al. (2007). "Dynamic discrimination analysis: a spatial-temporal SVM." *Neuroimage* 36(1): 88-99.

Palavras-Chave: Pattern Recognition, Machine Learning, Support Vector Machine

Apoio Financeiro: Kings College London

Multivariate Analysis of Genetic Influence on Brain Volume

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Objectives

In this abstract we present a feasibility study showing that, by using Kernel Correlation Analysis (KCCA), and a new genotypes representation, it is possible to analyse the relative associations of several genetic polymorphisms with brain structure. The implementation of the method is demonstrated on genetic and structural magnetic resonance imaging data acquired from a group of 16 healthy subjects by showing the multivariate genetic influence on grey and white matter.

Methods

KCCA (Hardoon et al., 2007) is a technique for finding pairs of basis vectors that maximize the correlation of a set of paired variables. In the following study we consider the SNPs (Single Nucleotide Polymorphism) of the genetic sequence and the segmented white or grey matter of structural MRI brain scans to be two “views” of the same object. KCCA seeks a pair of linear transformations one for each of the paired variables such that when the variables are transformed the corresponding coordinates are maximally correlated.

Results

We wish to avoid overinterpreting our results due to the small sample size, therefore we confine ourselves to represent each SNP's largest contributing (absolute) genotype value, this allows us to visualise the overall influence each SNP has on the grey and white matter. The outputs of the KCCA approach are the SNP “loading” profiles and the voxel weighting for the grey and white matter (figures excluded for brevity).

Conclusions

We should be very conservative in our interpretations of the results from a small, preliminary study. However, it appears (a) the method enables us to place some ranking on the possible importance of SNPs in driving structural brain change and (b) that the brain areas under apparent SNP-related influence fall into a number of broad regions. We conclude that KCCA with a linear kernel is capable of investigating the interactional effects of multiple genetic influences on brain structure. We hope that the possibilities raised by this method will be usefully exploited to clarify the nature of multiple gene influences on brain structure and function in large samples of healthy and diseased subjects.

References

David R. Hardoon, Janaina Mourao-Miranda, Michael Brammer, and John Shawe-Taylor. Unsupervised analysis of fmri data using kernel canonical correlation. *NeuroImage*, 37(4):1250–1259, 2007.

Palavras-Chave: multivariate analysis, Kernel Correlation Analysis

Apoio Financeiro: Kings College London and University College of London

G.03.012

Activity Analysis of Neuronal Reverberation during Slow-Wave Sleep after Novelty Exposition

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Objectives: Consolidation of memories after novelty exposition have been indicated to depend on neuronal reverberation during sleep. This reverberation process occurs in different brain regions during two main cyclical sleep stages: SWS (Slow-Wave Sleep) and REM (Rapid-Eye-Movement) sleep. The quantification of mnemonic reverberation pattern depends on the appropriate choice of mathematical methods to accurately correlate neuronal activity during novel waking experience with neuronal activity during post-experience sleep.

Methods: Using a Template Matching (TM) technique to calculate correlations of neuronal ensemble activity over time (1), we have demonstrated temporal asymmetry of neuronal reverberation pattern during SWS associated with tactile exploration of novel objects (2). However, another study raised doubts regarding the efficacy of the TM method to describe the reverberation process (3). In particular, Tatsuno and colleagues were not able to observe any temporal asymmetry of mnemonic reverberation by using TM method. We address the controversy on the use of TM method to describe neuronal reverberation activity.

Results: We analysed data recorded from primary somatosensory cortex and hippocampus of rats (n=5) (4). We compared regular with partial correlations, used to remove pre-experience effects from post-experience correlations. Partial correlation has shown more accuracy to display temporal asymmetry between pre and post SWS neuronal activity.

Conclusions: Our results suggest that the assessment of neuronal reverberation is greatly aided by removing past “baseline” effects from post-experience neuronal activity. Further investigation of this issue using Explained Variance and Mutual Information methods is ongoing.

References: 1 - Neuron 29:145, 2001; 2 - PloS Biology, 2:126, 2004; 3 - J. Neuroscience, 26(42):10727, 2006; 4 - FINS, 1:43, 2007. **Palavras-Chave:** Data Analysis, Slow-Wave Sleep, Neuronal Reverberation, Temporal Asymmetry

Apoio Financeiro: CNPq/Fapern

G.04.001

MATHEMATICAL MODELING OF THE CIRCADIAN TIMING SYSTEM OF MAMMALS

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The circadian timing system is structured in a way to receive information from the external and internal environments, and its function is the timing organization of the physiological and behavioral processes in a circadian pattern. In mammals the circadian timing system is constituted by a group of structures which includes the suprachiasmatic nucleus, the intergeniculate leaflet and the pineal gland. In suprachiasmatic nucleus are found neuron groups working as a biological pacemaker, forming a biological master clock. In present work we have simulated a simple model for the circadian timing system of mammals to reproduce two fundamental characteristics of biological rhythms: the endogenous generation and synchronization to the light-dark cycle. We have modeled the biological pacemaker of suprachiasmatic nucleus as a set of 1000 coupled oscillators with long-range coupling, forming spherical lattice. The characteristics of oscillator set were defined based on the Kuramoto's oscillator dynamics, but we have presented a new method for estimating the equilibrium order parameter. Simultaneous activities of the excitatory and inhibitory synapses on the elements of circadian timing circuit at each instant were modeled by specific equations to synaptic events. All simulation programs were written in *Fortran 77*, compiled and run on PC DOS computers. Our model has been able to exhibit responses in accordance with physiological patterns.

Palavras-Chave: Circadian cycle, coupling oscillators, suprachiasmatic nucleus.

Apoio Financeiro: FAPERJ

G.04.002

Constraints on a nonlinear model for sensorimotor synchronization

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Animals developed multiple timing mechanisms on scales that range more than 10 orders of magnitude, from microseconds to days. We focus on timing in the range of tens to hundreds of milliseconds, where many forms of sensory and motor processing occur –most notably speech and music recognition and production, and fine motor coordination. Sensorimotor synchronization, a robust and spontaneous behavior specific to humans, occurs within this range. Finger tapping experiments are usually performed to probe the mechanism in charge of keeping average synchrony. In these experiments, the subject is instructed to keep synchrony by tapping along with a periodic stimulus. A perturbation in the stimulus (a step change in period, for instance) may appear. The time difference between each stimulus and its corresponding response is recorded. In order to either keep average synchrony with a periodic stimulus or return to average synchrony after a perturbation, an error correction mechanism based on past performance should actively drive the subject's response.

We first review the literature on finger tapping time series (both isochronous and perturbations) in search of dynamical constraints on a model for the error correction mechanism. We found several qualitative features like correction overshooting that, although usually disregarded, impose strong constraints on both qualitative and quantitative aspects of a model. We interpret and arrange these published experimental findings into a dynamical framework, and develop an informed mathematical model for the error correction mechanism. We put forward several qualitative predictions, like the amount of overshoot in response to a positive step change should increase smoothly with the step size.

Palavras-Chave: finger tapping - milliseconds

Apoio Financeiro: CONICET - Universidad de Quilmes

G.04.003

Encoding of amplitude modulated sounds in the auditory nerve

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The aim of this work is to develop a detailed biophysical model of the inner ear and the transduction processes that take place in the hair cells and synapses with the auditory nerve. The output is a realistic spike train for the nerve fibers that preserve both temporal and place/frequency information and that could be used as input for the first relay of the auditory pathway: the Cochlear Nucleus [1]. We focus on the processing of amplitude modulated stimuli, and show how critical are the different stages of our cochlear and auditory nerve models for the encoding of this type of signals. This is a major topic, since the encoding of envelope modulations is one of the main cues used to extract useful percepts from speech signals and many environmental sounds.

Our model is made up of four parts: a) a hydrodynamic model of the cochlea, which takes account of the active processes that take place in it; b) a simplified model for the nonlinear transduction of the stimulus in the inner hair cells (mechano-electric transducers); c) a model for the ribbon synapses between the inner hair cells and the auditory fibers, which includes the dynamics of calcium and neurotransmitters; d) the generation of electric pulses in the auditory fibers through a dynamical stochastic process. The model was assembled using several previous biophysical models [2][3]. Some corrections are made based on recent experimental measurements [4], or results obtained in other cellular or neuronal systems, seeking to retain a comparable correlation with the real biological systems.

We are able to reproduce typical responses (tuning curves, inter-spike interval histograms (ISIH), and period histograms) for sinusoidal and amplitude modulated signals at various stages of signal processing. We also make a thorough study of the modulation transfer functions for our model, comparing to that obtained in experimental results [5]. Finally, we evaluate the fine temporal coding of the auditory nerve signals for modulated sounds, since it is crucial to have a well timed nerve spike train, with proper statistics, in order to further develop an accurate model of the cochlear nucleus [1]. Our model also has the advantage that all parameters are related to meaningful magnitudes within physiological ranges.

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Palavras-Chave: Auditory system-Temporal coding.

Apoio Financeiro: Agencia de Promoción Científica y Tecnológica (FONCyT - PICT 32391) and CONICET.

INVESTIGAÇÃO DOS MECANISMOS ENVOLVIDOS NA REFRATARIEDADE DE ATIVIDADES EPILEPTIFORMES DO TIPO NÃO-SINÁPTICAS SOB DIFERENTES CONDIÇÕES DE EXCITABILIDADE.

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Objetivos: Após uma atividade epileptiforme sustentada por conexões não – sinápticas (AENS) existe um período refratário absoluto (PRA) onde um estímulo elétrico não é capaz de evocar um novo evento epileptiforme. O presente trabalho tem como objetivo estudar, por meio de experimentos e simulações computacionais, os mecanismos envolvidos no PRA em diferentes condições de excitabilidade que são conseguidas variando-se o K^+ extracelular.

Metodologia: As AENS's foram induzidas com solução nutriente contendo 8.1 mM de K^+ e 0 mM de Ca^{2+} . O registro do potencial elétrico extracelular (PEE) das AENS's foi feito na camada granular do giro denteado (GD). Uma vez iniciadas as AENS, a solução nutriente foi trocada por outra com concentração menor de K^+ (6.6 mM e 6.0 mM). Pares de estímulos foram aplicados no hilus: o primeiro foi dado sempre após o fim do período refratário do tecido e o segundo em tempos diferentes após o fim do primeiro evento evocado. Do PEE foram analisados a duração dos eventos epileptiformes (DE) e a amplitude da variação lenta (DC). As simulações foram realizadas com um modelo computacional que compreende uma rede de células granulares e gliais, conectada a uma rede de compartimentos extracelulares justapostos, caracterizando a região do GD. A concentração de K^+ foi reduzida e os estímulos foram aplicados em cada neurônio simultaneamente, mimetizando a manobra experimental.

Resultados: Os resultados experimentais permitiram observar que para a concentração extracelular de K^+ em 6.6 mM e 6.0 mM houve respectivamente: (i) aumento de 1.13 ± 0.05 e 1.41 ± 0.11 no DC das AENS evocadas comparado às espontâneas; (ii) duração do PRA de 42.8 ± 1.5 s e 81.6 ± 1.6 s. Os resultados computacionais mostraram que diminuindo a concentração extracelular de K^+ houve: (i) aumento no DC; (ii) aumento na duração do PRA; (iii) aumento das correntes hiperpolarizantes de K^+ .

Conclusões: O aumento do DC se deve à elevação do efluxo de K^+ indicando um acréscimo das correntes hiperpolarizantes dependentes desse íon. Esse aumento é confirmado pela análise dos resultados computacionais. Uma maior hiperpolarização dificulta o aparecimento de novas AENS's prolongando o PRA.

Palavras-Chave: Atividades epileptiformes, Conexões não - sinápticas, Período refratário.

Apoio Financeiro: FAPEMIG

G.04.005

Análisis de la estabilidad de las Conexiones Recurrentes de una red con Selectividad a la Orientación, que fueron desarrolladas con Aprendizaje Hebbiano y Homeostasis

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La homeostasis es un mecanismo de control que permite regular las conexiones sinápticas y las propiedades intrínsecas de las neuronas, con el objetivo de mantener estable la actividad neuronal. Este tipo de plasticidad puede ser utilizado como complemento para la regla de Hebb, especialmente durante los procesos de aprendizaje y desarrollo del sistema nervioso.

En este trabajo se analiza en que medida pueden desarrollarse conexiones recurrentes y selectivas a la orientación, utilizando una regla de aprendizaje Hebbiano a la que se le incorporan diferentes tipos de homeostasis. A causa de que la simetría de traslación para las conexiones de la red se mantiene a lo largo de toda la evolución, fue posible realizar un estudio detallado de la evolución del sistema dinámico.

Palavras-Chave: Conexiones Recurrentes, Estabilidad, Homeostasis, Sistema Dinamico

G.04.006

Retina model calibration using multi-electrode array (MEA) data from *Octodon degus*, rodent

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The first step in the visual process is the transduction and encoding of incoming light into neural activity. This step occurs in the retina, a complex network of differentiated cells almost functionally isolated from other brain visual areas. This particularity allows to model them as an isolated process system [1]. In retina research, like in studies of other biological processes, models and simulators are becoming important tools. However, the validation of these models requires constant feeding from experimental data to improve simulator parameters and design.

In this work, we use and calibrate a retina simulator platform, developed at the Odysee laboratory, INRIA, France [1]. The general structure of the model consists in three biologically inspired processing levels: an outer plexiform layer composed of light receptors and horizontal cells, an inner plexiform layer which consists of bipolar and amacrine cells, and a ganglion layer of cells where spikes trains are generated in order to feed visual areas in the brain. Each of these levels contain a group of parameters, dependent on the biological species under study, that define among others the spatio-temporal stimuli convolution, the feed and feedback from one layer to another as well as cellular diversity, distribution and density. Each of these parameters are dependent on the biological species simulated.

Using available data and data obtained from *Octodon degus* retinas mounted on a MEA (plexon system) that were subjected to several visual stimuli[2], we introduce into the model reliable parameters for a rodent retina in general and *Octodon degus* retinas in particular. The improvement leads to a more realistic model which it is hoped will be further tested by future data.

References

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Palavras-Chave: Retina, Simulator platform, Multi-electrode array.

Apoio Financeiro: E.O. Is a Ph.D. students CONICYT grant 21080289

Memory strengthening and restructuring in a Boltzmann machine model of the cerebral cortex during different sleep states.

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Objectives: Cortical electrical activity has different statistical features during episodes of nondreaming sleep ("slow-wave" sleep, SWS) and dreaming sleep ("rapid-eye-movement" sleep, REM). SWS is marked by low frequency neural oscillations (delta waves, 0.5-4Hz) and high amplitude, while REM is characterized by fast frequency oscillations (>5 Hz) (1). By the same token, cortical neurons tend to fire more during REM than during SWS. Based on electrophysiological and molecular evidence, we have previously proposed that SWS and REM play distinct roles in the consolidation of memory traces, with memory strengthening during SWS and memory restructuring during REM (2). At present, little is known about the relationship between memory stability and the dynamics of neuronal firing. The aim of this work is to expose an Artificial Neural Network (ANN) to activation patterns of different firing rates and variances, and then assess memory stability over time, comparing initial and final ANN connectivity patterns. Our ultimate goal is to quantify the evolution of memory patterns across the characteristic statistical regimes of SWS and REM.

Methods: Neural firing codes were processed and transmitted from the first layer (input layer) to the last layer (output layer) of the ANN. We used a Boltzmann machine (3) as signal propagation model and Hebbian learning rules (4) to update the synaptic weights.

The ANN was initialized with a certain memory pattern (pre-defined synaptic weights) and the initial and final stages of the network were compared after several cycles of neural activity. Memory change was measured as the mean of the synaptic weight differences (dw).

Results: In general, an increasing gradient of memory change was detected as simulations ran from low to high mean firing rates (0.5–160Hz). Very small variations ($0 < dw \leq 0.03$, less than 3%) of synaptic weights were observed for low firing rates ($\leq 10\text{Hz}$). However, fast frequencies oscillations ($> 20\text{Hz}$) changed the initial synaptic weights up to 30%.

Conclusions: The results support the notion that neural activity with low frequency oscillations, characteristic of SWS, promote memory maintenance and stability; while high frequency oscillations, characteristic of REM, contribute to the restructuring of memory patterns.

References: 1-J. Neuroscience. 24(49):11137, 2004; 2- Learning and Memory, 11:686, 2004; 3- Cognitive Science, 9:147, 1985; 4- Hebb's, 1949. **Palavras-Chave:** Sleep, Memory, Artificial Neural Network, Boltzmann machine

Apoio Financeiro: CNPq

G.04.008

Effect of LTD Saturation on Pattern Recognition by Cerebellar Purkinje Cells

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Objectives: Many theories of cerebellar learning assume that long-term depression (LTD) of synapses between parallel fibres (PFs) and Purkinje cells (PCs) provides the basis for pattern recognition in the cerebellar cortex (1). Recent computer simulations and electrophysiological recordings in slices and awake mice have suggested that PCs can use a novel neural code based on the duration of silent periods, with shorter pauses in response to learned PF patterns (2). These simulations used a simplified learning rule, where the AMPA receptor conductance was halved each time a pattern was learned. However, experimental studies of LTD induction in cerebellar slices show that the mean AMPA receptor conductance saturates and is rarely reduced to less than 50% of its baseline value (3,4). Here we study the effect of LTD saturation at different minimal values on pattern recognition.

Methods: The simulations were performed using the GENESIS simulator. We used the multi-compartmental PC model with active dendrites and soma that is described in detail in (5,6). The pattern recognition performance was evaluated by calculating signal-to-noise ratios (2,7). Simulation data were analysed using C and MATLAB.

Results: We studied the effect of LTD saturation in our simulations and found that the pattern recognition performance was very sensitive to the value at which the synaptic weights saturated. The pause based pattern deteriorated drastically for smaller amounts of LTD and was affected strongly by the number of active PFs per pattern. In contrast, a corresponding artificial neural network (7) was unaffected by the value at which LTD saturated. To result in satisfactory pattern recognition, LTD had to depress the AMPA receptor conductances down to at least 70% of their baseline values, and optimal performance resulted from setting the weights to zero and silencing the synapses completely.

Conclusions: Our simulation results suggest that LTD based pattern recognition is improved by strong depression and optimized by setting the AMPA receptor conductances to zero. Interestingly, large numbers of silent synapses between PFs and PCs have been observed in cerebellar slices (8).

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Palavras-Chave: Cerebellar Learning, Purkinje cell, Pattern Recognition, LTD

Apoio Financeiro: University of Hertfordshire, BBSRC

G.04.009

Simulator for classical conditioning models: Rescorla-Wagner and Schmajuk-Di Carlo for Multiple Responses

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Objectives: Our objective is to construct a general purpose computer simulator for classical conditioning models to be used for both research and teaching. Here we describe the first version of the simulator, which implements two models: Rescorla-Wagner (R-W) (1) and Shcmajuk-Di Carlo for Multiple Responses (S-D) (2). The first is a linear trial level model in which learning occurs by discrepancy between expected prediction and stimuli in a trial. The second is a real time neural network model that simulates hippocampal and cortical activities in paradigms of classical conditioning. As far as we know this is the first program that implements these models together.

Methodology: The software was developed using object-oriented techniques implemented by a friendly visual java-applet interface (supported by any browser), which allows parameter modifications and results visualizations via graphs and tables. Users can choose classical conditioning paradigms either by strings or a binary code. Euler's method is the default for solving differential equations, but the interface has an advanced option that allows users to choose other methods.

Results: Our software reproduces well results from different simulations of the R-W and S-D models (2,3). In particular, the S-D model graphs show real-time behavior by plotting each response and unconditioned stimulus prediction moment-to-moment or trial-by-trial, allowing direct comparison with results from animal experiments.

Conclusions: The simulator is a differentiated scientific tool that allows an integrated analysis of the variables involved in the learning processes described by the R-W and S-D models in animal experiments. We believe that our simulator can be used by a wide range of researchers interested in animal behavior.

References:

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3-Behav. Res. Meths. Instr. Comput. 28:55, 1996.

Palavras-Chave: neural process, simulation tools, classical conditioning models, animal learning

G.04.010

Análise de sensibilidade de modelos biologicamente plausíveis de neurônios do córtex visual primário

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Objetivos: Estudos eletrofisiológicos mostram que os neurônios corticais do córtex visual primário podem ser classificados eletrofisiologicamente (2,3). O objetivo deste estudo é a modelagem de neurônios de seis dessas classes, a obtenção de respostas eletrofisiológicas satisfatórias diante a literatura e o uso de análise de sensibilidade para entender as características dinâmicas dos modelos e determinar a dependência do comportamento de cada modelo diante dos parâmetros usados.

Métodos: Os neurônios são modelados segundo o formalismo de Hodgkin-Huxley e a abordagem compartimental de Rall (1). Os parâmetros usados correspondem a características fisiológicas das células reais. Esses parâmetros foram ajustados para que os comportamentos eletrofisiológicos dos modelos reproduzam bem os comportamentos reportados na literatura. Uma vez construídos, os modelos foram investigados com o uso de técnicas de análise de sensibilidade sobre os parâmetros escolhidos e de análise de estabilidade de sistemas dinâmicos (4).

Resultados: Devido ao ajuste dos parâmetros dos modelos, suas respostas eletrofisiológicas estão de acordo com a literatura. Os modelos também foram classificados dinamicamente pela aplicação da análise de estabilidade. Por fim, a análise de sensibilidade permitiu a caracterização da dependência e robustez dessas classes com respeito a variações nos parâmetros usados. Foi possível entender como certos comportamentos como, por exemplo, a forma da curva de frequência *versus* corrente injetada (F-I), a adaptação dos disparos e o comportamento em rajada, ocorrem em termos dos parâmetros dos modelos.

Conclusões: As técnicas de análise de estabilidade e de sensibilidade são ferramentas úteis para a caracterização dinâmica de modelos de neurônios e possibilitam um entendimento dos mecanismos de transição repouso-disparo e de fenômenos mais complexos como adaptação e comportamento em rajada.

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1. Theoretical Neuroscience. MIT Press, 2001
2. J Neurosci. 23:96, 2003
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4. Dynamical Systems in Neuroscience: The Geometry of Excitability and Bursting. MIT Press, 2007

Palavras-Chave: Córtex Visual Primário, Neurociência Computacional, Sistemas Dinâmicos

Apoio Financeiro: CAPES

Otimização da Demanda Energética em Pequenos Endotermos

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Objetivos. Buscamos explorar, no campo teórico, variáveis relevantes do ponto de vista do controle fisiológico para a entrada em depressão metabólica (torpor diário) de pequenos endotermos. Nessa perspectiva, o estudo visa, também, buscar como se estabelece um balanço de pesos entre essas variáveis, para se gerarem possíveis minimizações/maximizações presentes no processo.

Métodos. Definimos um índice de performance que se caracteriza como um funcional relacionado ao acúmulo de estresse fisiológico e ao esforço de controle, ao longo do tempo:

$$J(U(t)) = \int (F(t)^2 + U(t)^2) dt$$

Através da teoria de controle ótimo, buscamos encontrar um controle para o sistema que minimizasse tal índice. Uma vez estabelecido um controle e, conseqüentemente, definidos os pesos que as variáveis envolvidas possuem neste balanço, simulamos o comportamento termorregulatório de animais expostos a temperaturas “frias” e “quentes”, e em condições que chamamos normais ou patológicas, caracterizadas pela correspondência ou não entre a temperatura ambiente e a condutância térmica adequada para tal temperatura. Para ambas as situações, as simulações são realizadas com entrada nula de alimento, indicando uma situação de privação alimentar.

Resultados. O controlador ótimo, resultado do processo minimização do funcional de estresse fisiológico, faz com que a taxa metabólica decresça até atingir um limite inferior, gerando uma entrada nula para o controlador de variação das reservas energéticas. Assim, surge uma indicação de como o sistema nervoso poderia atuar de forma a estabelecer uma possível minimização dos gastos energéticos e estresse fisiológico, numa situação de privação alimentar.

Conclusões. A presente modelagem consegue obter uma aproximação da resposta fisiológica do balanço energético de mamíferos heterotermos durante períodos de limitação de alimentos e/ou estresse térmico. Além dessa aproximação dos componentes de depressão metabólica observados nesses animais, o modelo é capaz de criar um simulacro de temperatura de *set-point* sem forçar a presença de tal valor como um *imprint* neural. Portanto, o *set-point* deixa de ser um valor imposto ao sistema e passa a ser uma variável que surge das relações do controle.

Palavras-Chave: depressão metabólica, controle ótimo, *set-point*

Apoio Financeiro: FAPESP - Processo: 06/05490-2

G.04.012

Modelagem da minimização do processamento na comunicação

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Objetivos: A comunicação entre indivíduos da mesma espécie está presente em todos os táxons conhecidos. Como existe uma capacidade limitada para processar todos os sinais vindos do ambiente, é interessante supor que ao longo da evolução exista uma tendência à minimização do processamento associado com a comunicação de modo a liberar atenção para outros fatores (e.g., presença de predadores). Como a minimização do processamento na comunicação pode ser associada a uma diminuição das possibilidades com as quais o sistema nervoso tem que lidar, podemos assim criar a hipótese que existe uma tendência à minimização de geração de entropia (σ) no sistema (i.e., um menor número de estados em que o sistema pode transitar e assim, uma menor carga de processamento). O objetivo do presente estudo é testar a hipótese descrita acima.

Métodos: Consideramos os organismos envolvidos na comunicação como osciladores descritos por equações diferenciais, nas quais a frequência e a fase são as variáveis de estado. “Comunicação” fica definida pelo acoplamento dos osciladores. σ é dada pelo produto do fluxo (frequência) pela diferença de potencial (parâmetro de forçamento do oscilador). O cálculo de σ foi realizado para as situações com 2 ou 3 osciladores com parâmetros diferentes e para uma situação generalizada com n osciladores iguais.

Resultados: Os resultados obtidos indicam que a situação com osciladores iguais minimiza a geração de entropia, e ainda que, dentro dessa estratégia, a geração de entropia é minimizada quando o parâmetro de acoplamento tende a zero ou ao infinito, sendo que a maior eficiência de fluxo é obtida quando esse parâmetro tende ao infinito.

Conclusões: Podemos concluir que a melhor estratégia para minimizar a geração de entropia é, na ausência de interações, manter o acoplamento alto (equivalente a prestar mais atenção no ambiente). Já com a presença de outros indivíduos, manter acoplamento menor que “1”, emitindo sinais próximos da frequência natural de cada oscilador passa a ser a melhor opção em termos de σ .

Palavras-Chave: processamento, osciladores, acoplamento, entropia

Apoio Financeiro: FAPESP (06/02176-5)

G.04.013

Mitral Cells Synchrony in an Olfactory Bulb Model based on Simplified Neurons

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Objectives: The main olfactory bulb (MOB) transforms signals received from the olfactory epithelium via the olfactory nerve into spatial-temporal patterns (1). Evidence suggest that a mechanism of indirect lateral inhibition between mitral cells (M) through dendodendritic synapses with granule cells (G) generates stochastic synchrony among MOB cells that is used in the discrimination process of diferent odor types (2). Our main objective is to investigate the synchrony and discrimination processes using a computational model of MOB consisting of populations of M and G cells and their synapses.

Methodology: According to (3) there are two types of M cell, which are named "bistable" and "nonbistable". Using Izhikevich's simplified neuron model equations (4) we implemented models of these two M cell types. Using the same formalism we also implemented a granule cell model based on data from a more detailed model (5). The excitatory synapses between M and G cells (mediated by NMDA and AMPA receptors), and the inhibitory synapses between G and M cells (using the GABA_A neurotransmitter) were modeled by alpha functions. Connections between M and G cells were set according to a probability density function so that, on the average, each M cell receives synapses from five G cells.

Results: Spike-times of M cells in response to different odor inputs with greater or shorter correlations show that M cell synchrony depends more on network connectivity than on odor input. Mitral cells exhibit similar responses for similar odor inputs, even if the inputs are presented together with background noise.

Conclusions: Our MOB model made of simplified neuron models can be used to test and validate experimental results or results from more detailed, Hodgkin-Huxley type models, at a relatively low computational cost.

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- 3-J Neurosci, 21:5311-5320,2001.
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G.04.014

Modelo compartimental reduzido da célula periglomerular do bulbo olfatório

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Objetivo: Este trabalho descreve um modelo computacional biologicamente plausível do neurônio periglomerular do bulbo olfatório que reproduz seu comportamento determinado experimentalmente. Conjectura-se que as células periglomerulares tenham um importante papel no processamento de informação pelo bulbo por constituírem uma via de interação entre células mitrais de glomérulos distintos (1). Portanto, a modelagem do neurônio periglomerular é fundamental para a compreensão do funcionamento do bulbo olfatório.

Métodos: O modelo computacional, baseado no formalismo de Hodgkin-Huxley e na técnica de compartimentalização de Rall (2), é constituído por quatro compartimentos, denominados “espinhas dendríticas”, “dendritos”, “soma” e “axônio”. O modelo foi desenvolvido com o uso do neurosimulador NEURON (3) com parâmetros obtidos da literatura (1,4) e ajustados visando a reprodução de medidas eletrofisiológicas dessa célula.

Resultados: Os resultados obtidos para o modelo proposto reproduzem o comportamento experimental, no qual ocorre disparo de um único potencial de ação seguido de um platô. As características apresentadas pelo modelo, tais como amplitude, potencial de repouso e valor do potencial no platô foram correspondentes ao esperado. Verifica-se também que o modelo responde a valores de corrente próximos aos da célula real.

Conclusão: Os resultados mostram que o modelo proposto foi bem sucedido, correspondendo aos resultados experimentais, com a vantagem de ser um modelo de poucos compartimentos com baixo custo computacional.

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- 3- The NEURON Book. Cambridge University Press, 2005
- 4- J. Neurophysiol. 86:1899, 2001.

Palavras-Chave: Neurociência computacional, célula periglomerular, bulbo olfatório

Apoio Financeiro: CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico

Modelagem do Controle Motor para Minimização de Geração de Entropia em Processos Mecânicos Periféricos

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Objetivos: Estudos clássicos de Hoyt e Taylor (1) mostraram que, na locomoção de vertebrados, a transição entre os tipos de passada ocorre justamente quando se inicia um aumento na demanda energética. Uma vez que o movimento muscular é realizado sob controle do sistema nervoso central, é este que determina o tipo de passada a ser utilizada. Portanto, essa estrutura consegue traduzir eventos mecânicos ligados a esses conceitos e, mais do que isso, consegue operar o sistema de modo a mantê-lo num ótimo de demanda.

Nossa abordagem pretende criar um mapeamento, no sistema nervoso central, das aferências e eferências de um sistema mecânico de maneira a se modelar uma rede de controle motor. Com isso, estabelece-se um modelo cânon para encontrar a região de operação que minimiza a geração de entropia no sistema mecânico.

Métodos: Modelos matemáticos para o funcionamento de músculos esqueléticos e tendões, acoplados em um sistema pendular e suas aferências, fusos e corpos tendinosos de Golgi, foram desenvolvidos e validados como plataforma biomecânica. Essas aferências foram então utilizadas como entradas para outro modelo descrevendo Redes Neurais Biológicas (BNNs, do inglês), o qual gera as eferências que são, então, utilizadas como sinais de controle para acionamento dos músculos. Todos os modelos foram baseados em equações diferenciais (ODEs), e sua resolução numérica realizada em ambiente Simulink®.

Resultados: A rede neural de controle proposta mostrou-se capaz de operar o sistema biomecânico levando-o a oscilar de forma similar a passadas, tendo como entrada sinais das fibras tipo Ib e Ia. Medidas de otimização baseadas no cálculo da geração de entalpia nos músculos mostraram que a rede é capaz de operar a maquinaria mecânica em regiões de melhor relação geração de entalpia por velocidade ou distância percorrida.

Conclusões: Os resultados teóricos desse trabalho fornecem algumas questões experimentais interessantes, como relações entre perfil de ativação muscular e escala biológica, além de embasar a visão de que existem arquiteturas neurais básicas envolvidas no controle dos processos biológicos.

Referências: 1- Nature 292: 239 – 241, 1981.

Palavras-Chave: Controle Motor, Redes Neurais, Entropia, Modelos Matemáticos.

Apoio Financeiro: Fundação de Amparo a Pesquisa do Estado de São Paulo - FAPESP

G.04.016

Simulação Computacional de um Modelo da Célula Mitral do Bulbo Olfatório

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Objetivos: O objetivo deste trabalho é implementar na linguagem de programação Java uma versão compartimental reduzida do modelo de Bhalla e Bower de 286 compartimentos para a célula mitral do bulbo olfatório (1), que foi desenvolvido no neurossimulador GENESIS (2). A versão reduzida foi baseada no modelo de 4 compartimentos de Davison et al. (3) implementado no neurossimulador NEURON (4). A construção do modelo reduzido em Java permite a realização de dois tipos de comparações: (i) entre o comportamento eletrofisiológico do nosso modelo em Java com o dos modelos de Bhalla e Bower (1) e Davison et al. (3); e (ii) entre os desempenhos das plataformas computacionais usadas nas diferentes implementações.

Métodos: O modelo foi implementado inicialmente com os mesmos compartimentos e parâmetros descritos por Davison et al. (3). Ele foi submetido aos mesmos tipos de estímulos de corrente constante usados por eles para produzir uma curva de disparos versus corrente injetada (F-I) que possa ser comparada à obtida por Davison et al. (3). Posteriormente, o modelo foi submetido a outros tipos de correntes injetadas não utilizados em (3) para verificar a ocorrência de novos tipos de comportamento.

Resultados: O modelo foi reimplementado e simulado como se pretendia. Para que ele se aproximasse dos resultados anteriores foi necessário retirar uma constante introduzida por Davison et al. (3) para ajustar seu modelo ao de Bhalla e Bower (1). Feita essa modificação, os resultados obtidos se aproximaram bastante dos encontrados por Davison et al. (3). Para tipos diferentes de corrente injetada o modelo apresentou novos comportamentos que podem ser caracterizados dinamicamente em um diagrama de fases.

Conclusões: É possível implementar o modelo de Davison et al. (3) em uma plataforma diferente, com resultados similares, desde que se façam pequenos ajustes nos parâmetros do modelo.

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- (1) J. Neurophysiol. 69:1948, 1993.
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- (3) Brain Res. Bull. 51:393, 2000.
- (4) The NEURON Book. Cambridge: Cambridge University Press, 2005.

Palavras-Chave: célula mitral, bulbo olfativo, neurociência computacional, modelo biofísico.

Apoio Financeiro: CAPES

H.01.001

S100B oxidation state interferes in a simple three steps ELISA.

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Objectives: The aim of this study was to standardize a high sensitivity ELISA for S100B and investigated the influence of oxidation state in immunorecognition.

Methods: Diluted samples of standard S100B (between 1.9 pg and 1 ng/mL) plus Tris buffer were incubated for 2 h on a microtiter plate previously coated with monoclonal anti-S100B (SH-B1). Polyclonal anti-S100B was incubated for 30 min and then peroxidase-conjugated anti-rabbit antibody was added for a further 30 min. A colorimetric reaction with *o*-phenylenediamine was measured at 492 nm. S100B solution at 1 mg/mL was incubated, or not, with 0.1mM H₂O₂, in the dark, for 30 min. Standard curves of S100B (from 0.015 to 0.25 ng/mL) were prepared and measured by ELISA. Another stronger procedure, utilizing cystein oxidation, was performed on purified S100B to investigate immunorecognition. A fresh solution of S100B was incubated with 5mM of sodium tetrathionate in the presence of 1mM CaCl₂ for 1h. Protein was dialyzed against 20mM Tris-HCl, pH 7.4 and analyzed by 11% SDS-PAGE for separation of S100B 10 kDa monomers (nonoxidized S100B) from 21 kDa dimeric S100B (oxidized S100B).

Results: This protocol showed a high sensitivity (1.9 ng/mL) and linearity (1 ng/mL) with a low intra-CV (2.5%) and inter-CV (4.9%) considering the concentration of 0.015 ng/mL of standard S100B. S100B oxidation by H₂O₂ and a vigorous oxidation by sodium tetrathionate clearly diminished S100B immunorecognition (60% and no detectable, respectively).

Conclusions: Our S100B showed a high sensitivity and linearity. We observe that the oxidation state of S100B affects antibody recognition. This aspect is quite important and should be considered, assuming that oxidized and reduced forms may coexist *in vivo* and that the equilibrium can be modified by oxidative stress of physiological or pathological conditions or even by obtaining sample conditions.

Palavras-Chave: Astrocyte; ELISA; Oxidative stress; S100B protein

Apoio Financeiro: CNPq, CAPES, FINEP/ Rede IBN 01.06.0842-00, FAPERGS.

H.01.002

Estabelecimento de método rápido e de alta eficiência para determinação de ácido γ -aminobutírico e glutamato em amostras de tecido cerebral de ratos

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A determinação de ácido γ -aminobutírico (GABA) e glutamato (GLU), neurotransmissores envolvidos em processos excitatórios e inibitórios no sistema nervoso central, constitui importante instrumento de estudo, pois, pode contribuir para o esclarecimento do papel fisiológico desses circuitos neuronais e das disfunções bioquímicas a eles associadas. A técnica de cromatografia líquida de alta eficiência (HPLC) associada à derivatização pré-coluna é amplamente utilizada na quantificação de GABA e GLU. Entretanto, a utilização de sistema não isocrático, tempo de análise longo e uso de derivatizantes tóxicos são inconvenientes dos métodos anteriormente descritos. O objetivo deste trabalho foi desenvolver um método rápido e eficiente para a quantificação de GABA e GLU, em tecido cerebral de ratos, através da técnica de HPLC associada à detecção por fluorescência. Usando coluna C18 (150mm×4.6mm), as condições estabelecidas foram: fase móvel constituída de 0,05M de acetato de sódio, tetrahydrofurano e metanol (50:1:49; pH4,0) com fluxo de 1mL/min e detecção nos comprimentos de onda 337 e 454 nm para excitação e emissão, respectivamente. As amostras após homogeneização em solução metanol/água (85:15 v/v) foram submetidas à derivatização pré-coluna com orto-ftaldeído, reagente de baixa toxicidade. Em um tempo inferior a 9 minutos, GABA e GLU foram eluídos em condição isocrática. A detecção de GABA e GLU foi linear e com coeficiente de correlação maior que 0,99. Os desvios padrões relativos para as reprodutibilidades intra-dia foram de 3% e 3,3% e inter-dia de 2,9% e 6,3% para GABA e GLU, respectivamente. A acurácia do método foi de 99,6% $\pm$ 2,3 para GABA e 101,9% $\pm$ 1,8 para GLU. Comparado com a literatura, o método de processamento das amostras mostrou-se mais eficiente para quantificação de GLU. Portanto, as concentrações médias de GLU obtida no hipocampo, tálamo e córtex pré-frontal foram superiores aos valores anteriormente descritos. Em conclusão, este trabalho descreveu um método isocrático de cromatografia líquida e processamento de amostra eficientes para determinação de GABA e GLU com alta acurácia, reprodutibilidade e pequeno tempo de análise.

Palavras-Chave: HPLC, glutamato, GABA,

Apoio Financeiro: CAPES

H.01.003

Co-localization of small GTPase Rnd1 and Stress-Inducible Protein 1 (STI1) on mice brain lipid rafts.

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Objectives: Lipid rafts are detergent resistant cholesterol-rich structures that have a multitude of functions. Recent evidences on literature have shown that these membrane structures have an important role during neuritogenesis. GPI anchored proteins and some GTPases such as Ras are acylated rafts proteins. The small GTPase Rnd1 is an acylated membrane protein and STI-1, a cellular prion protein (PrPc) ligand, are both involved with neurite outgrowth and cytoskeleton plasticity, suggesting a possible interaction. Based on results from literature we decided to investigate if Rnd1 and STI1 could specifically interact and could be observed on lipid rafts.

Methods: GFP-Rnd1 or only GFP expressed on 293 T cells and whole mice brain homogenate were used for pulldown assays using GST or GST-Rnd1 immobilized on glutathione-sepharose beads. Whole brain homogenate from mice were also used for Rnd1 co-immunoprecipitation assays with anti-STI1. Pulled proteins were resolved by SDS-PAGE and western blots were done using anti-STI1, anti-Rnd1, anti-GFP or anti-GST antibodies. Pull-down and co-immunoprecipitation assays suggest that Rnd1 and STI1 can interact *in vitro*. In order to investigate if STI1 and Rnd1 are localized in lipid rafts and if their distribution is dependent of PrPc expression, lipid rafts were purified by flotation assay from brains and cerebellum of wild-type and PrPc knock-out mice. Fractions were collected, resolved by SDS-PAGE and analyzed by western blots using anti-flotillin-1, anti-caveolin-1 and anti-PrPc antibodies as lipid rafts markers. Polyclonal antibodies anti-STI1 and anti-Rnd1 were used for protein distribution analysis.

Results: Pull-down and co-immunoprecipitation assays suggest that Rnd1 and STI1 can interact *in vitro*. Our results show that both STI1 and Rnd1 from mice brain are present on lipid rafts but also on soluble membrane fractions. The absence of PrPc did not interfere on STI1 distribution over the lipid rafts fractions of knock-out mice.

Conclusions: Taken together these results may suggest a putative cellular domain for Rnd1 and STI-1 interaction. Rnd1 may also be a lipid raft resident molecule and this compartmentalization may also regulate the GTPase activity. Biological relevance for these evidences is under investigation.

Palavras-Chave: Rnd1, STI1, lipid rafts.

Apoio Financeiro: CAPES/PROCAD, FAPESP, CNPq

ANÁLISE PROTÉICA DO CÉREBRO DE CAMUNDONGOS SUPLEMENTADOS COM GELÉIA REAL DE *Apis mellifera*

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A geléia real (GR) é uma secreção produzida pelas glândulas hipofaríngea e mandibular das abelhas operárias nutridoras. Estudos anteriores mostraram que animais suplementados com GR apresentaram aumento na expressão gênica do neurofilamento H e fatores neurotróficos. Desta forma, esse estudo tem como objetivo averiguar possíveis alterações na expressão de proteínas no cérebro de camundongos suplementados com GR. Camundongos Swiss foram suplementados oralmente com solução de GR a 10% em água por 10 dias. Posteriormente, os cérebros foram dissecados e homogeneizados em tampão HEPES pH 7.7 contendo quelantes e inibidores de proteases. As frações sobrenadantes foram submetidas à eletroforese em SDS-PAGE 5-16% e, posteriormente, realizou-se *Western blot* para investigar a expressão de neurofilamento. Observou-se no perfil protéico das amostras de homogeneizado de cérebros de camundongos jovens e adultos uma diferença em relação à intensidade de algumas bandas polipeptídicas de aproximadamente 70, 66, 60, 50, 40, 35, 20 e 5 kDa. Na fração sobrenadante de cérebro imunodetectou-se um polipeptídeo de 210 kDa que corresponde ao neurofilamento de cadeia pesada. Além disso, verificou-se que camundongos suplementados com GR apresentaram maior expressão desse neurofilamento e que não houve diferença significativa na expressão dessa proteína em camundongos adultos e jovens suplementados. Esse resultado sugere que alguns peptídeos se expressam diferencialmente em resposta à suplementação com GR oral. Deste modo, a continuidade do estudo utilizando análises bioquímicas e histológicas, assim como testes comportamentais é necessária para determinar os efeitos da GR no sistema nervoso. **Palavras-Chave:** geléia real, envelhecimento, camundongo, cérebro

Apoio Financeiro: CAPES, FAPEMIG, UFU e Apiário Girassol

Biochemical interaction between ADAM23 and cellular prion protein

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Objective: A Disintegrin And Metalloprotease (ADAM) transmembrane proteins have metalloprotease and adhesion activities, which are both important to the nervous system development. ADAM23 is an important member of this family predominantly expressed in the central nervous system (CNS) and can act in cell adhesion since it lacks metalloprotease activity. In addition, mice deficient in ADAM23 develop tremor and ataxia and die less than two weeks after birth. These observations suggest that ADAM23 plays a role in the development, maintenance and function of the CNS. However, the physiological role of ADAM23 is still unclear. Interested in ADAM23's roles, we have decided to investigate some interactions between ADAM23 and other molecules. Since cellular prion protein (PrPc) is a glycolipid-anchored protein expressed mainly in CNS, which can act as an adhesion molecule, we have decided to investigate the possible interaction between ADAM23 and PrPc.

Methodology: To test our hypothesis we carried out co-immunoprecipitation assays using extracts from HEK293 cells co-transfected with pEGFP-PrPc and pcDNA-HA-ADAM23 expression vectors that were incubated with anti-PrPc. The blotting reaction of the immunoprecipitated material was reacted with anti-HA antibody that recognizes ADAM23-HA. Pull-down assays was done to confirm whether ADAM23 interacts with both recombinant and endogenous PrPc. To show the role of glycosylation for this interaction, we did a co-immunoprecipitation assay as described above, but GFP-PrPc glycosylation was blocked with tunicamycin treatment. We also did a binding assay using GST-Disintegrin domain of ADAM23 and ₆His-PrPc.

Results: Co-immunoprecipitation and pull down assays showed that ADAM23 can interact in vitro with PrPc. In addition, binding assays showed that this interaction is direct and independent of glycosylation.

Conclusion: Our results describe for the first time an interaction between ADAM23 and PrPc and open new perspectives to elucidate ADAM23's biological roles.

Palavras-Chave: ADAM23; PrPc; biochemical interaction.

Apoio Financeiro: CAPES/PROCAD; FAPESP; CNPq

ORGANIZATION OF THE PROJECTIONS FROM THE PREFRONTAL CORTEX TO THE DORSAL RAPHE NUCLEUS

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Introduction: Several studies had shown that the prefrontal cortex (PFC) can influence the activity of the dorsal raphe nucleus (DRN) via efferent projections. These projections may regulate behavioral and physiologic functions of the DRN and also can regulate activity of PFC neurons via feedback mechanisms. Although some studies analyzed the projections from the PFC to the DRN, none presented a detailed description of the distribution of these projections. The present work injected the retrograde neuronal tracer CTb (cholera toxin b-subunit) in different parts of the DRN and analyzed the distribution of CTb+ cells through the anterior-posterior axis of the PFC.

Methods and results: CTb was injected by electrophoresis in the rostral, central and caudal parts of the DRN, and the animals were perfused after 7 days. The brains were removed from the skull and processed with immunohistochemical techniques against CTb. Our results showed CTb+ cells in all regions of the PFC. Higher cellular density was found in the rostral region of the PFC (medial and lateral polar frontal cortex) and in its medial wall (prelimbic, infralimbic and medial orbital cortex). Few CTb+ cells were observed in the precentral medial cortex and anterior dorsal cingulus. Injections of CTb in the rostral and central parts of the DRN presented significant differences in the number of CTb+ cells in the lateral and dorsolateral regions of the orbital prefrontal cortex. Injection in the caudal part of the DRN showed less CTb+ cells than injections in the rostral and central part of the DRN.

Conclusion: These results showed indications that subregions of the PFC, specially the medial, orbital and lateral, send heterogenic projections to the rostral, central and caudal parts of the DRN.

Palavras-Chave: Prefrontal cortex, dorsal raphe nucleus, Immunohistochemistry, CTb, retrograde neuronal tracer

Apoio Financeiro: FAPESP and CAPES

H.03.002

EN BUSCA DEL SUSTRATO NEURONAL DE LA MEMORIA

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Experimentos farmacológicos desarrollados previamente en el laboratorio con el cangrejo *Chasmagnathus granulatus*, basados en administraciones sistémicas, permitieron dilucidar mecanismos moleculares que sirven a diversas fases de la memoria. Como muestran numerosos trabajos en vertebrados, un abordaje localizado ayudaría a determinar la funcionalidad de distintas áreas del sistema nervioso central (SNC) en procesos mnésicos en este modelo. Un método utilizado para identificar estructuras implicadas en memoria se basa en la destrucción neuronal por medio de microinyecciones localizadas con sustancias neurotóxicas, como el ácido iboténico (agonista glutamatérgico que destruye cuerpos neuronales en el área de administración).

Considerando estos antecedentes, el objetivo del proyecto es determinar la función de diferentes neuropilos del SNC del cangrejo en diversas fases del proceso mnésico. Para ello está en desarrollo una técnica de microinyecciones localizadas y la puesta a punto de un método estereotáxico. El dispositivo diseñado para tal fin consta de dos rotores montados sobre un microscopio, que permiten girar al animal sobre sus ejes de forma cuantificable. Una vez seleccionado un sitio por el sistema de coordenadas, con un micromanipulador adosado al microscopio se realizan las microinyecciones. Se sabe que ciertos neuropilos del pedúnculo ocular están involucrados en procesos mnésicos, por esto se seleccionó dicha estructura como sitio de interés.

Los resultados muestran que es posible establecer un sistema de coordenadas en el pedúnculo ocular, considerando características anatómicas externas y basándose en la forma y ubicación de la pseudopupila. Se microinyectaron fluorocromos que interactúan con los ácidos nucleicos, y por microscopía confocal se localizó la zona afectada y se analizaron las dimensiones de la misma.

Utilizando las coordenadas para localizar los diferentes neuropilos, se administrará ácido iboténico, y se realizarán experimentos comportamentales con protocolos ya descritos en este modelo, para establecer correlaciones entre las lesiones causadas y el cambio observado en los animales.

Palavras-Chave: Acido Iboténico, Invertebrado, Estereotaxia, Memoria

Apoio Financeiro: IFIByNE-CONICET

H.03.003

Atenuação da resposta hemodinâmica no córtex motor de pacientes que sofreram Acidente Vascular Cerebral (AVC) detectada por método de análise paramétrica.

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Os mecanismos de reorganização cerebral, após um AVC, têm sido bastante estudados através da técnica de Ressonância Magnética Funcional, que se baseia no princípio da estabilidade do sinal BOLD (*Blood Oxygenation Level Dependent*) ao longo do tempo de aquisição da imagem. Entretanto, essa estabilidade pode não ocorrer em situações patológicas. Dessa forma, o objetivo deste estudo foi investigar a ocorrência de alterações dinâmicas na intensidade do sinal BOLD no córtex motor em pacientes que sofreram AVC. Estudamos 25 pacientes, sendo que 4 foram excluídos devido a artefatos de movimento. Utilizamos um protocolo composto por seis blocos de repouso (27 segundos) intercalados por cinco blocos de atividade (27 segundos), durante o qual o paciente deveria abrir e fechar a mão requerida. As imagens foram adquiridas em tomógrafo de 1.5T (Siemens, Magnetom Vision) e processadas utilizando o programa comercial BrainVoyager QX 1.9™. Os mapas estatísticos com a localização das áreas ativas durante a tarefa foram obtidos através do Modelo Linear Geral. Com o objetivo de determinar a presença de redução na intensidade do sinal BOLD, utilizamos a Modulação Paramétrica, que consiste em realizar uma análise de conjunção entre dois preditores, um codificando o efeito principal da estimulação e outro codificando o efeito paramétrico ortogonalizado. Nas regiões motora primária, pré-motora e motora suplementar, identificamos uma redução na intensidade do sinal BOLD em 15 pacientes, ao longo da execução da tarefa com a mão parética. Porém, o mesmo não ocorreu quando a tarefa era executada com a mão não afetada. As alterações observadas podem estar relacionadas à habituação do córtex motor, fadiga motora ou acoplamento neurovascular alterado. Independente da razão neurofisiológica, essas alterações dinâmicas do sinal BOLD podem impedir a detecção de ativação cortical pela imagem funcional por ressonância magnética em pacientes que sofreram AVC. Portanto, alguns aspectos da ativação BOLD em pacientes devem ser avaliados de maneira mais completa em estudos futuros.

Palavras-Chave: Ressonância Magnética Funcional, BOLD, AVC, Análise Paramétrica

Apoio Financeiro: FAPESP

OTIMIZAÇÃO E COMPARAÇÃO DE TRÊS ANTICORPOS ANTI ALFA- SINUCLEÍNA PARA O BANCO DE ENCÉFALOS HUMANOS DO GRUPO DE ESTUDOS EM ENVELHECIMENTO CEREBRAL (BEHGEEC) DA FM-USP

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Otimizar e comparar três diferentes anticorpos anti α - sinucleína (anti α -syn) para uso em imunohistoquímica no tecido encefálico pertencente ao Banco de Encéfalos Humanos do Grupo de Estudos em Envelhecimento Cerebral (BEHGEEC) Faculdade de Medicina da Universidade de São Paulo (FM-USP).

Foram utilizados três anticorpos anti α -sinucleína (α -syn): MDV-2 (região N-terminal do NACP/ α & β -syn), EQV-1 (região NAC, específico para α -syn) e PQE-3 (região C-terminal do NACP, específico para α -syn), todos são policlonais, fabricados em coelho e cedidos pelo Dr. Kenji Ueda do Inst. de Psiquiatria de Tóquio. Testou-se os anticorpos com diversas técnicas de recuperação antigênica, diluição dos anticorpos e tempos de incubação. Todas as reações foram realizadas com método de imunoperoxidase, em cortes seriais paralelos. Para os três anticorpos testados, a melhor técnica de recuperação antigênica foi o uso de imersão em citrato 10%, pH 6, na panela à vapor por 40 minutos. O melhor tempo de incubação do anticorpo primário foi por 12 horas, seguido por 1 hora no anticorpo secundário e complexo biotinilado. Todas as revelações foram feitas com o cromógeno DAB (Diaminobenzidina Tetrahydrocloro). Com os três anticorpos foi possível a detecção de corpúsculo de Lewy e neuritos de Lewy, característicos da doença de Parkinson, mesmo em casos pré-clínicos, sendo que o anticorpo EQV-1 apresentou a melhor marcação, com a diluição de 1:100 microlitros.

A α -sinucleína, uma proteína expressada nos terminais pré-sinápticos do sistema nervoso central é uma peça central na patogênese da doença de Parkinson. Desde a descoberta da α -sinucleína como componente principal dos corpúsculos de Lewy em 1998, esta proteína é utilizada como marcador-padrão de Doença de Parkinson e de Demência por Corpúsculo de Lewy. A utilização e otimização desse marcador é fundamental para o diagnóstico de doenças neurodegenerativas, em especial nas fases pré-clínicas, considerando-se o grande interesse na busca de biomarcadores precoces. Ademais, a detecção dos casos pré-clínicos para conseqüente comparação destes com casos controle, tem o potencial de revelar aspectos importantes da fisiopatologia inicial dessas doenças. **Palavras-Chave:** α -synucleína, imunoistoquímica, corpúsculo de Lewy, doenças neurodegenerativas.

Apoio Financeiro: CAPES, FAPESP, IIEP-AE

ANALYSIS OF THE DURA MATER-ARACHNOID INTERFACE IN THE SUPERIOR SAGITTAL SINUS REGION IN HUMANS

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Introduction: The dura mater-arachnoid interface has been the subject of many studies, and discrepancies exist among the results. These divergent opinions prompted us to carry out a study of the interface between the dura mater and the arachnoid in the vicinity of the superior sagittal sinus.

Materials and Methods: Sixty encephalons including the respective meninges were collected from cadavers of both sexes at the Service of Death Verification – USP – São Paulo. After collection, the material was washed in running water and fixed in 10% formalin for 72 hours. Small cubes were embedded in paraffin and stained, using azorcarmine to assess collagen fibers and of HE to visualize the cellular component. Those slides selected for documentation were photographed with a Zeiss photomicroscope.

Results: We determined that the portion of the arachnoid meninx which is near the superior sagittal sinus is thick, that it is primarily composed of small bundles of collagen fibers linked to cellular elements, and that it projects into thick bundles of connective tissue in the dura mater. Primarily among the thick bundles of collagen fibers in the dura mater, as well as in other adjoining areas, we observed fine collagen-fiber structures, appearing as either isolated structures or as extensions of the larger structures that compose the arachnoid granulations. In a frontal section, we noted that the inner layer of the dura mater, which constitutes the floor of the superior sagittal sinus, consists of bundles of connective tissue intermingled with tissue originating in the arachnoid meninx. We also ascertained that elements of the arachnoid meninx penetrate large bundles of collagen fibers in the area where the two inner folds of the dura mater converge, known as the cerebral falx, and even present formations similar to arachnoid granulations.

Conclusion: The arachnoid meninx projects into the inner layer of the dura mater, generating the arachnoid formations and granulations. In the vicinity of the collagen fiber bundles in the dura mater, which are in contact with the arachnoid meninx, the cellular elements predominate.

Palavras-Chave: Arachnoid, Dura Mater, Superior Sagittal Sinus

Apoio Financeiro: CAPES

NGF receptors interactions by fluorescence resonance energy transfer

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Nerve Growth Factor (NGF) is a peptide responsible for the regulation of survival, differentiation and maintenance of responsive neurons. It is released from the target tissue and interacts with receptors on the axon tips[1]. NGF engages two structurally distinct transmembrane receptors, p75 and TrkA, which have been proposed to create a "high affinity" NGF binding site through formation of a ternary NGF/p75-TrkA complex. To mediate cellular responses NGF must activate a series of signaling events from the terminal plasma membrane of the nerve to the nucleus. According to the signaling endosome model, NGF is bound to its receptors on the membrane surface, endocytosed via the clathrin mediated endocytic pathway, and transported to the soma by interaction with components of the cytoskeleton[2]. Our group reported the first direct observation of individual NGF-containing endosomes being retrogradely transported in living differentiated PC12 neurite-like processes using monobiotin-NGF coupled to streptavidin-quantum dots[3]. By quantitative single particle tracking of the mobile fraction, we resolved retrograde and anterograde movements into a series of "broken staircase steps", with regions exhibiting net advance, pauses, and reversals. The net displacement was in the direction of the cell body, i.e. retrograde, as a consequence of a bias to longer run lengths and a mean velocity of $0.054 \pm 0.020 \mu\text{m/s}$. We concluded that retrograde motion occurred via a facilitated transport, probably mediated by dynein.

To study NGF interactions with p75 and TrkA in the retrograde transport process, we fused the receptors to the visible fluorescent proteins mVenus[4] and Cerulean[5] which are suitable to determine protein-protein interactions by fluorescence resonance energy transfer (FRET)[6]. Images acquired in a confocal microscope are quantitatively analyzed. Fluorescence parameters are exploitable to assess receptor interactions as well as localization, processing and retrograde transport of the NGF-receptor complexes. These studies will allow us to correlate the kinetics with functional responses of the neurons to NGF.

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Palavras-Chave: NGF, NGF Receptors, Retrograde Transport, FRET

Defined Displacement Algorithms in Combination with Robust Segmentation Procedures Create Structure Sensitive Random Scenarios for Statistical Evaluation of Colocalization in Confined Cellular Compartments

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Objective: The term colocalization refers to the detection of biological structures at the same physical location. So far, methods which estimate statistical significance of correlation or colocalization in a given biological sample do not respect the interconnectivity of adjacent pixels in response to the biological structure and the respective point spread function of the fluorescence channels (Biophys J. 86:3993-4003, 2004).

Methods: We use synthetic image series with defined degrees of colocalizing point signals in combination with different biological systems to test the robustness of a Defined Displacement Algorithm (DDA). DDA is developed on the basis of IDL (TT, Boulder, CO) in SCIAN-Lab and is presently installed on our web page for remote image processing (www.scian.cl).

Results: The comparison of the suggested DDA with previously suggested box-scrambling (BS) provides direct evidence that BS overestimates the random component due to the rupture of structure intrinsic intensity pattern. The gauss-like probability density functions show that DDAs generate 2σ -values that are a factor of 2-4 wider than those generated by BS. In consequence statistical significance for a colocalization scenario is overestimated significantly by BS. In addition we confine the DDA to segmented cellular compartments which leads to the generation of case sensitive random scenarios which directly model the signal distributions inside cellular compartments.

Conclusion: The evaluation of DDA in synthetic image series and different biological systems suggest DDA to be a method for the statistical evaluation of colocalization in confined cellular compartments.

Palavras-Chave: Colocalization, Defined Displacement Algorithms (DDA)

Apoio Financeiro: FONDECYT 1060890, 1071001, 1040824, PBCT AC47

H.03.008

Local Colocalization Indices in Combination with Defined Displacement Algorithms Quantify Colocalization of Point Sources in Confocal Microscopy

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Objective: In fluorescence microscopy, the term colocalization refers to the detection of biological molecules at the same physical location. Its application however bears two major drawbacks: First, the visual quantification of colocalizing biological structures is often ambiguous due to variations in the human perception. Second, existing analytical methods work with rather simple, colocalization or correlation coefficients (Manders or Pearson), which do not consider the diffraction limited intensity pattern of the point spread function (Biophys J. 86:3993-4003,2004).

Methods: We have developed an algorithm, which quantifies global and local colocalization reliably and leads a robust statistical estimation in different subcellular compartments by a defined displacement algorithm (Cardiovasc Res. 77:387-97, 2008).

Results: Here we present a series of local colocalization indices: (i) linear correlation functions (LPC), (ii) Scaling Indices Method (SIM), sensitive to geometrical image properties, (iii) Correlation Coefficient of Raw Images (CCRI), and (iv) Joint Moment of Standardized Images (JMSI). We combine the local colocalization indices with our defined displacement algorithm to validate statistical significance. We apply the method to synthetic data with well defined colocalization of fluorescent point signals and to glutamate receptors (GABA_BR1/R2) in nascent synapses of cultured hippocampus neurons.

Conclusion: Local colocalization indices in combination with defined displacement algorithms lead to a robust and statistically solid quantification of colocalization of sub-cellular structures in confocal microscopy.

Palavras-Chave: Local Colocalization Indices, Defined Displacement Algorithms (DDA)

Apoio Financeiro: FONDECYT 1060890, 1071001, 1040804, PBCT ACT47, FONDAP 15010006

H.03.009

Distribuição da atividade da NADPH diaforase no sistema nervoso central de caracóis terrestres jovens e adultos *Megalobulimus abbreviatus*.

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Objetivo: Com o objetivo de darmos continuidade à caracterização dos neurônios do SNC de *M. abbreviatus* e sabendo que o ON e sua enzima de síntese, a NOS, expressam-se muito inicialmente na filogenia, foi analisada a expressão e a distribuição do óxido nítrico através de histoquímica para NADPH diaforase em animais jovens e adultos. **Métodos:** Animais jovens e adultos (6 em cada grupo) foram anestesiados em uma solução de mentol saturada em solução fisiológica por 30 min, logo após retirada a concha foram retirados os diferentes gânglios que formam o SNC e fixados em paraformaldeído 4% em tampão fosfato (TF) 0,1M pH 7,4, por 4 h a temperatura ambiente. Logo, foram crioprotetidos em sacarose 30% em TF a 4°C. Os gânglios foram incluídos em meio de congelação e congelados em nitrogênio líquido. Os cortes (50 µm) foram realizados em criostato (Leitz) e recolhidos em tampão fosfato (TF) 0,1M pH 7,4 e processados para histoquímica. Foi feita uma pré- incubação dos cortes à temperatura ambiente por 5 min em uma solução contendo 12µl de triton X-100 (Sigma) diluído em 100ml de TF 0,1M pH 7,4. Após, os cortes foram incubados a 37°C em uma solução contendo 5mg de NADPH (Sigma) e 3mg de NBT (Sigma), diluídos em TF 0,1M pH 7,4. Os cortes permaneceram em estufa por 1h e 30 min. Após a reação, os cortes foram lavados com TF 0,1M pH 7,4. Posteriormente, os cortes foram montados em lâminas gelatinizadas, desidratados em uma série alcoólica crescente, diafanizados em xilol e cobertos com Permount. **Resultados:** Células nervosas coradas foram encontradas na maioria das regiões dos gânglios constituintes do SNC do caracol *M. abbreviatus*. Fibras NADPH-d positivas foram fortemente coradas e estão distribuídas extensivamente através de regiões neuropilares dos gânglios e presentes em todos os conectivos interganglionares. **Conclusão:** Podemos afirmar a existência de um sistema nitrérgico no SNC de *M. abbreviatus* e, a partir da correlação dos neurônios e fibras NADPH-d encontrados com células e fibras contendo neuropeptídeos envolvidos na nocicepção, o próximo passo de nosso estudo é verificar o possível envolvimento destes neurônios na modulação de seu comportamento nociceptivo.

Palavras-Chave: NADPH-d, óxido nítrico, caracol terrestre

Apoio Financeiro: CNPq

H.04.001

First Nicotine Cage Compound for neurochemical research.

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Nicotine is an [alkaloid](#) found in the [nightshade](#) family of plants. Classical nicotine signaling occurs in the brain, spinal cord, and autonomic nervous system and is mediated by nicotinic cholinergic receptors. Nicotine receptors also exist outside the nervous system and are related to cell survival. A better understanding of nicotine's functions using alternative techniques in short and long term experiments is worthwhile. We have developed a stable caged nicotine compound, suitable for biological uses such as single cell experiments or receptor mapping.

In this work we describe the synthesis, chemical and characterization of $[\text{Ru}(\text{bpy})_2(\text{Nic})_2](\text{PF}_6)_2$ (Nic: Nicotine), a caged nicotine that can be released using harmless blue light instead of the UV used in traditional caged compounds.

NMR analysis shows only nicotine release after a pulse of visible light is applied, with no traces of other photoproducts. The caged nicotine shows maximal absorbance at 443nm and quantum yield of uncaging in aqueous solution at 293 K an pH 7 was $\Phi = 0,25$.

Retzius neurons in leech ganglia show an increase in firing frequency after a laser pulse (473nm) when neurons are bathed in 100 μM caged nicotine. Firing frequency is measured in current clamp mode using sharp microelectrodes. Furthermore, the compound is very stable in the dark and does not show toxicity to leech ganglia or cultured mammalian cells (SHSY-5Y and NIH-3T3) under several hours.

All these properties make this caged nicotine an important tool for circuitry determination, receptor mapping and highly localized application of nicotine in biological models.

Palavras-Chave: caged compound, nicotine, circuitry, electrophysiology

Apoio Financeiro: CONICET, ANPCyT, UBA

H.04.002

Sensory deprivation by vibrissae removal long-lastingly facilitates cortical spreading depression propagation in rats.

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Objectives: Deprivation of sensory input from whiskers, at various stages of development, can induce physiological, and often structural, changes that modify the circuitry of these sensory systems, altering their response properties following prolonged alterations in input activity. Here we investigated whether such morpho-physiological alterations would influence the propagation features of cortical spreading depression (CSD), in just-weaned and adult rats.

Methods: Male Wistar rat pups had the right mystacial vibrissae removed followed by electro-cauterization at postnatal days 2-3 (n=20), and were submitted to CSD recordings, during 4h, on 2 cortical points of both hemispheres at 30-40 days of life (n=10; young rats) and 90-120 days (n=10; adult rats). CSD velocities of propagation were calculated based on the time required for CSD to spread along the inter-electrode distance.

Results: In young rats, CSD velocities (in mm/min) in the hemisphere contralateral to the vibrissae removal was higher (4.52 ± 0.36), as compared to those in the ipsilateral hemisphere (3.84 ± 0.20 ; $P < 0.05$). This effect persisted until adult life, when the mean velocities for lesioned and controls were respectively 4.13 ± 0.12 and 3.35 ± 0.06 . In age-matched controls, no inter-hemispheric differences in CSD velocities were found (mean values for the right and left hemispheres, respectively, of 3.80 ± 0.22 and 3.77 ± 0.20 for the young rats, and 3.41 ± 0.10 and 3.40 ± 0.10 for the adults).

Conclusions: The results suggest that deprivation of sensory input from whiskers during brain development facilitates cortical spreading depression propagation in rats. Since this effect persisted until adulthood, it is tempting to suggest that it is permanent, or at least long-lasting.

Palavras-Chave: Sensory deprivation; Vibrissae; Cortical Spreading Depression

Apoio Financeiro: CNPq, IBN-Net, Propesq/UFPE.

H.04.003

CARACTERIZACION ELECTROMIOGRAFICA DE LOS MUSCULOS ISQUIOTIBIALES Y GEMELOS EN EL DESARROLLO DEL SALTO VERTICAL.

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Describir cualitativa y cuantitativamente el comportamiento electromiográfico de 2 grupos musculares involucrados en el desarrollo del salto vertical tipo Abalakov y contribuir a establecer un patrón de medición que permita la evaluación objetiva del gesto motor.

METODOS

Empleando electromiografía de superficie (EMGS) se estudió un tipo de salto vertical en adultos jóvenes sanos. El salto vertical Abalakov, fue realizado sobre una plataforma de contacto marca AXON JUMP(R) que, a partir de la medición del tiempo de vuelo, permite determinar la altura alcanzada en cada salto. Para la descripción de las características EMGS se registraron los músculos isquiotibiales y gemelos de la pierna dominante, y posteriormente se realizó el análisis espectral del comportamiento en cuanto a frecuencia y amplitud de los músculos estudiados. Cada uno de los saltos fue subdividido en 6 fases para facilitar la descripción técnica y espectral electromiográfica. Para la EMGS se empleó el equipo de adquisición de señales POWERLAB 8/30(R). Para la división de las fases y el cálculo de la duración de cada una de ellas se empleó el software de análisis de movimiento SIMI MOTION (R), previa filmación y edición del evento.

RESULTADOS

El salto se dividió cualitativamente en 6 fases: 0 Reposo, 1 Impulso, 2 Despegue, 3 Vuelo de ascenso, 4 Vuelo de descenso, 5 Amortiguación, 6 Recuperación. El análisis electromiográfico mostró la mayor amplitud en la fase 1 y la frecuencias osciló entre 0 y 40 Hz en cada una de las fases.

CONCLUSIONES

Desde el punto de vista electromiográfico se encontró actividad sincronizada en los músculos isquiotibiales y gemelos del miembro dominante, con mayor actividad en la fase 1 del salto. La duración de las fases muestran similitudes en el desarrollo de cada uno de ellas. La descripción del salto vertical en fases de movimiento y el análisis electromiográfico pueden ser tomados como una medición standard, objetiva de referencia para estudios posteriores del gesto motor.

Palavras-Chave: Electromiografía de superficie, análisis espectral, salto vertical, gesto motor

Apoio Financeiro: Universidad de Pamplona, Grupo Actividad física, deporte y recreación en el desarrollo humano

ESTUDO DA CONDUÇÃO NERVOSA DO NERVO CAUDAL EM RATOS: UMA TÉCNICA PRECISA, SIMPLES E BARATA

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Objetivos: Desenvolver uma técnica precisa, simples e barata para o estudo da condução nervosa do nervo caudal em ratos. Os métodos para o estudo da condução nervosa no rato variam com o nervo estudado e a metodologia utilizada para o estímulo do nervo e o registro dos potenciais. Os nervos estudados incluem o safeno, o ciático e o nervo caudal. O nervo caudal é de acesso mais fácil e seu longo percurso o torna adequado ao estudo de neuropatias. As técnicas que usam eletrodos de superfície são mais simples e baratas que as que usam eletrodos de agulha.

Métodos: 20 Ratos *Wistar*, machos, com 60 dias foram utilizados. O sistema de estimulação e registro era constituído por uma tábua com 6 garras “jacaré” fixadas em linha a cada 3 centímetros. Os 2 eletrodos distais foram utilizados para estimulação, os 2 proximais para registro e os 2 centrais como terra. Os ratos foram anestesiados e posicionados ventralmente, de forma que sua cauda ficasse livre. A pele da cauda foi limpa, desengordurada e fixada nas garras “jacaré”. A temperatura do terço médio da cauda foi mantida em uma média de 31,5°. Os potenciais foram registrados e analisados com um eletroneuromiógrafo clínico convencional na cauda do animal através de técnica ortodrômica. A velocidade de condução (VC) e a amplitude pico-a-pico do potencial de nervo sensitivo foram determinadas durante 7 semanas consecutivas. As equações das retas de regressão linear e seus coeficientes de correlação foram calculados.

Resultados: A VC e a amplitude cresceram linearmente entre a 8ª e a 15ª semana de vida do animal de 34 ± 3 m/s a 44 ± 4 m/s e de 29 ± 6 μ V a 85 ± 13 μ V, respectivamente. As equações de regressão linear foram: $VCN(m/s) = 1,2 \text{ 'semanas} + 25$ ($R^2 = 0.86$) e $Amplitude(\mu V) = 8.1 \text{ 'semanas} - 34$ ($R^2 = 0.99$).

Conclusão: O estudo da condução nervosa do nervo caudal de ratos pode ser realizado de modo preciso, fácil e barato utilizando garras comuns “jacaré” como eletrodos de superfície.

Palavras-Chave: Condução nervosa, Nervo caudal, Eletrodos de superfície

H.04.005

Contagem estereológica de neurônios parvalbumina-positivos no hipocampo de primatas não humanos da espécie *Cebus Apella* (Humbolt 1812)

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As proteínas ligantes de cálcio têm sido objeto de muitos estudos, devido a sua capacidade reguladora de múltiplas funções dos íons cálcio. A utilização desses marcadores celulares para o mapeamento de regiões cerebrais tem resultado em importantes avanços no estudo da organização morfo-funcional do sistema nervoso. No presente trabalho foi empregada a técnica de imuno-histoquímica para a proteína ligante de cálcio parvalbumina (PV) com o propósito de estimar o número total de neurônios marcados (PV-positivos) no hipocampo de primatas-não-humanos da espécie *Cebus apella* ("macaco prego"). A contagem das células marcadas foi realizada através de neuroestereologia, que é definida como um conjunto de métodos matemáticos que permitem relacionar parâmetros tridimensionais das estruturas, a partir de mensurações realizadas em duas dimensões. A formação hipocampal de dois animais foi inteiramente seccionada no plano coronal, em fatias de 50 micrômetros, e observada em aumento de 400X. A contagem das células PV-positivas foi realizada sob visão direta e o número total de células foi obtido através da fórmula: $N_T = Q \times (1/ssf \times 1/asf \times 1/tsf)$, onde Q é o número de células contadas, ssf (amostragem das fatias) = 1:10, asf (amostragem da área) e tsf (amostragem da espessura) = 1. O número total de neurônios PV-positivos foi de 172.800 na região de CA1 e 276.800 na região do subiculum, com um coeficiente de erro no valor de 0,027. Sugere-se o uso ético desses animais como modelos experimentais em investigações biomédicas, pois particularmente em neurologia, as semelhanças entre sistema nervoso de macacos e humanos abrem uma possibilidade única para estudarmos a fisiologia e a patologia de diversos distúrbios neurológicos, ampliando assim as discussões e possíveis contribuições na área primatológica, bem como, na medicina humana.

Palavras-Chave: Cebus Apella, Células Hipocampais, Imuno-histoquímica, Contagem celular

Apoio Financeiro: CAPES, CNPq, FAPESP

H.04.006

Ritmo *mu* e o estudo do sistema de neurônios espelho em humanos: uma metanálise

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O ritmo *mu* é uma oscilação sincronizada com frequências dominantes entre 8 e 13 Hz, e 15 e 25 Hz, limitada a breves períodos de 0,2 a 0,5 segundos de duração, que pode ser registrada no córtex sensoriomotor de humanos, na ausência de movimentos. Este ritmo dessincroniza durante a execução ou observação de movimentos e durante a estimulação somatossensorial direta. Como as propriedades motoras dos neurônios espelho são eletrofisiologicamente indistinguíveis das relacionadas aos neurônios motores de áreas vizinhas, incluindo área pré-motora, motora e sensoriomotora, a dessincronização do ritmo *mu*, expressa pela supressão das ondas durante a execução de ações, é atribuída a ativação de sistemas neuronais dos córtices pré-motor e sensoriomotor. Entretanto, durante a observação de movimentos das mãos, o sistema de neurônios espelho parece ser a única rede ativa identificada nestas áreas corticais. Assim, tem sido sugerido que a supressão das ondas *mu* durante a observação de ações corresponderia a um correlato fisiológico da ativação do sistema de neurônios espelho. Foram pesquisados, nas bases PubMed, Scopus e Scirus, todos os artigos publicados até 2008 usando “mu rhythm” e “mirror neurons” como descritores. Dos 19 artigos encontrados, 12 que utilizavam métodos e desenhos experimentais similares foram analisados. Estes artigos relatam experimentos em que voluntários deveriam observar a ação executada por outra pessoa e, a seguir, imitar a ação executando-a; na condição controle, os voluntários apenas observaram movimentos não biológicos. Todos os artigos analisados relatam supressão progressiva das ondas *mu* relacionada a imitação das ações humanas. Parece haver consenso de que a dessincronização do ritmo *mu* durante a observação de movimentos é produto da atividade de vários domínios-alfa independentes (visual, auditivo e somatossensorial) que se acoplam para a tradução percepção-ação. Este sistema de tradução seria o principal mecanismo subjacente aos neurônios espelho, que estaria envolvido no reconhecimento de uma ação e na representação motora interna da mesma. **Palavras-Chave:** ritmo *mu*, neurônios espelho, dessincronização, metanálise

Apoio Financeiro: CNPq, Capes e Fapesp

H.04.007

Electrophysiological recordings of the hippocampus in rats: Preliminary findings

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We developed a high-density micro-electrode array for electrophysiological recordings in the rat hippocampal inner circuitry. The array consisted of 32 electrodes spaced 360µm in the antero-posterior and 500µm in the medio-lateral axis, with two depths spaced 1,0mm apart (16 electrodes for CA1/CA2 and 16 for DG/CA3, respectively). The animal was anesthetized with Xylazine (10 mg/kg) and Ketamine (100 mg/kg), following pre-medication with halothane (2% in O₂ for 5 min). After a small window was opened on the skull, the dura-mater was removed and the array implanted at the right hemisphere according to stereotaxic coordinates (based on Paxinos and Watson, 2005: Center of the array at approximately AP - 3.0mm; ML -2.5mm; DV -2.8 and -3.8mm, from bregma). After euthanasia and perfusion, forty micrometers frozen sections were cut through the whole hippocampus, and stained with Neo-TIMM and Nissl in order to allow hippocampal subregions to be correctly identified and electrode tips precisely localized. Following the proximo-distal axis, 2 electrode tips were at the DG polymorphic layer, 3 at CA3 proximal, 11 at CA3 distal, 2 at CA2, 5 at CA1 proximal, 8 at CA1 distal and one outside the hippocampus. Distances from pyramidal/polymorphic cell layers were (Mean + SEM in µm): 65.7 + 25.4 (DG polymorphic), 120.1 + 27.8 (CA3 proximal), 176.4 + 31.6 (CA3 distal), 158.0 + 158.0 (CA2), 121.3 + 31.1 (CA1 proximal) and 94.6 + 24.9 (CA1 distal).

Recordings were performed in the awake animal. Spike sorting was performed using template matching and PCA, both online and offline, using Sort Client® and Offline Sorter® (Plexon Inc – Dallas, Texas). Waveforms with artifacts were removed offline, and spikes aligned by their minimum, which allow better cluster separation in PCA (PNAS 2005 102:6125-6130). Sixty-seven units were identified, 3 in DG polymorphic layer, 5 in CA3 proximal, 27 in CA3 distal, 5 in CA2, 10 in CA1 proximal and 17 in CA1 distal.

We were able to precisely record multiunit activity from different hippocampal subregions, thus allowing for future recordings during learning and memory tasks in free-moving rats.

Palavras-Chave: hippocampus, CA1, CA2, CA3, electrophysiology

Apoio Financeiro: AASDAP, CAPES, FAPESP, IEP-HSL

H.04.008

Design and development of a microelectrode array for simultaneous recording of multiple brain regions

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Objective: The microelectrode array has properties of conventional microwire electrodes with the additional features of multichannel recordings. This work describes the design and development of a microelectrode array for simultaneous recording of multiple brain regions.

Methods: Using micromachining techniques, we fabricated electrodes array that consists of 4 bundles of 2 by 4 Teflon coated tungsten sharp microwire electrodes (diameter 50 μm ; impedance 1-3 MW at 1 kHz measured in saline). Each bundle was built with specific coordinates to implant in the septal area, anterior thalamic nuclei, cingulate cortex and mamillary body. The microwires were precisely positioned under guide plates using a drill bit sized to the wire. The drill bit was used to make holes in guide plates with an accuracy of 0.001 mm. The microwires of each bundle have the same length of 8.18mm, 6.7mm, 3.0 mm and 10.5 mm. The distances among bundles were 1.2 mm, 1.4 mm and 0.5 mm in the antero-posterior direction and 0.2, 1.4 e 0.2 in the latero-lateral direction. Each electrode in the bundle was separated by 300 μm in both directions. The bundles were fixed with epoxy paste, soldered in a printed circuit board (PCB) and connected to miniature connector (Omnetics, MN, USA) attached to the opposite side of the PCB.

Results: The microelectrode array has been successfully used in Wistar rats to record action potentials and local field potentials. We have successfully recorded up to four neurons per microwire electrode channel based on the quantification of multiple parameters (waveform voltage, waveform shape and interspike interval).

Conclusion: The study of information processing among brain regions requires monitoring large ensembles of neuronal populations simultaneously. Therefore, the development of microelectrode arrays with different shapes and sizes is essential for the comprehension of the neural mechanisms underlying behavior.

Palavras-Chave: microelectrode array, multiunitary recordings, local field potential, spike

Apoio Financeiro: Alberto Santos Dumont Research Support Association (AASDAP), Sirio Libanês Hospital Teaching and Research Institute/ (HSL-IEP), FINEP, Duke University, FAPESP, CNPq, CAPES

H.04.009

Identificação computadorizada de crises tipo ausência no eletrocorticograma de ratos

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Objetivos: Crises tipo epilepsia de ausência são expressas no eletrocorticograma de ratos como surtos paroxísticos de descargas espícula-onda. Essas crises têm frequência intrínseca na faixa de 7 a 11 Hz e voltagem entre 300 e 1000 µV (3 a 10 vezes a potência de base). O objetivo do presente trabalho foi desenvolver e testar um sistema automatizado de identificação de crises.

Métodos: O princípio do sistema é diferenciar sinais de crise de outros sinais pela análise da potência na faixa de frequência entre 7 e 11 Hz, que é alta apenas durante as crises. A rotina, elaborada no Matlab[®], é informada com um intervalo de tempo em que não ocorreram crises (crivo), a fim de estabelecer o critério de identificação: potências maiores que a média do crivo acrescida de 3 desvios-padrão são identificadas como crise. O sistema compara, por meio da transformada rápida de Fourier, o crivo com o registro inteiro, que é dividido em intervalos de 1 s. A saída do programa é uma tabela informando a presença de crises nesses intervalos. Quatro ratos Wistar geneticamente portadores de crises tipo ausência receberam implante de 8 eletrodos corticais e, após uma semana de recuperação, registrou-se o eletrocorticograma por 80 min. As crises foram quantificadas manualmente minuto a minuto. O registro e a quantificação das crises de um animal foram usados no refinamento do sistema.

Resultados: Os valores da quantificação automatizada foram bastante similares aos da quantificação manual para o animal usado na elaboração do sistema (a equação da reta é $\text{quantificação_automatizada} = 0.4207 + 0.8625 * \text{quantificação_manual}$; $r=0.9842$). Os registros dos demais animais foram usados para testar o sistema apresentaram padrão similar ($\text{quantificação_automatizada} = 2.0132 + 0.8213 * \text{quantificação_manual}$; $r=0.9544$).

Conclusões: O sistema é capaz de quantificar com grande fidelidade as descargas eletrocorticográficas espícula-onda, mostrando-se como potente instrumento na análise de registros em estudos com modelos animais desta patologia. Adicionalmente, pequenas adaptações permitem identificar outros padrões encontrados em registros eletroencefalográficos (sono, vigília, manifestações patológicas).

Palavras-Chave: EEG, identificação computadorizada de padrão, Fourier, descargas espícula-onda, epilepsia de ausência.

Apoio Financeiro: CNPq

H.04.010

Monitoreo de función cerebral durante la ocurrencia de hipoxias reiteradas breves o prolongadas en el cerdo recién nacido y su correlación con la hemodinamia sistémica y el estado ácido base

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Objetivos

El monitoreo de función cerebral (MFC) se ha utilizado para valorar el estado de afectación de la funcionalidad neurológica luego del agravo hipóxico/isquémico en modelos de experimentación neonatal así como en el recién nacido. El estudio de las variables influyentes sobre su alteración puede ayudar a detectar marcadores de sufrimiento precoz, con perspectiva de ensayar terapéuticas eficaces aún no existentes.

El objetivo del presente trabajo ha sido identificar aquellas variables (presión arterial sistémica (PAS), frecuencia cardíaca (FC), pH, exceso de base (BE), lactato en sangre, que se asocian a las alteraciones severas del MFC durante el establecimiento de agravo neurológico hipóxico.

Métodos

Se utilizaron cerdos recién nacidos (n=20), menores de 48 hs, anestesiados y ventilados, expuestos a 1) hipoxias reiteradas y 2) hipoxia prolongada hasta lograr agravo electroencefalográfico definitivo, con valoración de parámetros gasométricos, metabólicos, PAS y MFC. Los animales fueron anestesiados con midazolam, ketamina y fentanyl i/v que no modifican el patrón basal del MFC en vigilia, se colocó catéter arterial femoral para la monitorización de PAS. MFC, pulsioximetría y temperatura central fueron monitorizados.

Resultados

La PAS se relaciona en forma directamente proporcional al voltaje del MFC, lo cual señala la trascendencia en el mantenimiento de la hemodinamia para impedir mayor deterioro de la actividad eléctrica encefálica.

La duración de las hipoxias, el tiempo total en hipoxia y en hipotensión así como el ácido láctico son parámetros que inciden directamente en las alteraciones del voltaje encefálicas. El BE menor a -20 y el lactato en aumento señalan el daño por MFC irreversible.

Conclusiones

El MFC muestra buena correlación con la entidad del agravo hipóxico.

Las variables hemodinámicas y metabólicas se afectan en cualquiera de los 2 tipos de hipoxia, sin embargo las mismas se alteran según la severidad del insulto, pudiendo ser reversibles frente a hipoxias de menor duración o que se intercalan con períodos de normoxia. En este caso el MFC acompaña la reversibilidad del daño, no sucediendo así con las hipoxias mantenidas por períodos mayores a 30 minutos.

Palavras-Chave: hipoxia, MFC, hemodinamia

Apoio Financeiro: Lucia Vaamonde

H.06.001

Establishment of an acute ocular hypertension model to test experimental gene therapy for glaucoma

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Objective: To establish an experimental model that mimics natural acute glaucoma, for pre-clinical testing of experimental gene therapy

Methods: Newborn Lister hooded rats were injected with Retrobeads (1:2 in PSB, Lumafluor) in the superior coliculus. After one month, the same rats were injected intravitreously with 2uL (10^{12} particles/mL) rAAV containing either the *GFP* or the *Max* gene. Acute intraocular pressure (IOP) is induced by introducing a 27-gauge needle, connected to a reservoir containing 500mL sterile saline, into the anterior chamber of the eyes of young adult rats (8-10 weeks old). The container was raised to 1,496m to elevate the IOP to 110mmHg². After 2 weeks post induction, the eyes were fixed, the retinas were flat mounted and DNA labeled with Sytox Green (5uM in PBS, Invitrogen). The processed retinas were examined by epifluorescence microscopy (Axiophot, Carl Zeiss) and cells were counted in the retinal ganglion cell layer. The data were statistically analysed using T-students test.

Results: The data show 30-50% cell death among retinal ganglion cells. The injections in the superior coliculus label the RGC and intravitreal injections are under experimentation.

Conclusion: Intraocular hypertension has been shown to be a good model to mimic acute glaucoma. This model should be useful for pre-clinical testing of a gene therapy protocol previously shown to be effective against retrograde degeneration of optic nerve damaged RGCs.

References: [1] Petrs-Silva, H., et al. Braz. J. Med. Biol. Res. 38(3): 375-379, 2005. [2] Huang, Y., et al. Exp. Eye Res. 85(5): 659-666, 2007.

Palavras-Chave: Gene Therapy, Glaucoma, retinal ganglion cells

Apoio Financeiro: CNPq, Instituto do Milênio

H.07.001

Anxiolytic effects of Cannabidiol in a Posttraumatic stress disorder model in rats

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Introduction: Posttraumatic stress disorder (PTSD) is an incapacitating chronic syndrome that reflects cognitive, emotional, and physiological processing disorders that follows an initial reaction to a traumatic experience. Activation of serotonin 1-A (5HT1A) and cannabinoid-1 (CB1) receptors decrease defensive behaviors. Moreover, CB1 receptors participate in extinction of aversive memories Cannabidiol (CBD), a nonpsychotomimetic constituent of Cannabis sativa plant, produces anxiolytic-like effects in animal models and humans. Although its mechanisms of action are not clear, CBD could facilitate 5HT1A- and CB1-mediated neurotransmission. However the effects of CBD in PTSD models have not yet been investigated. Thus, the aim of this study was to investigate the effects of CBD in rats submitted to a PTSD model. **Methods:** Male Wistar rats (210-230g) were placed into a box where they were exposed to a toy or a live cat for ten minutes. After the exposure the rats received daily systemic injections of vehicle (V) or CBD (5mg/Kg) for seven days. Twenty four h after the last injection the rats were submitted to the Elevated plus maze test (EPM), an animal model of anxiety.

Results: Exposure to a live cat decreased the time spent in the open arms of the EPM (cat versus drug interaction: 165 ± 30 , $F_{(1,24)} = 5.6$). CBD (5mg/Kg) was able to increase the number of entries (toy cat: $V = 17.9 \pm 4.8$; $CBD = 20.4 \pm 6.8$; live cat: $V = 7.8 \pm 3.7$; $CBD = 24.0 \pm 4.9$) and the time spent (toy cat: $V = 9.9 \pm 2.7$; $CBD = 5.2 \pm 1.5$; live cat: $V = 0.9 \pm 0.7$; $CBD = 5.9 \pm 2.6$) in the open arms of the EPM of rats exposed to the live cat seven days before. (Results were expressed in mean $\pm$ SE, $p < 0.05$; $n = 7$).

Conclusions: The results suggest that CBD can attenuate the behavioral consequences of traumatic events.

References: [Physiol. Behav.](#) 54:101-9, 1993; *Nature* 418: 530-4, 2002; *Neuropsychopharmacology* 31:2610-8, 2006.

Palavras-Chave: anxiety, cannabinoids, predator, stress, elevated plus maze

Apoio Financeiro: FAPESP

H.07.002

Interferência de exposições múltiplas sobre os parâmetros comportamentais avaliados em modelos experimentais de ansiedade em camundongos.

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Introdução: Há evidências na literatura de que o efeito da exposição repetida de camundongos ao labirinto em cruz elevado(LCE) altera o número de entradas nos braços abertos. No entanto, não há concordância sobre a interferência de exposições múltiplas em outros modelos experimentais de ansiedade como a caixa claro-escuro(CCE). Um protocolo que reúna vários testes para refinar a avaliação da atividade ansiolítica é de grande importância, por implicar na redução do número de animais de experimentação e no melhor aproveitamento das preparações em teste.

Objetivo: Estabelecer uma bateria de testes para avaliação da atividade ansiolítica que permita o uso do mesmo animal em vários procedimentos sucessivos.

Métodos: Grupos independentes de camundongos swiss machos (n=7-10) foram expostos ao LCE e depois à CCE(grupo LC) ou à CCE seguido do LCE(grupo CL), com intervalo de 30 min. entre as exposições. Foram analisados os parâmetros clássicos destes procedimentos; e as comparações foram feitas entre os resultados obtidos na primeira e segunda exposição ao mesmo modelo experimental. Os resultados, apresentados como [mediana(Q1-Q3)], foram analisados por Mann-Whitney, com diferenças significantes com $p \leq 0,05$.

Resultados: Na CCE não foram observadas diferenças significantes em nenhum dos parâmetros avaliados entre os grupos submetidos ao procedimento na primeira (CL) e na segunda exposição (LC). No entanto, no LCE, houve diferença no tempo de permanência nos braços abertos entre o grupo LC submetido ao modelo na primeira exposição [14(2-19)] e o grupo CL submetido ao modelo na segunda exposição [0(0-0)].

Conclusão: A exposição prévia à CCE exerce efeito ansiogênico que leva a alteração na resposta dos animais no LCE, evidenciada no principal parâmetro deste modelo, mostrando que, de fato, a resposta no LCE é dependente da experiência prévia do animal. Por outro lado, os comportamentos avaliados na CCE parecem ser mais robustos, pois não sofreram a interferência da experiência prévia no LCE. A continuidade deste estudo com diferentes intervalos entre as exposições e outros procedimentos é necessária para refinar essa avaliação e estabelecer uma bateria de testes para ansiedade.

Palavras-Chave: Bateria de testes comportamentais; labirinto em cruz elevado; caixa claro-escuro

Apoio Financeiro: CAPES

H.07.003

Efeito da silimarina sobre a memória e a atividade antioxidante em cérebro de ratos da espécie *Rattus norvegicus* Berkenhout, 1769) (Mammalia: Rodentia)

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O estresse oxidativo (EO) é causado pelo desbalanço entre as defesas antioxidantes e a produção de espécies reativas de oxigênio (ERO), causando dano oxidativo a macromoléculas como lipídios de membrana, enzimas, DNA. As ERO podem modular a consolidação da memória. Flavonóides são moléculas consideradas antioxidantes capazes de interceptar ERO. Silimarina (Sili) é um complexo de flavonóides utilizado para hepatoproteção, câncer epitelial, etc. O objetivo deste trabalho foi avaliar o efeito da Sili sobre aprendizado e memória na tarefa de esquiwa inibitória (EI), correlacionando com parâmetros bioquímicos que avaliam EO. Para o estudo de EO foram utilizadas as medidas de capacidade antioxidante total (CA), através da concentração de espécies reativas de oxigênio por fluorescência e de peroxidação lipídica (LPO), através da formação do complexo Fe (III)-laranja de xilenol por absorbância. Foram utilizados Ratos Wistar de 2-4 meses (250-350g), tratados com Sili nas doses de 200mg/Kg/dia e 400mg/kg/dia (Sili 200 e 400), o grupo controle (CTR) recebeu veículo, por gavagem, durante 15 dias. Os resultados da EI foram analisados pelos testes de Kruskal-wallis e Mann-Whitney, e os demais parâmetros foram analisados por ANOVA ($p < 0,05$). As duas doses de Sili utilizadas demonstraram ser facilitatórias das memórias de curta duração (STM) e de longa duração (LTM) dados expressos em medianas e intervalos interquartis (CTR: STM-23,97/21,7-60,75; LTM-72/57, 1-96, 09, Sili200: STM-180/78, 95-180; LTM-180/180-180, Sili400: STM-74,50/59,02-161,4; LTM-180/107, 7-180). A CA aumentou somente no córtex dos animais tratados com Sili 200 (CTR: 14415000 ± 95410 ; Sili 200: 1110000 ± 79770 ; Sili 400: 1190000 ± 61070) e a LPO diminuiu somente no córtex dos animais tratados com Sili 200 (CTR: $129,6 \pm 9,044$; Sili 200: $95,40 \pm 24,81$; Sili 400: $108,1 \pm 54,23$), expressos em média e erro padrão. Estes resultados sugerem que a dose de Sili 200 é eficaz contra dano oxidativo, além de favorecer o aprendizado e a memória.

Palavras-Chave: Silimarina - Memória - Estresse Oxidativo

Apoio Financeiro: Capes - FAPERGS - CNPq

AUTOMAÇÃO DO PARADIGMA DA EXPLORAÇÃO LIVRE II: O USO DAS ZEOLITAS.

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Objetivos: O Paradigma da Exploração Livre, por ser, até o momento, o único modelo animal proposto como modelo de ansiedade-traço, tem representado um papel muito importante no estudo da ansiedade. E para que seus resultados sejam confiáveis, é imprescindível a precisa quantificação dos comportamentos apresentados pelos animais testados. Neste contexto, um sistema computadorizado de rastreamento de animais pode ser de grande valor científico. Dessa forma, o objetivo do presente estudo foi dar continuidade ao processo de validação da automação do Paradigma da Exploração Livre (PEL), explorando o uso de zeolitas como forração da caixa de exploração.

Métodos e Resultados: Inicialmente, 15 ratos Wistar machos, adultos foram submetidos ao PEL, sendo que o lado familiar da caixa de exploração recebeu forração de zeolitas em substituição à maravalha, para que o rastreamento por vídeo fosse possível. Os resultados obtidos por um software de vídeo-rastreamento, Anymaze©(Stoelting, USA) foram comparados aos obtidos manualmente por dois observadores através de estudo correlacional. O coeficiente de correlação intraclassa (CCI) foi calculado para os parâmetros porcentagem de tempo no ambiente não-familiar (%TNF; média $\pm$ dp: Anymaze= 61,9 $\pm$ 17,5; Observador 1= 63,0 $\pm$ 18,0; Observador 2= 63,9 $\pm$ 17,6) e número total de transições no aparato (TT; média $\pm$ dp: Anymaze = 124,5 $\pm$ 24,2; Observador 1= 123,8 $\pm$ 27,7; Observador 2= 127,1 $\pm$ 25,5). As análises mostraram uma alta correlação entre os dados coletados pelo Anymaze e pelos observadores, tanto para %TNF (CCI= 0,9961; $p < 0,0001$) como para TT (CCI= 0,9595; $p < 0,0001$). A seguir, a fim de verificar uma possível interferência do uso das zeolitas nos resultados do modelo, 18 animais foram aleatoriamente alocados para dois grupos: PEL com maravalha e PEL com zeolitas, ambos avaliados manualmente. Não foram encontradas diferenças significativas entre os grupos para %TNF (média $\pm$ dp: zeolitas = 80,8 $\pm$ 8,7; maravalha = 82,0 $\pm$ 7,2), nem para TT (média $\pm$ dp: zeolitas = 74,7 $\pm$ 25,4; maravalha = 67,9 $\pm$ 11,6).

Conclusão: Os resultados obtidos trazem mais evidências acerca da confiabilidade da versão automatizada do PEL. **Palavras-Chave:** Paradigma da Exploração Livre, Automação, Zeolitas

**DIETHYL-2-PHENYL-2-TELLUROPHENYL VINYLPHOSPHONATE EXERTS
ANTIDEPRESSANT-LIKE EFFECT IN MICE**

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Organotellurium compounds have been reported as excellent antioxidants in several models of oxidative stress. Between them, it is a vinylic telluride, the diethyl-2-phenyl-2-tellurophenyl vinylphosphonate (DPTVP) and a simple diaryl diselenide, the diphenyl diselenide (PhSe)₂, which did not present significant toxic effects to mice [1,2,3,4,5]. It's important to notify that, as organochalcogens and belonging to the group 6A in the periodic table, the selenium and tellurium compounds have similar chemical characteristics. Moreover, it has been reported in literature that PhSe₂ exerts antidepressant-like and anxiolytic effects in mice [6]. Therefore, based on the considerations above, the present study was undertaken to investigate if DPTVP causes antidepressant-like effect, employing forced swimming test (FST) and tail suspension test (TST). The duration of immobility time was scored during the 6 min test period. The locomotor and exploratory behavior was accessed in an open-field test. The behavioral experiments were conducted using adult Swiss mice (25-35 g) from both sex and DPTVP intraperitoneal administration at doses from 1 to 100 µmol/Kg. The control group received canola oil and the positive control group, amitriptyline 2mg/kg. The animals presented no significant difference in the number of crossings between the groups control and DPTVP in the open-field test. The following data represents immobility time (seconds) during the FST test. Values are represented with the mean±S.E.M. Control: 216,111±7,008; Positive control: 108,38±18,9; DPTVP 1 µmol/kg: 113,5±25,5; DPTVP 10 µmol/kg: 159,33±15,19; DPTVP 25 µmol/kg: 147±13,97; DPTVP 50 µmol/kg: 173,83±9,63; DPTVP 75 µmol/kg: 109,50±38,47; DPTVP 100 µmol/kg: 142,83±17,37. The TST test depicted similar results. Our results suggest that DPTVP is not a psychomotor stimulatory and that this vinylic telluride has antidepressant-like effect in mice similarly as (PhSe)₂ and the positive control utilized.

1- Toxicology 129:29 (2003b)

2- Chem Rev 104:6255 (2004)

3- Toxicology 224:100 (2006)

4- Life Sciences 80:1865 (2007)

5- Brain Research Bulletin 76:114 (2008)

6- Pharm, Biochem and Beh 88:418 (2008)

Palavras-Chave: Antidepressant-like; Tellurium; diethyl-2-phenyl-2-tellurophenyl vinylphosphonate

Apoio Financeiro: CAPES, CNPq, UFSM

Antidepressant Effect and Changes of GABA_A Receptor Subunits mRNA After Hippocampal and Striatal Administration of Allopregnanolone in Rats.

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Brain areas related with the humor involve the limbic-talamic-cortical or limbic-striatal-palid-talamic-cortical circuits, where there is high concentration of GABA neurons. The aims of the present study are to verify the effect of bilateral intra-hippocampus and intra-striatum administration of the neurosteroid allopregnanolone (ALLO), a GABAergic positive modulator, in the modified forced swimming test (MFST) and the involvement of the alpha-4 and gamma-2 GABA_A receptor subunits gene expression on the hippocampal effect. Wistar male rats were treated with control solution (SolC): 2-hydroxypropil-β-cyclodextrin 20% w/v in artificial cefalo-rachidian liquid fluid; ALLO 1.25 mcg/rat; ALLO 2.5 mcg/rat or ALLO 5.0 mcg/rat. The microinjection was carried through by bilateral cannulae, at 0.5 µl/side and 0.25 µl/min. Each rat received the ALLO or SolC, 24, 5 and 1 h before the test section of the test, and they were videotaped during 300 s for posterior behavior analysis. At the end of the experiments the animals were eutanazied and their brains were removed and freezed for posterior histological analysis. Results showed that bilateral intra-hippocampal ALLO administration of 2.5 mcg/rat reduced immobile behavior and increased climbing behavior in the FST test ($F_{(3, 27)} = 4.56$; $p = 0.011$). The intra-striatal administration did not affect the immobility duration. Overall, for all doses of ALLO tested in the hippocampal administration, an increase of gamma-2 GABA_A subunit mRNA was observed ($F_{(3, 61)} = 9.649$, $p = 0.003$). There was a higher increase in the gamma-2 gene expression in the right hemisphere than in the left hemisphere ($F_{(3, 61)} = 4.33$, $p = 0.008$) after intra-hippocampal ALLO treatment. ALLO did not change the expression of the alpha-4 subunit. In conclusion, the intra-striatal administration of ALLO, at these three concentrations, has not presented antidepressive-like effect at the MFST. However, the intra-hippocampal administration of ALLO produces this effect, at an intermediate dose, confirming the potential of neurosteroids as a new class of antidepressant drugs. Our findings suggest that the gamma-2, but not the alpha-4 GABA_A subunit, is involved in the antidepressant-like effect of ALLO in the hippocampus and that there is a hemispheric diversity in the biochemical effect of the drug.

Palavras-Chave: Depression, Allopregnanolone, GABA_A receptor, gamma-2, alpha-4, FST

Apoio Financeiro: CAPES, CNPq, UFCSPA

Efecto de lesiones en la Corteza Prefrontal sobre el síndrome comportamental inducido por el antagonista NMDA, Dizocilpina, y su bloqueo por Clozapina.

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Dizocilpina (MK-801), antagonista del receptor N-metyl-D-aspartato (NMDA), es usado como modelo farmacológico para estudiar la neurobiología de la esquizofrenia y del mecanismo de acción de los fármacos antipsicóticos (AT). La administración sistémica de MK-801 en roedores, produce un síndrome comportamental de hiperlocomoción, estereotipias y ataxia; aumenta la actividad y la liberación de glutamato de la corteza prefrontal medial (CPFm), efectos antagonizados por el AT Clozapina (Clz). Así, la CPF parece participar en la acción del antagonista NMDA. Sin embargo, los elementos celulares, circuitos y regiones involucrados en su mecanismo aún no se conocen completamente.

En el presente trabajo estudiamos el papel de la CPF en la generación del síndrome conductual inducido por MK-801 y su antagonismo por Clz.

Ratas macho cepa-Wistar fueron lesionadas en la CPFm con lesión electrolítica (LE, corriente constante de 1mA/10s) o transección cortical (TC). Animales sham (S, operados-sin lesión) se usaron como control. A los 7 días, los grupos fueron tratados con MK-801 (0.2 mg/kg) y/o Clz (1 mg/kg); 30 min. después se evaluaron las conductas en el modelo *Open Field*. Se registró en forma automática la actividad locomotora y por observación directa se cuantificaron estereotipias, *rearings* y signos de ataxia. El nivel de significación fue $p < 0.05$.

MK-801 indujo un aumento significativo de la locomoción en animales S similar al encontrado en animales con LE y TC ($p < 0.001$). La co-administración de Clz bloqueó la hiperlocomoción producida por MK-801 tanto en animales S como lesionados. La inducción de estereotipias y ataxia por MK-801, y su bloqueo por Clz, no fueron modificados por la LE y TC. MK-801 disminuyó significativamente la conducta de *rearings* ($p < 0.001$), la cual no fue bloqueada por Clz y apareció disminuida basalmente en los animales con LE y TC ($p < 0.05$).

Los resultados muestran que el síndrome inducido por MK-801 y su bloqueo por Clz son mayormente independientes de la funcionalidad de la CPF. La conducta de *rearings* usualmente asociada con la transmisión dopaminérgica, estaría regulada además por el sistema glutamatérgico vía receptor NMDA y por la CPFm.

Palavras-Chave: Corteza prefrontal, antagonistas NMDA, comportamiento

Apoio Financeiro: Proyecto Fondo Clemente Estable 10.029

Cotinine: neuroactive actions upon extinction of conditioned learning and oxidative stress.

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The present work aimed to evaluate the effect of cotinine over extinction of conditioned learning and its possible connection with lipid oxidative damage levels in the hippocampus. Young male rats were anesthetized and implanted bilaterally with cannulae (hippocampus CA1 area). Conditioned learning was performed in a step-down inhibitory avoidance apparatus. Extinction was performed in 24h intervals, up to six days. Animals were infused, 15 min. pre-trial, with saline, nicotine 18mM, cotinine (6mM or 18mM), methyllycaconitine 34mM (MLA) or dihydro-b-eritroidine 18mM (DHbE). After, hippocampi were dissected and TBARS assay was performed (it quantifies malondialdehyde - MDA- a product of free radical damage to lipids). Results are expressed as median and interquartile range. Saline-treated animals consolidated extinction after 5 trials (34.7/17.3-52.5). Nicotine group consolidated extinction after 3 trials (23.4/9.9-134.9). For both cotinine groups (21.2/3.6-11.5 and 25.7/13.7-25.9, respectively) and DHbE animals (22.6/17.4-92.8) this consolidation occurred by the 4th day. MLA impaired the consolidation of conditioned learning extinction (180/51.8-0). Lipid oxidative damage (nmoles MDA/g tissue) was increased by MLA (23091.7 ± 4683.7) and DHbE (34356.9 ± 4056.2), compared to saline (1583.3 ± 4683.7). Nicotine also elevated these levels (75750 ± 5736.4), but cotinine did not (487.5 ± 8112.5 and 1470.8 ± 57.36.4, respectively). Our results showed that cotinine, as well as nicotine might facilitate the consolidation of conditioned learning extinction and that effect could be mediated by a7 nicotinic acetylcholine receptors, since MLA (but not DHbE) impaired consolidation. Interestingly, these alkaloids effect upon lipid oxidative damage is divergent. Also, no relation of lipid oxidative damage levels with extinction consolidation could be outlined. Studying cotinine effects over nervous system is very important since this nicotine metabolite figures as a possible non-aggressive treatment for smoking or even a potential drug for cognitive or neurodegenerative diseases caused by oxidative damage. **Palavras-Chave:** Cotinine, memory extinction, oxidative stress

Apoio Financeiro: FURG, CNPq

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SEROTONIN, VENTROMEDIAL HYPOTHALAMUS AND DEFENSE.

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INTRODUCTION AND PURPOSE: The dorsomedial part of the ventromedial hypothalamus (VMHdm) is part of the medial hypothalamic defensive system, critically involved in the organization of defensive behavior. The purpose of the present study was to investigate the effect of serotonin (5-HT) intra-VMHdm on defensive behavioral responses in rats submitted to the elevated T maze model of anxiety (ETM). The model allows the measurement of two behavioral defensive responses in the same rat: one-way escape and inhibitory avoidance. In clinical terms, these responses have been related to panic and generalized anxiety, respectively. **METHOD:** Male Wistar rats (300 g) were treated intra-VMHdm with 5-HT (20 nmols/0.5 µl) or saline (0.5 µl) and 10 min after were submitted to the ETM. Avoidance was measured by placing the rat at the end of the enclosed arm and recording the time to withdraw from this arm in 3 consecutive trials (Baseline, avoidance 1 and 2). Soon afterwards, escape was measured by recording the time taken by the animals to withdraw from the open arm again in 3 trials (escape 1, 2 and 3). Immediately after, animals were tested in the open-field for locomotor assessment. Data was analysed by ANOVA followed by the Duncan test. **RESULTS AND DISCUSSION:** No significant differences were found among the groups of treatment in the measurements performed in the ETM ($P > 0.05$). These results agree with previous observations performed in our laboratory showing the intra-VMHdm 8-OH-DPAT, a 5-HT_{1A} agonist, does not alter defense responses in the ETM. However, it is also important to point out that, in the present study, a significant decrease in the number of rearings was found in the open field (5-HT: 51.4 ± 8.7 , SALINE: 81.4 ± 10.8). It is, thus, possible that at least some of the negative results observed might have been related to motor impairment. **Palavras-Chave:** ventromedial hypothalamus, serotonin, defense, anxiety, elevated T-maze

Apoio Financeiro: CAPES and FAPESP (Brazil)

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Acute dehydroepiandrosterone treatment exerts differential effects on dopamine and serotonin metabolism in several brain regions

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Introduction: It has been shown that dehydroepiandrosterone (DHEA) interacts with dopamine (DA) and serotonin (5-HT) neurotransmitter systems, which are in turn involved in the pathophysiology of neurological and psychiatric disorders.

Objective: To explore if acute DHEA treatment modulates DA and 5-HT metabolism we analyzed the content of those neurotransmitters and their metabolites in the rat corpus striatum (CS), nucleus accumbens (NAc), frontal cortex (FCx) and hippocampus (Hip)

Methods: DHEA was i.p. injected (30, 60 and 120 mg/kg) and animals were sacrificed 2 hours after steroid administration as reported in the literature. The content of DA and 5-HT as well as their metabolites was analyzed by high-performance liquid chromatography coupled to electrochemical detection.

Results: DHEA treatment significantly reduced DOPAC content (control: 0.917 ± 0.073 pg/mg tissue, DHEA 120 mg/kg: 0.655 ± 0.056 pg/mg tissue; $p=0.022$) as well as DOPAC/DA (control: 0.142 ± 0.011 , DHEA 120 mg/kg: 0.096 ± 0.004 ; $p=0.003$) and (DOPAC+HVA)/DA ratios (control: 0.231 ± 0.015 , DHEA 120 mg/kg: 0.172 ± 0.009 ; $p=0.013$) in the CS, while DA ($p=0.840$) and HVA ($p=0.288$) contents, as well as HVA/DA ratio ($p=0.050$) remained unchanged. DA markers were not significantly altered in the NAc under our experimental conditions ($p>0.064$). 5-HT content was only altered in the FCx showing reduced levels after treatment (control: 0.5362 ± 0.035 pg/mg tissue, DHEA 60 mg/kg 0.3934 ± 0.021 pg/mg tissue; $p=0.021$). 5-HIAA/5-HT ratio was increased in the CS (control: 0.581 ± 0.114 , DHEA 60 mg/kg: 1.025 ± 0.087 ; $p=0.029$), NAc (control: 0.592 ± 0.051 , DHEA 120 mg/kg: 0.927 ± 0.056 ; $p=0.001$) and FCx (control: 0.3407 ± 0.031 , DHEA 60 mg/kg: 0.4569 ± 0.018 , $p=0.034$; DHEA 120 mg/kg: 0.4739 ± 0.026 , $p=0.014$) following treatment. No significant effects were observed in the Hip ($p>0.073$).

Discussion: Our results suggest that DHEA inhibits monoamine oxidase (MAO) activity while stimulates the serotonergic system. Results from our acute model shed some light on the mechanism of action of DHEA on DA and 5-HT neurotransmitter systems, the relevance of those acute effects to chronic disorders awaits further investigation. Our results support the hypothesis that DHEA might protect the nigrostriatal system through MAO inhibition. Also, DHEA may improve mood and attention/cognition as well as reduce aggression through the stimulation of the serotonergic system in the NAc and FCx. **Palavras-Chave:** dehydroepiandrosterone, dopamine, serotonin, behavior

H.07.011

Evaluation of pyridoxine in rats exposed to two models of depression.

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Objective: Evaluate the effect of pyridoxine in rats exposed to two models of depression.

Method: In experiment I, 30 male Wistar rats were submitted in a modified rat forced swimming test (FSTM). 24h prior to the experiment all animals were exposed to a pretest for fifteen minutes. The animals received intraperitoneal injections (ip) of pyridoxine at a dose of 10 Mg/ Kg, imipramine 15Mg/ Kg or saline at 23 h, 5 h and 1 h prior the test. At each 5 seconds, the occurrence of immobility, swimming or climbing behavior was measured for a total of 300 seconds. In experiment II, 40 male Wistar rats were submitted to the chronic mild stress protocol (CMS). In a period of three weeks, a baseline was developed to measure the consumption of water and sucrose 2% which consisted in a water privation period of 24h, followed by an exposure of a bottle containing 100 ml of water and another one containing 100 ml of sucrose during one hour. The CMS was conducted during eleven weeks by using 11 stressors. After seven weeks, the groups were injected ip daily of pyridoxine 10 Mg/ Kg, imipramine 15 Mg/ Kg or saline. The tests of consumption were realized once a week until the end of CMS.

Results: In experiment I an increase in the swimming frequency was observed only by the administration of imipramine (mean 33,6, $\pm$ 6,4 $p < 0,01$). Animals treated with pyridoxine significantly increased climbing behavior (mean 16,8, $\pm$ 4,3, $p < 0,01$). In experiment II, the pyridoxine administration increased the consumption of sucrose during the last three weeks. An increase on the consumption of sucrose wasn't noticed through imipramine. However, no statistically significant difference was found in the last week for sucrose for all groups ($p = 0,3$).

Conclusion: In experiment I pyridoxine raised climbing behavior which displayed antidepressant-like effect through catecholamine system. In experiment II, although it had been observed a progressive increase in the consumption of sucrose for the group treated with pyridoxine, on the last week this event occurred in all groups, which suggests an adaptation process of the animals regarding the CMS model due to difficult to reply this protocol.

Palavras-Chave: pyridoxine, depression, animal models

ACUTE TREATMENT WITH DIAZEPAM BUT NOT CHRONIC TREATMENT WITH FLUOXETINE, IMIPRAMINE AND DESIPRAMINE REVERSED THE ANXIOTIC PROFILE OF SHORT-TERM ISOLATION IN RATS TESTED IN THE ELEVATED PLUS-MAZE MODEL OF ANXIETY

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Objective: The aim of this study was to verify if acute treatment with diazepam and chronic (21 days) treatment with fluoxetine, imipramine and desipramine would reverse the anxiogenic profile of short-term isolation in rats tested in the elevated X-maze model of anxiety.

Methods and Results: Male Wistar rats (250-300 g) were isolated for a period of 12 or 24 h and their behaviour on the X-maze was studied. Acute treatment with diazepam (5 mg/kg;ip) (N=10) increased in the percentage of open-arm entries [$F(1,49) = 5,50$; $p < 0,05$; 12 h isolated], [$F(1,47) = 12,78$; $p < 0,05$; 24 h isolated] and percentage of time spent in the open arms [$F(1,49) = 13,18$; $p < 0,05$; 12 h isolated], [$F(1,47) = 6,3$; $p < 0,05$; 24 h isolated]. Regarding ethological measures, it was observed an increase in the categories inversely related to anxiety: end-arm exploration [$F(1,49) = 11,28$; $p < 0,05$], and head-dipping [$F(1,49) = 12,06$; $p < 0,05$] in rats isolated for 12 h. Chronic treatment with fluoxetine (10mg/kg;ip) (N=10), imipramine (10mg/kg;ip) (N=10) and desipramine (10mg/kg;ip) (N=10) showed no significant effect in the percentage of open-arm entries [$F(1,49) = 0,78$; $p > 0,05$], [$F(1,49) = 0,72$; $p > 0,05$], [$F(1,49) = 0,04$; $p > 0,06$], respectively and percentage of time spent in the open arms, [$F(1,49) = 0,78$; $p > 0,05$], [$F(1,49) = 0,07$; $p > 0,05$], [$F(1,49) = 0,14$; $p > 0,05$] respectively in rats isolated for 12 h. In rats isolated for 24 h, chronic fluoxetine [$F(1,47) = 7,65$; $p < 0,05$] and imipramine [$F(1,48) = 6,13$; $p < 0,05$] decreased the percentage of open-arm entries, and percentage of time spent in the open arms [$F(1,48) = 5,38$; $p < 0,05$], [$F(1,48) = 5,48$; $p < 0,05$]. Regarding ethological measures, no significant effect were seen in any of the categories in both studies. Results of experiments were analysed by one-way analyses of variance (ANOVA). $P < 0.05$.

Conclusion: The anxiolytic effect of diazepam corroborate with the effect of this treatment in the clinic. The lack of anxiolytic effects of chronic fluoxetine, imipramine and desipramine also conforms with the poor efficacy of this drugs in anxiety. Although diazepam has been used to treat anxiety, fluoxetine, imipramine and desipramine are a mainstay in the treatment of depression.

Palavras-Chave: anxiety, elevated plus-maze, rats, diazepam, fluoxetine, imipramine, desipramine

Apoio Financeiro: Faperj

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Antipsychotics drugs alter acetylcholinesterase activity in zebrafish brain

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Antipsychotics drugs have been increasingly used for the management of patients with a variety of psychotic disorders and severe behavioral disturbances. It has been demonstrated that cognitive impairment including attention, memory, and executive functioning impairment may be related with cholinergic dysfunction in patients with brain injury, such as in schizophrenia. Considering that cholinergic system has been characterized in zebrafish brain and that antipsychotics can modulate the cholinesterase activity in rat brain and plasma, we evaluated the *in vitro* and *in vivo* effects of sulpiride, haloperidol, and olanzapine on acetylcholinesterase (AChE) activity from zebrafish brain. For *in vitro* assay, we have tested concentrations of sulpiride, haloperidol, and olanzapine varying from 1 to 250 μM . For acute treatment, fish were exposed to water with sulpiride (250 μM), olanzapine (100 μM) and haloperidol (5 and 9 μM) for 2 h. Zebrafish brain homogenates were prepared and the determination of AChE activity was performed as previously described (Biochem. Pharmacol. 7: 88, 1961). All antipsychotic drugs tested *in vitro* were able to promote significant changes on AChE activity from zebrafish brain. There was a significant decrease of acetylcholinesterase activity in the presence of 250 μM haloperidol (39.4 %; $24.8 \pm 2.1 \mu\text{mol thiocholine. h}^{-1}. \text{mg}^{-1}$) or 250 μM olanzapine (25.1%; $27.7 \pm 2.8 \mu\text{mol thiocholine. h}^{-1}. \text{mg}^{-1}$) when compared to control (41 ± 4.04 and $37.07 \pm 1.9 \mu\text{mol thiocholine. h}^{-1}. \text{mg}^{-1}$, respectively). Sulpiride presented a significant effect on AChE activity, inhibiting at 10, 50, 100, and 250 μM (24.5%, 33.8%, 36.4%, and 61.8%, respectively) when compared to control ($37.5 \pm 2.9 \mu\text{mol thiocholine. h}^{-1}. \text{mg}^{-1}$). Olanzapine and sulpiride *in vivo* did not alter AChE activity, but this enzyme activity was significantly increased at 5 and 9 μM haloperidol (29.9% and 20.4%, respectively), when compared to control group ($32.1 \pm 2.06 \mu\text{mol thiocholine. h}^{-1}. \text{mg}^{-1}$). Therefore, our results indicate that the acetylcholine metabolism can be modulated by these drugs in this species.

Palavras-Chave: acetylcholinesterase; sulpiride; olanzapine; haloperidol; zebrafish

Apoio Financeiro: CNPq, FAPERGS, FINEP (IBN-Net 01.06.0842-00).

PRE-FRONTAL CORTEX, 5-HT_{1A} AND 5-HT_{1B} RECEPTORS, SOCIAL INSTIGATION AND AGGRESSIVE BEHAVIOR IN MALE MICE

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Rationale: Social instigation is used in rodents to induce high levels of aggression, a pattern of behavior similar to that of violent individuals (Eur J Pharmacol. 526:125, 2005). Studies using 5-HT_{1A} and 5-HT_{1B} agonist receptors show an effective reduction in aggressive behavior (Neuropsychopharmacology. 23:20, 2000). An important site of action for these drugs is the ventral orbito frontal cortex (VO PFC), an area of the brain which is particularly relevant in the inhibitory control of aggressive and impulsive behavior (Brain Cogn. 55: 198, 2004).

Objectives: To assess the anti-aggressive effects of 5-HT_{1A} and 5-HT_{1B} agonist receptors (8-OH-DPAT and CP-93,129) when microinjected into the VO PFC of socially provoked male mice and to confirm the specificity of the receptor using 5-HT_{1A} and 5-HT_{1B} antagonist receptors (WAY-100,635 and SB-224,289).

Methods: Male mice (residents, $n=110$) were implanted with a unilateral guide cannula into VO CPF for the subsequent microinjection of 8-OH-DPAT or CP-93,129 (0.1; 0.56 e 1.0 µg/0.2 µl dose). In antagonism studies, WAY-100,635 (10.0 µg/0.2 µl) or SB-224,289 (1.0 µg/0.2 µl) were microinjected before specific agonist receptors. After the microinjections, the animals were provoked socially for five minutes by exposition of a male mouse (instigator) behind a protective glass screen. Past a short interval, an intruder male (without protection) was directly confronted with the resident.

Results: 8-OH-DPAT (0.56 µg and 1.0 µg doses) reduced the frequency of attack bites (8.4 ± 3.4 ; 4.8 ± 2.1 , respectively). The lowest dose of CP-93,129 (0.1 µg) also decreased the number of attack bites (11.9 ± 2.4) and lateral threats (14.5 ± 2.9). Specific participation of the 5-HT_{1A} and 5-HT_{1B} receptors was verified by reversal of anti-aggressive effects using selective antagonists WAY-100,635 (14.4 ± 4.3) and SB-224,289 (20.5 ± 3.4).

Conclusions: The decrease on aggressiveness observed with microinjections of 5-HT_{1A} and 5-HT_{1B} receptor agonists into the VO PFC of socially provoked mice supports the hypothesis that activation of these receptors modulates escalated levels of aggression in a behaviorally specific manner.

Palavras-Chave: aggression, social instigation, serotonin, 5-HT_{1A} receptor, 5-HT_{1B} receptor, prefrontal cortex

Apoio Financeiro: CNPq, FAPESP

GABAERGIC CHANGES AND HORMONAL INFLUENCE IN METHYLPHENIDATE SENSITIZED FEMALE RAT

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Repeated use and sensitization to psychostimulants are recognized to modulate the dopaminergic system and to induce changes in GABAA receptors subunits and GABA synthesis enzymes. The objective of this study was to verify if the GABAergic system changes by repeated use of Methylphenidate (Mp) is influenced by female sexual hormones in the sensitization process in female rats. Ovariectomized (OVX) or intact (INT) Wistar female rats were used and randomized to the treatment groups (n=12). The controls (CTR) and acute treatment (ACT) received saline from day 1 to day 5 of the protocol while Mp 2.5 mg/kg was administered ip in the repeated treatment group (RPT). Both ACT and RPT groups received a Mp 2.5 mg/kg challenge in the 12th day; CTR received saline. Locomotion was assessed on day 1 and day 12 of the protocol. After behavioral observation, the animals were decapitated and striatum, hippocampus and pre-frontal cortex were dissected. GAD65 and GAD67 mRNA were estimated by RT-PCR. The data were analyzed by ANOVA-2W, followed by Student-Newman-Keuls, and are represented as mean $\pm$ sem. Differences were considered when $p < 0.05$. Mp significantly increases locomotion (ACT 139.33 ± 12.33) and repeated treatment induces sensitization (RPT 297.58 ± 17.79) in comparison to CTR 62.33 ± 6.42 ; ($F(2,66)=109.812$; $p < 0.001$). There was an interaction ($F(2,66)=6.606$, $p=0.002$) due to hormonal status. INT rats had greater locomotion than OVX after RPT ($F(1,66)=10.287$, $p=0.002$). There was a significant decrease in striatal GAD65 mRNA in RPT females in comparison to CTR and ACT in OVX rats ($F(2,13)=4.344$, $P=0.036$). In INT, RPT or ACT treatment decreases GAD65 mRNA in respect to CTR ($F(1,9)=13.211$, $P=0.007$). It was concluded that treatment with Mp leads to increased locomotor activity of female rats and its repeated use leads to behavioral sensitization, which is higher than in the presence of the female sexual hormones. Sensitization to Mp is associated to decreased in levels of GABA synthesis enzymes mRNA in the striatum of female rats and the presence of hormones seems to be associated to a more prompt response in presence of the psychostimulant.

Palavras-Chave: Methylphenidate, sensitization, GABA, sexual hormones, female

Apoio Financeiro: CNPq, AAPeFaTo, UFCSPA

Nicotine intrahippocampal infusion: Effects upon induced stress

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This work aimed to evaluate the effect of nicotine over induced stress in young male rats. Animals were anesthetized and implanted bilaterally with cannulae above hippocampus CA1 area. After recovery, they were tested in an Elevated Plus Maze (EPM) apparatus to access anxiety levels. A week after, animals were stressed by immobilization for 60 min. they were allowed to recover for 45 min. and infused with saline (CTR) or Nicotine (6mM – NIC06 or 18mM – NIC18) 15 min. before the new EPM test. In the first exposure to EPM, no significant differences were found in the percentage of time NIC18-treated animals spent into open (39.8 ± 3.1) and closed (40.7 ± 2.8) arms compared to CTR (39.4 ± 2.5 and 40.5 ± 2.3 , respectively). Subjects treated with NIC06 spent more time, compared to CTR, into closed arms (46.3 ± 2.8). In the second test, NIC06 and NIC18 treated animals spent more time in the open arms (38.3 ± 3.6 and 35.6 ± 3.6 , respectively) and less time in closed arms (44.5 ± 3.9 and 47.4 ± 3.9) compared to CTR (open – 28.2 ± 2.9 ; closed – 56.4 ± 3.2). No differences were found in the number of entries into open (CTR– 5.9 ± 0.5 ; NIC06– 6.6 ± 0.6 ; NIC18– 5.3 ± 0.6) or closed arms (CTR– 6.1 ± 0.5 ; NIC06– 6.2 ± 0.6 ; NIC18– 5.9 ± 0.6) nor rearings (CTR– 12.8 ± 1.0 ; NIC06– 15.4 ± 1.2 ; NIC18– 15.4 ± 1.2) in the first and second EPM tests (open arms entries: CTR– 5.9 ± 0.7 , NIC06– 7.9 ± 0.9 , NIC18– 5.6 ± 0.9 ; closed arms entries: CTR– 6.8 ± 0.5 , NIC06– 6.1 ± 0.6 , NIC18– 6.9 ± 0.6 ; rearings: CTR– 16.8 ± 1.1 , NIC06– 18.6 ± 1.3 , NIC18– 17.2 ± 1.3). Nicotine is believed to be the main responsible for cigarette addiction. It is possible that nicotine's effect over anxiety be one of the reasons for its popularity. Our results showed that animals infused with nicotine spent a higher percentage of time in the open arms and a lower time in the closed arms even after induced stress, results that suggest an anxiolytic effect of this alkaloid in young male rats. To evaluate the mechanisms by which nicotine acts in the emotional pathways within the nervous system is crucial to understand its mechanisms of addiction and the possible treatments for this worldwide health issue. **Palavras-Chave:** Nicotine - induced stress - plus maze

Apoio Financeiro: CNPq

Involvement of 5-HT_{2C} receptors of the basolateral amygdala in the mediation of anxiety-related defensive behaviors

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Introduction: Evidence in the literature is suggestive that 5-HT_{2A} and 5-HT_{2C} receptors in the basolateral amygdala (BLA) play opposed roles in the modulation of anxiety-like behavior. For instance, whereas activation of 5-HT_{2A} receptors in the BLA impairs inhibitory avoidance acquisition in the elevated T maze (ETM), an anxiolytic-like effect, stimulation of 5-HT_{2C} receptors in the same brain area is reported to increase anxiety in the elevated plus-maze [1]. Here, we further addressed the involvement of BLA 5-HT_{2C} receptors in the modulation of anxiety-like behavior by investigating the effects of local administration of the 5-HT_{2C} receptor agonist MK-212 in rats exposed to the ETM and the light-dark transition test. *Methods:* Male *Wistar* rats were tested in these models 10 minutes after the intra-BLA injection of the endogenous agonist 5-HT (16 nmol) or the 5-HT_{2C} receptor agonist MK-212 (0.05, 0.5 or 5 nmol). The ETM consists of three elevated arms, one enclosed and two open. Inhibitory avoidance was measured by placing the rat at the end of the enclosed arm and recording the latency to withdraw from this arm in three consecutive trials. Escape was measured by recording the time taken by animals to withdraw from the end of one of the open arms. The light-dark transition apparatus consists of a box with two compartments, one brightly lighted and other dark. The number of transitions between these compartments and time spent in the lighted area were measured during a 5 min session. *Results:* Intra-BLA injection of 5-HT and MK-212 facilitated inhibitory avoidance acquisition, an anxiogenic effect [Mean + EPM (s) – sal=95.7±35.0, 5-HT=233.4±34.7, MK-212(0.05)=233.2±35.4], without changing escape performance in the ETM. In the light-dark transition test, both drugs [sal= 15.4±2.3, 5-HT=8.6±1.8, MK-212 (0.5)=7.7±1.3] decreased the time spent in the lighted compartment. *Conclusion:* The results support previous evidence showing that, in contrast with 5-HT_{1A} receptors, activation of 5-HT_{2C} receptors in the BLA causes anxiogenic-like effects.

References: [1] Psychopharmacology, 182:345, 2005.

Palavras-Chave: serotonin, anxiety, basolateral amygdala, 5-HT_{2C} receptors, elevated T maze

Apoio Financeiro: CNPq

MANIFESTAÇÕES DOS COMPORTAMENTOS MATERNAL E PREDATÓRIO EM RATAS LACTANTES TRATADAS COM SULFATO DE MORFINA NO TERÇO FINAL DA GESTAÇÃO.

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OBJETIVO: Este estudo teve como objetivo testar o efeito da sensibilização à morfina em baixas doses na preferência por cuidar da prole ou caçar insetos. **MÉTODOS:** Ratas da linhagem Wistar, idade média de 90 dias, foram acasaladas e mantidas em salas com sistema de ventilação (23⁰C), ciclo de luz de 12 horas, comida e água *ad libitum*. Do 17^o ao 21^o dias de gestação, os animais foram tratados com sulfato de morfina 3,5mg/Kg/dia, s.c. (M) ou solução salina s.c. (S). No 5^o dia de lactação, as ratas previamente habituadas, foram colocadas na caixa experimental 1h antes do início do teste. Trinta minutos após, receberam diferentes doses desafio com sulfato de morfina 0,5mg/kg (MM0,5 n=10 e SM0,5 n=9); 1,0 mg/kg (MM1,0 n=10 e SM1,0 n=6) e 1,5 mg/kg (MM1,5 n=10 e SM1,5 n=9) ou solução salina (SS n=10 e MS n=10). Foram introduzidos na caixa experimental com a rata mãe 8 filhotes (4 machos e 4 fêmeas) juntamente com 5 baratas (*Periplaneta americana*) após 30 minutos da dose desafio. Os comportamentos foram filmados durante 30 minutos consecutivos e posteriormente analisados através do software Etholog 2.2®. **RESULTADOS:** O comportamento de *Nursing* foi prejudicado nas três doses desafio, sendo estatisticamente significantes entre os grupos SM0,5 (77%) e MM0,5 (10%); SS (70%) e MM1,0 (0%); MM1,5 (0%) e MS (40%); SM1,5 (10%) e SS (70%), segundo o Teste de Fischer, $p < 0,05$. Não foram encontradas diferenças estatisticamente significantes com relação à da captura da 1^a à 5^a barata, observando-se apenas uma tendência a esta diferença entre os grupos SS (6,6±0,4) e MM1,5 (4,4±0,7); MS (6,2±0,5) e SM1,5 (3,6±0,6), após análise estatística Kruskal-Wallis. **CONCLUSÕES:** O pré-tratamento com morfina foi decisivo no comprometimento dos parâmetros do comportamento maternal, demonstrando-se assim o fenômeno de sensibilização comportamental. Em relação aos parâmetros do comportamento predatório, as baixas doses de morfina aqui testadas não foram capazes de promover estimulação à caça predatória.

Palavras-Chave: comportamento maternal, comportamento predatório, morfina

Apoio Financeiro: FAPESP

EFEITO ANTIOXIDANTE DO RESVERATROL E DO VINHO TINTO NO SISTEMA NERVOSO CENTRAL DE RATOS DIABÉTICOS

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Estudos demonstram correlação entre hiperglicemia crônica e produção de espécies reativas de oxigênio no sistema nervoso central em indivíduos diabéticos. Substâncias antioxidantes como vinho tinto e resveratrol poderiam reduzir danos centrais pelo estresse oxidativo. Foi nosso objetivo avaliar o efeito antioxidante do resveratrol e do vinho tinto no hipocampo e estriado de ratos diabéticos. Ratos Wistar machos, adultos, foram divididos em dois grupos: controle (CTR) e diabéticos induzidos por estreptozotocina (STZ), 60 mg/kg, i.p. Após confirmado o diabetes, os grupos foram subdivididos segundo o tratamento (n=8/grupo): vinho tinto (VIN) (4 mL/Kg), resveratrol (RES) (20 mg/Kg) e solução salina (4 mL/Kg), administrados via oral, por 21 dias consecutivos. No 21º dia, 1h após a última administração, os animais foram sacrificados, o hipocampo e o estriado foram dissecados para análise do estresse oxidativo pelo método das substâncias reativas ao ácido tiobarbitúrico e expressão das enzimas antioxidantes superóxido dismutase e catalase. Nossos resultados mostraram que tanto o resveratrol quanto o vinho tinto apresentam efeito antioxidante no hipocampo de ratos diabéticos, demonstrado pela redução do estresse oxidativo (CTR: 0,058±0,032; CTR/RES: 0,043±0,027; CTR/VIN: 0,044±0,022; STZ: 0,067±0,026; STZ/RES: 0,028±0,018; STZ/VIN: 0,030±0,013 nmol MDA/mg proteína, p=0,012). Ainda no hipocampo de ratos diabéticos, o vinho tinto aumentou a produção da enzima antioxidante catalase (p<0,001). No estriado, o tratamento com resveratrol aumentou significativamente a produção da enzima superóxido dismutase em animais diabéticos, não modificando o estresse oxidativo ou a catalase (p=0,013). Nossos resultados mostram que o resveratrol, mesmo em baixas concentrações como aquela presente em dois cálices de vinho tinto (resveratrol ~ 3.2 mg/mL), apresenta efeito antioxidante de acordo com a área cerebral. **Palavras-Chave:** estresse oxidativo, diabetes, resveratrol, vinho tinto

Apoio Financeiro: CNPq - CAPES - UFCSPA - PUCRS - IPA

INVESTIGAÇÃO DO EFEITO ANTIDEPRESSIVO DA *POLYGALA PANICULATA* NO TESTE DO NADO FORÇADO: ENVOLVIMENTO DO SISTEMA MONOAMINÉRGICO

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A *Polygala paniculata* (Polygalaceae) é utilizada popularmente como planta medicinal. Há relatos de que plantas do gênero *Polygala* são dotadas de propriedades farmacológicas como efeito neuroprotetor (Am. J. Chin. Med. 32:599, 2004), anticonvulsivante e ansiolítico (Psychopharmacology 197:351, 2008), ação anti-inflamatória (Phytomedicine 15:321, 2008) e atividade antioxidante ([Chem. Biodivers.](#) 1:415, 2004). Este trabalho investigou o efeito da *P. paniculata* no teste do nado forçado (TNF), modelo preditivo de ação antidepressiva, e o mecanismo de ação envolvido no seu efeito. Camundongos Swiss adultos (30-45 g) foram tratados (p.o.) com veículo ou extrato hexânico da planta (0,001-300 mg/kg) 60 min antes de serem submetidos ao TNF. Houve redução significativa no tempo de imobilidade nas doses de 0,1 mg/kg ($85,10 \pm 4,27\%$), 10 mg/kg ($88,56 \pm 1,82\%$), 30 mg/kg ($82,05 \pm 1,24\%$), 100 mg/kg ($84,79 \pm 3,41\%$) e 300 mg/kg ($85,66 \pm 4,79\%$) em comparação ao grupo controle (100%), sem causar alteração significativa na atividade locomotora no teste do campo aberto. A fim de investigar o mecanismo de ação da *P. paniculata*, os animais foram pré-tratados com NAN-190 (0,5 mg/kg, i.p.; antagonista de receptor 5-HT_{1A}), prazosina (1 mg/kg, i.p.; antagonista de receptor α_1 -adrenérgico), ioimbina (1 mg/kg, i.p.; antagonista de receptor α_2 -adrenérgico), propranolol (1 mg/kg, i.p.; antagonista de receptor β -adrenérgico), SCH-23390 (0,05 mg/kg, s.c.; antagonista de receptor dopaminérgico D₁) ou sulpirida (50 mg/kg, i.p.; antagonista de receptor dopaminérgico D₂). O efeito antidepressivo da *P. paniculata* no TNF foi prevenido pelo pré-tratamento dos animais com ioimbina ($98,81 \pm 4\%$), propranolol ($103,11 \pm 6,97\%$), SCH-23390 ($95,99 \pm 1,24\%$) e sulpirida ($113,40 \pm 1,83\%$), mas não com NAN-190 e prazosina. Os resultados demonstraram que a *P. paniculata* possui efeito antidepressivo no TNF, o qual envolve uma interação com receptores α_2 e β -adrenérgicos e dopaminérgicos D₁ e D₂. **Palavras-Chave:** depressão; *Polygala paniculata*; antidepressivo; teste do nado forçado; noradrenalina; dopamina

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O Uso Associado Da Rivastigmina e Sinvastatina: Estudo de dois casos clínicos

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A doença de Alzheimer está cada dia mais freqüente em nossa sociedade, tornando uma preocupação constante na busca de tratamentos mais eficazes, visando a melhoria da qualidade de vida destes pacientes, bem como aumentar as perspectivas de vida, mas seu diagnóstico, apesar dos avanços tecnológicos somente se dá, com certeza, através da biopsia *post-mortem*. Isto não impossibilita que sejam feitas hipóteses diagnósticas através de tomografia, ressonância magnética computadorizada, relato de familiares e provas neuropsicológicas. Um dos maiores avanços é o surgimento de novas drogas que retardam a evolução da doença em sua fase inicial, fazendo com que seu uso se torne uma referência quando se trata de diagnóstico de demência. O presente estudo visa apresentar dois casos clínicos, que após avaliação neuropsicológica, suscitaram dúvidas quanto as intervenções farmacológicas e seus efeitos colaterais. Os pacientes eram do sexo masculino, nível superior, 67 anos, fazendo uso de Rivastigmina e Sinvastatina. Ambos os casos, os familiares relataram apatia, desmotivação, desinteresse e declínio cognitivo, sendo confirmados estes dados através da avaliação. Na avaliação *follow-up*, um paciente apresentou melhora significativa após a suspensão da Sinvastatina devido a uma neuropatia periférica. Foi sugerido ao neurologista a suspensão também da Sinvastatina por um curto período, do outro paciente, para investigar se ocorreria melhora neste paciente, e em dois meses sem este medicamento, ele apresentou melhora significativa. Isto levou a pesquisar possíveis causas da melhora. Diversos autores relatam as vantagens do uso concomitante da Rivastigmina e Sinvastatina em pacientes com Alzheimer, mas a Sinvastatina pode induzir depressão favorecendo o surgimento de pseudodemência, que pode confundir o profissional, induzindo a um diagnóstico inadequado. Tal fato nos estimula a pesquisar as possíveis conseqüências da polifarmácia em pacientes demenciados.

Palavras-Chave: Neuropsicofarmacologia, Avaliação Neuropsicológica, Demência, Rivastigmina, Sinvastatina

Metilfenidato melhora déficit em curva de posição serial de recordação livre de palavras no Transtorno de Déficit de Atenção e Hiperatividade

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Objetivo. Este estudo examinou o desempenho de crianças e adolescentes com TDAH em curva de posição serial de recordação livre de palavras antes e após 6 e 12 m de tratamento com metilfenidato (MPH). **Métodos.** 70 crianças e adolescentes (7 a 14 anos) procedentes de escolas públicas e privadas, com TDAH (DSM-IV), foram comparadas a 39 crianças e adolescentes sem TDAH (controle) pareadas por sexo, idade e escolaridade. As memórias de curto (MCP) e longo (MLP) prazos foram examinadas empregando-se o teste de recordação livre de palavras, sendo 6 delas com (listas pares) e 6 sem (listas ímpares) relacionamento semântico entre as 3 palavras centrais. O grupo TDAH recebeu MPH após a 1ª avaliação cognitiva, via oral, nas doses de 0,6-0,9 mg/kg/dia em 2 tomadas por 12 m. Aos 6 e 12 m foi reavaliado em testes cognitivos 90 minutos após a administração do MPH. **Resultados.** Os grupos apresentaram perfis similares de desempenho nas curvas de posição serial em forma de U, com maior evocação das 3 primeiras (primazia) e das 3 últimas (recência) palavras das listas, porém houve um rebaixamento significativo desses efeitos no grupo TDAH (primazia: $3,1 \pm 0,3$; recência: $12,3 \pm 0,3$) comparado ao controle (primazia: $5,6 \pm 0,5$; recência: $13,3 \pm 0,4$). A relação semântica entre as três palavras centrais facilitou a evocação das mesmas, mas, no grupo TDAH houve menor evocação mesmo usando desse recurso (TDAH: $5,3 \pm 0,4$; controle: $6,1 \pm 0,7$). O tratamento com MPH aumentou significativamente a evocação das três primeiras palavras aos 6 e 12 m de tratamento das listas pares (0 m: $2,6 \pm 0,4$; 6 m: $3,9 \pm 0,5$; 12 m: $4,7 \pm 0,7$) e das listas ímpares (0 m: $3,5 \pm 0,5$; 6 m: $5,6 \pm 0,5$; 12 m: $6,2 \pm 0,6$), das três palavras centrais com relação semântica nas listas pares (0 m: $4,6 \pm 0,6$; 6 m: $5,9 \pm 0,6$; 12 m: $5,7 \pm 0,8$), e também das três últimas palavras nas listas ímpares aos 12 m (0 m: $12,2 \pm 0,5$; 12 m: $14 \pm 0,5$). **Conclusões.** As crianças e adolescentes com TDAH apresentam prejuízos significativos das memórias de curto (recência) e longo prazo (primazia), e da retenção e/ou evocação das palavras relacionadas semanticamente. Estes prejuízos foram revertidos pelo tratamento com o MPH por 6 a 12 m.

Palavras-Chave: TDAH, Metilfenidato, Posição serial, Teste de recordação livre de palavras, MCP, MLP

Apoio Financeiro: FACITEC - Fundo de Apoio à Ciência e Tecnologia

ADMINISTRAÇÕES AGUDAS DO ANTAGONISTA SELETIVO DO RECEPTOR DOPAMINÉRGICO D₂ (SULPIRIDA) NO CÓRTEX PRÉ-FRONTAL MEDIAL REDUZ OS PREJUÍZOS DO ÁLCOOL SOBRE A MEMÓRIA OPERACIONAL ESPACIAL DE RATOS.

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Objetivos: Investigar os efeitos agudos da administração intracortical de diferentes doses do antagonista seletivo de receptores D₂, sulpirida, no córtex pré-frontal medial (CPFm), sobre os efeitos de prejuízo da memória operacional produzidos pelo etanol (EtOH), envolvendo tarefa de retardo prolongado. **Métodos:** Ratos Wistar machos (n = 9, 250-300 g; $\approx$ 3 meses), previamente treinados no labirinto radial de 8 braços, com cânulas bilaterais implantadas no CPFm (B: 2,5 mm A, +/-1 mm L, 2,7 mm V), receberam administrações intracorticais (IC) de sulpirida (0,32, 1,0 e 3,2 μ g) ou ácido clorídrico 0,05 M (HCl) 10 minutos antes da administração IC de SAL ou EtOH 100 μ g. Cinco minutos após a última administração, os animais foram submetidos ao teste de desempenho no labirinto com retardos de 1 hora. No desempenho do pós-retardo de 1 hora foram considerados erros tipo 1 ou 2 que correspondem a repetição dos braços visitados no pré-retardo ou pós-retardo, respectivamente. **Resultados:** O EtOH IC após HCl (2,2 $\pm$ 0,5) ou sulpirida (3,2 μ g) (2,2 $\pm$ 0,5) IC produziu significativamente ($p < 0,01$) maior número de erros tipo 1 quando comparado à combinação controle HCl e SAL (1 $\pm$ 0,3). Animais tratados com a sulpirida (0,32 μ g: 0,9 $\pm$ 0,4; e 1 μ g: 1,2 $\pm$ 0,2) combinada ao EtOH apresentaram significativamente ($p < 0,01$) menor número de erros tipo 1 comparado ao tratamento combinado de HCl/EtOH (100 μ g) ou sulpirida (3,2 μ g)/EtOH (100 μ g) no pós-retardo de 1 hora. Os erros tipo 1 predominaram em todos os tratamentos, sendo pouco freqüentes ou mesmo ausentes os erros do tipo 2. **Conclusões:** O antagonista dopaminérgico D₂ (sulpirida) administrado no CPFm reduziu, de forma dose-dependente, o prejuízo da MO produzida pelo EtOH administrado IC, sugerindo a participação dos receptores do tipo D₂ do CPFm nos efeitos de prejuízos cognitivos do etanol, especialmente sobre a MO. A prevalência do erro tipo 1 sugere que o etanol pode interferir na retenção e/ou evocação da informação, componentes essenciais da memória operacional, sendo este prejuízo revertido pelo antagonista dopaminérgico D₂. **Palavras-Chave:** 1. Córtex Pré-Frontal Medial. 2. Sistema Dopaminérgico. 3. Etanol. 4. Memória Operacional. 5. Labirinto Radial. 6. Sulpirida.

SULPIRIDA ADMINISTRADA PREVIAMENTE NO CPFm BLOQUEIA OS EFEITOS DE PREJUÍZO DO EtOH SOBRE A MEMÓRIA OPERACIONAL ESPACIAL DE RATOS APÓS LATÊNCIA DE 15 DIAS

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Objetivos: Investigar o efeito de repetidas doses do antagonista seletivo D₂, sulpirida, administrada no CPFm após latência de 15 dias sobre os efeitos de prejuízo na memória operacional produzido pelo etanol (EtOH) também administrado no CPFm. **Métodos:** Ratos Wistar machos (n = 21, 250-300 g; 3 meses), previamente treinados no labirinto radial de 8 braços, com cânulas bilaterais implantadas no CPFm (B: 2,5 mm A, +/-1 mm L, 2,7 mm V), receberam administrações intracorticais (IC) de sulpirida 1,0 µg ou ácido clorídrico 0,05 M (HCl) por quatro dias. Após a última administração aguardou-se 15 dias sem tratamento, no 16º dia os animais receberam administrações IC agudas de EtOH 100 µg e foram submetidos ao teste de desempenho no labirinto radial com retardos de 1 hora. **Resultados:** Animais tratados previamente com sulpirida IC por quatro dias, apresentaram no desempenho do pós-retardo de 1 hora significativamente (p < 0,01) menor número de erros do tipo 1 após administração IC aguda do EtOH 100 mg (0,6 ± 0,2) quando comparados ao desempenho dos animais tratados com EtOH 100 mg no 16º sob administração prévia de HCl (2,0 ± 0,4). Os erros observados sob a administração de EtOH 100 µg foram predominantemente do tipo 1 (repetição dos braços visitados no pré-retardo) e os erros tipo 2 (repetição dos braços visitados no pós-retardo) apresentaram-se com menor frequência ou foram ausentes. **Conclusões:** Administrações prévias de Sulpirida no CPFm reduzem os prejuízos do EtOH sobre a memória operacional após latência de 15 dias, sugerindo que a diminuição prévia da função dos receptores D₂ pode modificar os efeitos do EtOH no CPFm. A prevalência dos erros tipo 1 sugere que o etanol pode interferir na retenção e/ou evocação da informação, componentes essenciais da memória operacional, e este prejuízo foi prevenido pela administração repetida do antagonista dopaminérgico do tipo D₂ seguido de um intervalo de 15 dias.

Palavras-Chave: 1. Córtex Pré-Frontal Medial. 2. Sistema Dopaminérgico. 3. Etanol. 4. Memória Operacional. 5. Labirinto Radial. 6. Sulpirida.

Efeito de clordiazepóxido e pentilenotetrazol sobre o comportamento de ratos submetidos a sessões de 60 minutos no labirinto em cruz elevado padrão ou com todos os braços fechados.

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Objetivo:

Caracterizar os comportamentos do rato no labirinto em cruz elevado (LCE) (com dois braços abertos e dois fechados ou com quatro braços fechados), visando fornecer subsídios para um modelo computacional de simulação de comportamento de ratos no LCE. Visa também descrever a dinâmica temporal dos comportamentos do rato nesses labirintos, sob efeito de drogas, em sessões de uma hora de duração.

Método: Ratos machos de uma derivação Wistar foram distribuídos em dois grupos, segundo o tipo de labirinto no qual foram testados (padrão ou totalmente fechado). Cada grupo foi ainda dividido em três subgrupos, segundo o tratamento farmacológico: salina, clordiazepóxido (CDP, 5 mg/Kg) ou pentilenotetrazol (PTZ, 30 mg/Kg). As drogas foram injetadas i.p. em um volume de 1 ml/Kg. Todos os ratos foram submetidos a uma sessão de 60 min. Foram registrados o número de entradas, tempo gasto e distância percorrida neles.

Resultados: Nos dois tipos de labirinto, independente do tratamento farmacológico, observou-se que após os primeiros 5 min a atividade dos animais decaí drasticamente, esse decaimento é especialmente acentuado nos braços abertos. A análise dos 60 min de registro revelou que o efeito do PTZ foi contínuo ao longo da sessão, gerando diferenças significativas no número de entradas e distância percorrida em comparação com os grupos controle e CDP. O efeito do CDP, ao contrário, tendeu a diminuir depois do minuto 30, provavelmente devido ao efeito hipnótico da droga, igualando o desempenho desse grupo ao do grupo controle.

Conclusões: A dinâmica temporal dos efeitos das duas drogas é diferente, e não depende necessariamente da meia vida destas. Essa dinâmica é observável tanto no LCE padrão quanto naquele com todos os braços fechados, sendo os primeiros 10 minutos os com maior concentração de comportamentos, após os quais há um acentuado declínio.

Palavras-Chave: Labirinto em cruz elevado, clordiazepóxido, pentilenotetrazol, comportamento exploratório

Apoio Financeiro: FAPESP

Déficit de memória operacional no Transtorno de Déficit de Atenção e Hiperatividade e sua reversão pelo metilfenidato

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Objetivo. Este estudo examinou o desempenho de crianças e adolescentes com TDAH no teste de repetição de pseudopalavras e no teste de dígitos antes e após 6 e 12 m de tratamento com metilfenidato (MPH). **Métodos.** 70 crianças e adolescentes (7 a 14 anos) procedentes de escolas públicas e privadas, com TDAH (DSM-IV), foram comparadas a 39 crianças e adolescentes sem TDAH (controle) pareadas por sexo, idade e escolaridade. A alça fonológica da memória operacional (MO) foi avaliada empregando-se o teste de repetição de pseudopalavras para crianças brasileiras (BCPR) e o teste de dígitos. O grupo TDAH recebeu MPH após a 1ª avaliação cognitiva, via oral, nas doses de 0,6 – 0,9 mg/kg/dia em 2 tomadas por 12 m. Aos 6 e 12 m foi reavaliado em testes cognitivos 90 minutos após a administração do MPH. **Resultados.** No BCPR o grupo TDAH ($37,4 \pm 0,3$) apresentou significativa ($p < 0,001$) redução na média total de acertos comparado ao controle ($39,2 \pm 0,2$), apresentando maiores dificuldades na repetição das pseudopalavras longas (3-5 sílabas). No teste de dígitos, o grupo TDAH mostrou significante prejuízo na evocação direta ($p < 0,001$) ($4,5 \pm 0,1$) e inversa ($p < 0,001$) ($3,3 \pm 0,1$) de dígitos comparado ao controle ($5,2 \pm 0,1$ e $3,9 \pm 0,1$, respectivamente). Tomados em conjunto, os resultados obtidos por medidas distintas evidenciaram significativo comprometimento no grupo TDAH tanto na quantidade quanto no comprimento dos itens armazenados na alça fonológica da MO. O grupo TDAH apresentou melhora ($p < 0,001$) na média total de repetições corretas de pseudopalavras com o uso do MPH por 6 ($38,8 \pm 0,3$) e 12 m ($39,3 \pm 0,3$) comparado ao início do tratamento ($37 \pm 0,5$). Além disso, observou-se melhora na repetição de pseudopalavras de maior quantidade de sílabas (3-5 sílabas). O MPH também melhorou o desempenho do grupo TDAH aos 12 m no teste de dígitos direto ($p < 0,001$) ($5,1 \pm 0,2$) e inverso ($p < 0,01$) ($3,8 \pm 0,1$) comparado à avaliação inicial ($4,1 \pm 0,2$ e $3,2 \pm 0,1$, respectivamente). **Conclusão.** As crianças e adolescentes com TDAH apresentam déficits no armazenamento e no processamento da informação na alça fonológica da MO, que são revertidos pelo tratamento com MPH por 6 a 12 m.

Palavras-Chave: TDAH, Memória Operacional, Metilfenidato, BCPR, Teste de Dígitos

Apoio Financeiro: FACITEC - Fundo de Apoio à Ciência e Tecnologia

PERFIL NEUROADAPTATIVO DA MODULAÇÃO COLINÉRGICA MUSCARÍNICA DOS RECEPTORES M4 DURANTE OS PROCESSOS DE CONSOLIDAÇÃO, EVOCAÇÃO E RECONSOLIDAÇÃO DE UMA MEMÓRIA AVERSIVA EM RATOS.

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Objetivo: verificar os efeitos da MT3 (antagonista muscarínico seletivo para o receptor M4) intra-hipocampal sobre os processos de consolidação, evocação e reconsolidação de condicionamento aversivo contextual (CAC) em ratos; comparando com o CNQX, antagonista glutamatérgicos ionotrópico não-NMDA na consolidação e reconsolidação de CAC.

Métodos e resultados: ratos Wistar machos foram canulados bilateralmente no hipocampo dorsal e treinados na tarefa de CAC (2 choques 0,5mA/2s). O teste (4 min.) dos animais ocorreu 24 h. após e as respostas de congelamento foram registradas em porcentagem. A consolidação foi investigada com a infusão 0 min. pós-treino de MT3 (2,0 ug/lado), CNQX (0,5 ou 1,5 ug/lado) ou com TFS (controle). A evocação foi com a infusão 20 min. pré-teste de MT3 (2,0 ug/lado) ou de TFS. A reconsolidação foi com a infusão 0 min. pós-reativação (1º teste 3 min.) com MT3 (2,0 ug/lado) ou TFS, com o inibidor de transcrição gênica DRB (2,0 ng/lado) ou DMSO 8% em TFS, ou com CNQX (0,5 ug/lado) ou TFS. No tratamento pós-treino, os grupos infundidos com MT3 (56[8,4] N=9, sempre média[DP]) e CNQX (15,5[4,7] N=9) (0,5ug/lado) foram diferente dos respectivos TFSs (90,5[1,9] e 46,7[9,4] Ns=10, respectivamente) (P=0,001 e P=0,021, Teste T e ANOVA uma via e post hoc de Tukey). No pré-teste, o grupo infundido com MT3 (71,1[5,2] N=9) foi diferente do TFS (46,7[4,3] N=8) (P=0,003, Teste T). No pós-reativação, somente os grupos infundidos com MT3 e CNQX (83,1[3,6] N=10 e 71,8[2,8] N=11) foram diferentes dos respectivos TFSs (67,7[3,6] N=11 e 93[1,3] N=9) no teste 1 ocorrido 24 pós-reativação (P=0,038 e P=0,001, respectivamente, Testes ANOVA uma via com post hoc de Tukey) e no teste 2 (85,8[3,3] e 58,6[6,3] – MT3 e CNQX; 63,6[3,5] e 89,9[2,8] – TFSs) ocorrido 5 dias após (P=0,01 e P=0,000, respectivamente).

Conclusões: A MT3 foi amnésica sobre a consolidação, porém, mostrou-se facilitatória sobre a evocação e a reconsolidação. O subsistema M4 muscarínico deve modular positivamente a consolidação, porém devido a alterações plásticas passa a modular negativamente a evocação e a reconsolidação dessa memória aversiva. O CNQX foi amnésico na consolidação e reconsolidação.

Palavras-Chave: Memória; MT3; receptor M4; Consolidação; Evocação; Reconsolidação; Rato

Apoio Financeiro: CNPq, CAPES, FAPERGS, PROPESQ/UFRGS, IFS.

A MUDANÇA DE EFEITOS DA MT3 DURANTE O PROCESSO DE CONSOLIDAÇÃO DA MEMÓRIA DA ESQUIVA INIBITÓRIA É DEPENDENTE DE TRANSCRIÇÃO GÊNICA: INDÍCIO DE PLASTICIDADE DE RECEPTORES M4 HIPOCAMPAIS.

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Objetivos: A MT3 (antagonista muscarínico seletivo para receptores M4) apresenta efeitos antagônicos sobre a consolidação (amnésico) e a evocação (facilitatório). Portanto, os objetivos deste trabalho são investigar em que momento da consolidação ocorre a mudança de efeitos e verificar se isso é dependente de transcrição gênica.

Métodos e resultados: ratos Wistar machos com 250-350g foram operados para a implantação de cânulas no hipocampo dorsal. Após a recuperação, eles foram treinados na esQUIVA INIBITÓRIA (EI) com choque de 0,5 mA/3s. No 1º experimento os ratos foram infundidos (0,5 µl) 0, 45, 90, 180 ou 360 min. pós-treino com MT3 (2,0 µg/lado) ou TFS (controle). No 2º os ratos foram infundidos 0 min. pós-treino com o inibidor de transcrição gênica DRB (0,8 µg/lado ou 2,0 µg/lado) ou DMSO 8% em TFS (controle). No 3º os ratos foram infundidos 0 min. pós-treino com DRB (0,8 µg/lado) ou DMSO 8% e 20 min. pré-teste com MT3 (2,0 µg/lado) ou TFS. O teste ocorreu 24 h. após o treino. No 1º somente os grupos com MT3 0, 90 e 180 min. pós-treino foram diferentes em relação ao controle (Teste U de Mann-Whitney, P=0,018, 0,044 e 0,014, respectivamente): 20[12,5/104,5] N=13 e 180[36,5/180] N=10; 72[27,5/180] N=17 e 23[15/37] N=15; 35,5[10,75/102,75] N=14 e 10[5/19,5] N=13 (medianas[IQ25/IQ75]). No 2º somente o grupo com DRB (2,0 µg/lado) foi diferente em relação ao controle (P=0,004, Teste de Kruskal-Wallis e post hoc de Dunn): 18[7,5/28,25] N=14 e 35[23,5/145,5] N=13. No 3º somente o grupo com DMSO 8% pós-treino e MT3 pré-teste foi diferente em relação ao controle (P=0,022, Teste de Kruskal-Wallis e post hoc de Dunn): 121[38/180] N=11 e 26[16,75/53] N=12. O grupo com DRB pós-treino e MT3 pré-teste (32[16,5/125,5] N=13) não foi diferente.

Conclusões: A MT3 passou de um efeito amnésico (0 min. pós-treino) para um facilitatório (90 e 180 min. após). O DRB foi amnésico, demonstrando que a transcrição gênica é essencial à consolidação. Além disso, a administração pós-treino de DRB (subdose) reverteu o efeito facilitatório da MT3 pré-teste. Este é um indício de que ocorrem fenômenos de plasticidade sobre os receptores M4 hipocampus na consolidação da EI.

Palavras-Chave: memória, consolidação, MT3, receptor M4, plasticidade sináptica, rato

Apoio Financeiro: CNPq, CAPES, FAPERGS, PROPESQ/UFRGS, IFS

Efeito da cotinina sobre a memória, habituação e ansiedade em ratos

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Este trabalho objetivou verificar o efeito da cotinina (COT) sobre a evocação da memória aversiva. Cânulas foram implantadas bilateralmente na região CA1 do hipocampo de ratos machos jovens. Salina, cotinina (COT - 3µM ou 18µM) ou metil-licaconitina (MLA - 34mM) foram infundidos 15 min. pré-teste em todas as tarefas. Os animais foram submetidos à esQUIVA INIBITÓRIA (EI), em uma sessão de treino e uma de teste, 24h após. Foram também realizados os testes de campo aberto (CA), em duas sessões (treino e teste) com intervalo de 24h, e de labirinto em cruz elevado (LCE) para avaliação do grau de ansiedade induzido pela droga. No teste de EI (resultados expressos como mediana e intervalo interquartis), os animais tratados com COT 18µM apresentaram maior latência para descida da plataforma (130.7/83.19-171.2) comparado ao grupo salina (77.5/53.85-109.7). O grupo MLA apresentou a menor latência para descida da plataforma (20.44/15.66-32.88). Na tarefa de CA (resultados expressos em média e erro padrão), o grupo salina apresentou uma redução no número de cruzamentos entre o treino (61.6 ± 8.3) e o teste (39.25 ± 7.9). O mesmo ocorreu nos grupos COT 18µM (treino 85 ± 8.7; teste 60.63 ± 8.2) e COT 3µM (treino 66.18 ± 8.7; teste 49.27 ± 8.2). O grupo MLA não apresentou redução no número de cruzamentos (treino 89.7 ± 8.4; teste 108.3 ± 8.9). Na tarefa de LCE foi observada uma permanência significativamente maior do grupo MLA nos braços abertos (148.93 ± 14.1) em relação aos demais grupos: COT 3µM (106.7 ± 12.7), COT 18µM (102.41 ± 12.2) e Salina (102.8 ± 12.7). Foi observado um efeito facilitatório da cotinina sobre a evocação da memória na dose de 18µM, reforçando descobertas recentes que mostram a possível ação desse fármaco sobre receptores nicotínicos neuronais, uma vez que o antagonista de receptores nicotínicos α7 MLA parece prejudicar o processo de evocação na EI. Os indícios de que a ação da cotinina é realmente sobre os mecanismos de evocação é reforçado pelo fato de que esse alcalóide não alterou a atividade locomotora e tão pouco a capacidade de habituação dos animais, bem como o potencial anti e pró-conflito, analisado no LCE.

Palavras-Chave: Cotinina, Memória, EsQUIVA INIBITÓRIA

Apoio Financeiro: Cnpq, Capes

H.07.030

Efeito da aplicação de Ecstasy e Fluoxetina sobre a exploração do Labirinto em Cruz Elevado

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Benzodiazepínicos e barbitúricos têm sido utilizados no tratamento da ansiedade. Recentemente agonistas serotoninérgicos 5-HT_{1A} assim como inibidores seletivos da reabsorção de 5-HT (ISRS) têm sido também utilizados. Os estímulos aversivos ativam dois tipos de resposta: fuga e congelamento, segundo Graeff essas respostas correspondem ao pânico e à ansiedade generalizada, respectivamente (1). Sabe-se que o MDMA, o principal composto do Ecstasy, causa a liberação maciça de 5-HT, o que permite supor um possível papel como agente útil na reversão de alguns sintomas de ansiedade, de forma semelhante aos ISRSs. Este estudo comparou as alterações comportamentais causadas pelo tratamento crônico com uma dose moderada de MDMA ou Fluoxetina sobre a exploração no Labirinto em Cruz Elevado (LCE) de ratos previamente submetidos a Estresse Crônico Moderado (ECM).

Metodologia

45 ratos machos Wistar (300±50g) receberam o implante de uma cânula no ventrículo lateral. Depois foram divididos em 2 grupos: SEM ECM e COM ECM, protocolo de 7 dias (2). Cada grupo foi, pela sua vez, dividido em 3 subgrupos que receberam (a partir do quarto dia do protocolo) um total de 4 injeções de 1ul de fluoxetina (2ug/ul), MDMA (0,2 ug/ul) ou solução salina (0,9%). Após o tratamento os ratos foram avaliados no LCE. Os resultados foram analisados utilizando uma ANOVA de duas vias, seguida de Student Newman-Keuls.

Resultados

Houve diferenças significativas na interação entre os fatores tratamento e condição de estresse; o grupo MDMA–COM ECM mostrou uma percentagem maior de entradas e maior tempo no braço aberto comparado com o grupo MDMA–SEM ECM ($F_{[2,28]}=3,48$, $P=0048$, $F_{[2,28]}=3,85$, $P=0036$, respectivamente). Também os sujeitos MDMA–COM ECM permaneceram mais tempo nos braços abertos que os SAL–COM ECM e FLX–COM ECM

Discussão

Os efeitos do MDMA não foram devidos ao aumento da locomotricidade já que não houve diferença nos cruzamentos nem nas visitas aos braços fechados. Assim, o ECM perdeu seu efeito ansiogênico nos ratos tratados com MDMA, talvez devido ao efeito empatomimético. É possível então que o MDMA tenha feito que o ECM fosse entendido como um ambiente enriquecido, diminuindo assim a ansiedade.

Palavras-Chave: MDMA, Ecstasy, Fluoxetina, Estresse Crônico Moderado, Ansiedade, Labirinto em Cruz Elevado

Apoio Financeiro: FAPA-Universidad de los Andes, Bogotá

INFLUÊNCIA DO CICLO ESTRAL NA RESPOSTA ANSIOLÍTICA DE DIAZEPAM E IMIPRAMINA EM CAMUNDONGOS

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Introdução: Fêmeas são diferentes dos machos frente a reações de estresse e comportamento emocional. Sabe-se que desordens do humor, entre elas os transtornos de ansiedade, são mais frequentes em mulheres que em homens e que mudanças emocionais nas fêmeas são associadas com os hormônios esteroidais ovarianos. No entanto, a grande maioria das pesquisas básicas para os distúrbios ansiosos e drogas ansiolíticas têm seu foco em animais machos.

Objetivo: Avaliar a influência das diferentes fases do ciclo estral na resposta ansiolítica em camundongos.

Métodos e Resultados: Fêmeas (60 dias) foram tratadas com Tween (TW-2%, vo), Diazepam (DZP-1mg/Kg, ip) ou Imipramina (IM-30mg/Kg, ip) 30 min. antes da exposição à Caixa Claro/Escuro (CCE) ou ao Teste de Esconder Esferas (TEE) para avaliação de parâmetros relacionados aos transtornos de ansiedade generalizada (TAG) e obsessivo compulsivo (TOC), respectivamente. Ao final das sessões experimentais o esfregaço vaginal determinou o estágio do ciclo estral de cada fêmea que foi, então, alocada nos grupos: Estro (estro/proestro) e Não-estro (diestro/metaestro). Os resultados, expressos como [mediana (Q1-Q3)], foram comparados pelo Teste de Mann-Whitney e as diferenças em relação ao grupo TW consideradas significantes quando $p \leq 0,05$. Na CCE, apenas as fêmeas em Estro tratadas com a droga-padrão DZP mostraram aumento significativo no tempo (s) de permanência no compartimento claro [153(144-218)] quando comparadas ao grupo TW [47(45-90)]. O mesmo ocorreu para o TEE no qual as fêmeas em Estro tratadas com IM demonstraram diminuição no número de esferas escondidas [4(0-11)], em relação ao grupo TW [22(17-24)].

Conclusão: Na CCE e no TEE, as drogas-padrão, sob as mesmas condições experimentais, mostraram efeito apenas nas fêmeas em Estro reforçando a contribuição do nível endógeno de estrogênio na resposta a estímulos ansiogênicos. Na fase Não-Estro a resposta comportamental basal apresenta-se alterada tanto para estímulos relativos a TAG quanto ao TOC, pressupondo ajustes na dose de drogas ansiolíticas e endossando a importância destas flutuações na gênese de desordens ansiosas, mais prevalentes em fêmeas.

Palavras-Chave: TAG TOC fêmeas ciclo estral ansiedade

Apoio Financeiro: CAPES e FAPESP

CHRONIC PRETREATMENT WITH AMPHEPRAMONE INDUCES BEHAVIORAL CROSS-SENSITIZATION WITH METHYLPHENIDATE

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Women have fewer symptoms of aggression/impulsivity and low rates of conduct disorder than males and are less frequently diagnosed to have Attention Deficit Hyperactivity Disorder (ADHD). Procrastinated treatment with Methylphenidate (MP) is more common amongst females. In frequent cases, amphetamine-type agents, such as Amphepramone (AMP) were previously used by young women and adolescents in search of its anorectic effects. Thus, females are at risk of suffering cross-sensitization between different psychostimulants. The objective of this work was to verify if chronic treatment with different doses of AMP develops sensitization to MP. Thirty-five female Wistar rats were used (n=7). Amphepramone 5, 10, 15 and 20mg/kg or control solution was administered to rats during 21 days. A wash-out period of 30 days was allowed and right after all animals received a single dose of Methylphenidate 2.5mg/kg. Animals were placed in the open field for 5 minutes and their locomotor activity was video-taped recorded. The results are presented as mean±SEM. The statistical test used was One Way-ANOVA, followed *post-hoc* by the Student-Newman-Keuls Method. Differences of $P < 0.05$ were considered significant. The horizontal activity of rats chronically treated with AMP 10mg/kg (217 ± 15.73), 15mg/kg (198.29 ± 10.08) and 20mg/kg (206.43 ± 18.89) submitted to challenge dose of MP was significantly higher than those treated acutely with MP (153.71 ± 6.98), ($F(4.30) = 3.159$, $p = 0.028$), with higher response at AMP 10mg/kg. Increased vertical activity was also observed in rats pretreated with AMP 10mg/kg (31.71 ± 3.29) in comparison to acute MP (13.14 ± 2.01), ($F(4.30) = 9.841$, $p < 0.001$). There was no difference in respect to the horizontal activity in AMP 5mg/kg, in vertical activity of AMP 5, 15 and 20mg/kg, grooming or defecation. It may be concluded that chronic pretreatment with an anorectic agent induces behavioral cross-sensitization with agent used for ADHD treatment.

Palavras-Chave: Methylphenidate, Amphepramone, cross-sensitization

Apoio Financeiro: AAPeFato, UFCSPA, CNPq.

Effects of the putative antipsychotic alstonine on brain amines.

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Introduction: Alstonine (AL) is an indole alkaloid identified as the major component of a plant-based remedy used in Nigeria to treat the mentally ill. In mice, this alkaloid presents a clear antipsychotic profile, closer to atypical agents and, apparently, differentially affects distinct dopaminergic pathways. Moreover, we previously reported that AL shows anxiolytic properties, likely to result from its inverse antagonist effect on 5HT_{2A/C} receptors. **Objective:** The aim of this study was to complement the antipsychotic profile of AL, verifying its effects on mice brain amines in the frontal cortex and striatum. **Methods:** Male (CF1) adult albino mice were treated (N=10) with saline or AL 1.0mg/Kg (i.p.), and 30min later sacrificed by decapitation. The striatum and frontal cortex were removed, weighed and immediately stored in liquid nitrogen, until samples were prepared in 0.1M perchloric acid by manual sonication. DA and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), as well as 5-HT and its metabolite 5-hydroxyindole acetic acid (5-HIAA) were measured by reverse-phase HPLC (electrochemical detection). Results were analyzed by independent T-test. **Results:** DA levels were found to be decreased ($t=4.96$, $p<0.01$) in the frontal cortex (but not striatum), with a concomitant increase in DOPAC ($t=-2.22$, $p<0.05$), also increased ($t=-3.62$, $p<0.01$) at the striatum; there were no changes in HVA. 5HT levels are increased only in the frontal cortex ($t=-3.74$, $p<0.05$), whereas increases in 5-HIAA are seen in the frontal cortex ($t=-2.68$, $p<0.01$) and striatum ($t=-2.5$, $p<0.05$). **Discussion:** The suggestion that AL acts as a 5HT_{2A/C} inverse agonist is compatible with the observed increases in 5HT and 5HIAA, since pre-synaptic 5HT_{2A/C} receptors modulate 5HT release. Increased DOPAC levels without changes in HVA indicates augmented intra neural DA degradation. The elucidation of the precise mechanism(s) by which AL induce these alterations requires further investigation. Such unusual neurochemical profile may be useful to open innovative scenarios for developing antipsychotics. **Palavras-Chave:** Alstonine, antipsychotics, brain amines. alkaloid

ACÇÃO OPOSTA DOS RECEPTORES CB1 HIPOCAMPAIS NA RECONSOLIDAÇÃO E EXTINÇÃO DE MEMÓRIAS AVERSIVAS.

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OBJETIVOS: Durante a evocação, dois processos antagônicos podem ocorrer, dependendo, dentre outros fatores, da duração da sessão de reativação: se esta é breve, a memória será reconsolidada, se é longa, será extinta. O objetivo deste trabalho é estudar a participação do sistema endocanabinóide sobre a extinção e reconsolidação de memórias aversivas na região CA1 do hipocampo dorsal.

MÉTODOS: Ratos Wistar machos foram canulados bilateralmente no hipocampo e submetidos à tarefa comportamental do condicionamento aversivo ao contexto (2 choques de 0,7mA). Imediatamente após as sessões de reativação (3 min) ou extinção (25min), os animais eram tratados com uma, ou duas das seguintes drogas: antagonista seletivo dos receptores CB1, AM251 (5,5 ou 0,27ng/lado), veículo (DMSO 8%/TFS), inibidor de síntese proteica, DRB (10ng/lado), ou agonista CB1, anandamida (0,17ng/lado). Após 24h, os animais eram testados e as respostas de medo (congelamento) eram avaliadas.

RESULTADOS: A dose de 5,5ng/lado de AM251 facilitou a reconsolidação da memória, e a administração de DRB, a bloqueou ($p < 0,05$, $n = 7-11$). Por outro lado, a anandamida inibiu a reconsolidação, efeito que foi revertido pela infusão concomitante de uma dose de AM251 sem efeito próprio (0,27ng/lado), ($p < 0,05$, $n = 6-8$). A extinção da memória foi inibida pelo AM251 ($p > 0,05$, $n = 8$) e facilitada pela anandamida ($p < 0,05$, $n = 7$), a não ser quando esta foi administrada conjuntamente com a subdose de AM251, tendo seu efeito revertido. Quando a sessão de reativação foi omitida, o AM251, o DRB e a anandamida não apresentaram efeito ($p > 0,05$, $n = 6$), mostrando que a evocação da memória é necessária para que ela se torne lábil.

CONCLUSÃO: Nossos resultados mostram a importância do sistema endocanabinóide nos processos de extinção e reconsolidação da memória. Neste caso, esse sistema atuaria facilitando a extinção e inibindo a reconsolidação (pelo menos no hipocampo).

Compreendendo como esse sistema funciona, poderíamos interferir com esses processos para modificar memórias já consolidadas, como memórias mal-adaptativas (traumas, por exemplo).

Palavras-Chave: Endocanabinoides, Reconsolidação, Extinção, Memórias Aversivas

Apoio Financeiro: CNPq, FAPERGS

PARTICIPAÇÃO DO SISTEMA BAUNILHÓIDE (VANILLOID) NO PROCESSO DE CONSOLIDAÇÃO DE MEMÓRIAS AVERSIVAS EM RATOS

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Objetivos: os receptores baunilhóides (TRPV1 ou VR1) estão presentes em grandes quantidades no sistema nervoso periférico e têm sido amplamente estudados como integradores de estímulos nocivos. A detecção desse sistema baunilhóide também no sistema nervoso central leva ao questionamento de qual seria o papel fisiológico dos receptores VR1 localizados no encéfalo. No presente estudo foi investigada a participação do sistema baunilhóide hipocampal sobre a consolidação de memórias aversivas em ratos.

Métodos e resultados: foram estudados os efeitos da administração bilateral intra-hipocampal de *capsaicina*, um agonista baunilhóide e de *capsazepina*, um antagonista dos receptores baunilhóides VR1 sobre o processo de consolidação da memória em ratos machos Wistar adultos. Foram utilizadas as tarefas de Esquiva Inibitória (EI) e Tarefa de Condicionamento Aversivo ao Contexto (CAC). Os animais foram infundidos imediatamente após o treino com capsaicina (agonista) ou capsazepina (antagonista) no volume de 0,5µl/lado e as concentrações de 0.1, 1 ou 10µM ou 2, 10 ou 20µM, respectivamente. O grupo controle de ambos os tratamentos foi infundido com DMSO 8% em TFS. Efeitos da capsaicina: não houve diferença significativa entre os grupos no teste em EI (P=0,392, ANOVA de Kruskal-Wallis, N=11, 10, 12, 11, respectivamente para controle e drogas) e CAC (P=0,712, ANOVA de uma via, N=9, 11, 13, 9). Efeitos da capsazepina: não houve diferença significativa entre os grupos no teste em EI (P=0,277, ANOVA de Kruskal-Wallis, N=12, 10, 11 e 11); porém, houve diferença significativa entre os grupos no teste em CAC (P=0,034, teste ANOVA de uma via, N=11, 9, 9, 10), o teste *post-hoc* de Tukey, mostrou que somente o grupo infundido com 10µM era significativamente diferente do grupo controle (P<0,05).

Conclusões: O antagonista capsazepina, na concentração de 10µM teve um evidente efeito amnésico sobre a consolidação somente na tarefa de CAC. Estes resultados sugerem que o sistema baunilhóide endógeno participa somente nos processos de consolidação da memória de experiências com um forte componente aversivo.

Palavras-Chave: baunilhóide (vanilloid), memória, rato

Apoio Financeiro: CNPq, CAPES, FAPERGS, Propesq/UFRGS, IFS.

Neonatal morphine exposure alters the ectonucleotidase activities from spinal cord and cerebral cortex synaptosomes of rats.

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Objectives: There are many systems that modulate nociceptive neurotransmission in the central nervous system. It has been proposed that the extracellular adenosine is involved in the physiological control of pain and in opioid antinociception. Otherwise, ATP is a neurotransmitter released from the terminals of afferent sensory nerves to act in the central pain pathway. Extracellular nucleotides can be hydrolyzed by E-NTPDase and 5' nucleotidase. This cascade presents a function of removing a signal of ATP and generating adenosine. Here, we evaluated ATP, ADP and AMP hydrolysis from spinal cord and cerebral cortex synaptosomes of rats at different ages following repeated morphine exposure in early life.

Methods: male Wistar rats were divided into two groups: saline (C: n=30) and morphine (M: n = 29). Each animal received saline or morphine (5µg s.c. in the midi-scapular area) once a day at P8 until P14. At P16 and P30 were killed and they structures were removed for enzyme assays. The synaptosomes were isolated as described by Nagy *et al.* (1984) and reaction medium used to enzyme assays was essentially as described by Battastini *et al.* (1991) and Heymann *et al.* (1984). Enzymatic activity was expressed as nmol of Pi/min/mg of protein. **Results:** at P16 we observed an increase in ATP hydrolysis in the cerebral cortex synaptosomes (C=161.6±27.5, M=213±30.9; Student's *t* test, *P*<0.05) and a decrease ADP hydrolysis in the spinal cord synaptosomes (C=82.5±1.6, M=51.8±10.3; Student's *t* test, *P*<0.05) for morphine group. There is no difference in other nucleotides hydrolysis at P16 and in all nucleotides hydrolysis at P30 for both structures (Student's *t* test, *P*>0.05). **Conclusions:** Our findings suggested that the repeated morphine exposure in early life has effect in the purinergic system of young rats. The different activities are possibly due to different NTPDases types present in both structures, such as NTPDase 1 and NTPDase 2 in spinal cord and in cerebral cortex, respectively. These results may constitute one of the mechanisms that mediate the development of some of the side effects associated with repeated morphine exposure in early life.

Palavras-Chave: opioid antinociception; Enzymatic activity ; synaptosomes

Apoio Financeiro: CNPq, FAPERGS and PROPESQ/UFRGS

Diferenciação dos comportamentos apresentados por camundongos no Teste do Nado Forçado após tratamento com Imipramina, Nortriptilina ou Fluoxetina.

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Introdução: O escasso rendimento em preparações de origem vegetal prescinde de procedimento adequado para avaliar atividade antidepressiva em camundongos. No mais utilizado destes procedimentos, o Teste do Nado Forçado (TNF), a redução no tempo de imobilidade (I) é associada à atividade antidepressiva e, em ratos, a execução dos comportamentos de escalar (E) ou nadar (N) parece estar associado a drogas que atuam sobre noradrenalina (NOR) e serotonina (5-HT), respectivamente (1).

Objetivo: Avaliar a ocorrência de comportamento diferencial no TNF em camundongos pré-tratados com antidepressivos que atuam com maior seletividade sobre NOR (NTP: Nortriptilina), 5-HT (FLU: Fluoxetina) ou sem seletividade prevalente (IMI: Imipramina).

Métodos: Camundongos (n=6-9), machos, adultos foram pré-tratados com IMI, NTP ou FLU (ip, 24h, 5h e 1h antes do teste). Uma sessão de treino (15 min) precedeu em 24 h a sessão de teste (5 min) nas quais cada animal é colocado em um recipiente (h: 21 cm, $\varnothing$: 20 cm) com 13 cm de água. O comportamento executado (I, E ou N) é registrado a cada 10 s na sessão de teste.

Resultados e Discussão: Os resultados, apresentados como [mediana(Q1-Q3)] foram comparados pelo teste de Mann-Whitney, e as diferenças consideradas significantes quando $p < 0,05$. Os valores de referência para o grupo controle são: I: [26(24-27)]; N: [3(2-5)], E: [1(0-2)]. Após tratamento com IMI, com ação inespecífica sobre NOR e 5-HT, ocorreu redução de I [18(16-20)] e aumento em E [7(3-8)] e N [7(5-8)]. Por outro lado, o tratamento com NTP, inibidor seletivo da recaptação de NOR, reduziu I [22(19-25)] e aumentou apenas E [3(1-6)], enquanto o tratamento com FLU, inibidor seletivo da recaptação de 5-HT, reduziu I [14(12-17)] e aumentou apenas N: [11(11-12)].

Conclusão: Os resultados obtidos em camundongos com as drogas-padrão que atuam de modo diferencial sobre a recaptação de NOR e 5-HT reproduzem o comportamento relatado para ratos. Deste modo, o uso do TNF, em camundongos, pode beneficiar a investigação da atividade diferencial de potenciais antidepressivos de origem vegetal sobre estes sistemas de neurotransmissão.

Referências: 1- Tips. 23(5):238, 2002.

Palavras-Chave: modelo experimental, antidepressivos, camundongos, teste do nado forçado

Apoio Financeiro: CAPES

Ácidos Graxos Poliinsaturados *n*-3 modificam o Comportamento Exploratório e a Atividade Locomotora de Ratos.

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Objetivos: Ratos submetidos à dietas com diferentes fontes de ácidos graxos podem apresentar alterações comportamentais importantes, incluindo distúrbios de emocionalidade, interação social, atividade exploratória e déficits relacionados à memória. O objetivo do trabalho foi investigar o comportamento exploratório e a atividade locomotora em ratos consumindo ração elaborada com óleo de soja ou óleo de peixe durante o período de gestação e lactação, e após o desmame.

Métodos: Os animais foram mantidos em biotério com temperatura e luminosidade controladas. Aos 40 dias de vida foi avaliado o comportamento dos filhotes do grupo soja (n=16) e peixe (n=14), separados pelo gênero, em campo aberto. No dia do teste, cada rato foi colocado dentro de uma caixa de madeira medindo 60x60x50cm, cujo chão foi dividido por linhas pretas formando um total de 49 quadrados. Os ratos permaneceram dentro da caixa por 5 minutos. A cada troca de animal o aparato era limpo e seco. Todo o experimento foi filmado para que posteriormente fossem quantificados os seguintes parâmetros: (1) número de quadrados percorridos, (2) número de vezes que os ratos levantam as patas anteriores (*rearing*) e (3) tempo (s) de *freezing*. Os resultados foram avaliados pelo teste *t* student com significância de $P<0,05$.

Resultados: Foi observado que os filhotes machos tratados com ração a base de óleo de peixe apresentaram maior atividade locomotora durante os primeiros 4 minutos do teste, perfazendo um total de $147,5\pm 21,5$ quadrados percorridos, enquanto que o grupo controle percorreu $55,8\pm 4,5$. Os machos também apresentaram maior atividade exploratória comprovada pelo maior número de *rearing* que foi de $18,7\pm 2,5$ e $10,4\pm 1,0$ no grupo peixe e soja, respectivamente. Quanto as fêmeas, não houve diferença significativa em relação a atividade locomotora e exploratória, no entanto as fêmeas do grupo peixe mostraram menor tempo de *freezing* ($38,6\pm 3,9$) quando comparadas ao grupo controle ($60,3\pm 9,1$).

Conclusões: Os resultados mostraram que ratos machos consumindo dieta elaborada com óleo de peixe rico em ácidos graxos *n*-3 apresentam importante aumento da atividade locomotora e exploratória no teste do campo aberto.

Palavras-Chave: Ácidos graxos poliinsaturados *n*-3, campo aberto, ratos.

Apoio Financeiro: Faperj.

H.07.039

D-serine administration improves working memory in mice

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Objectives: D-serine is an endogenous co-agonist of the N-methyl-D-aspartate (NMDA) type of glutamate receptor. Oral administration of D-serine leads to cognitive improvement in subjects with schizophrenia. Functional magnetic resonance imaging study in rodents reveals that parenteral administration of D-serine induces hippocampal activation. These previous findings suggest that D-serine modulates NMDA receptors in circuits related to cognition. The present study investigates the effects of D-serine administration on delayed alternation test in mice using a T-maze protocol.

Methods: The T-maze is widely used for the cognitive assessment of working memory. This simple labyrinth consists of a start box opening on a main stem and two side arms, perpendicular to the main stem. For each trial, the animal was released from the start box and freely choosed either the left or the right arm. Only one arm contained a food reward, which was systematically alternated between consecutive trials. Hence, the animal had to learn that the reward is alternating sides. Animals were maintained on a slightly restrictive diet so that they remain motivated. Adult male Balb-C mice (n = 18) received intraperitoneal injections of D-serine (50mg/kg) or saline (50ml/kg) before each session.

Results: Animals treated with D-serine had better performances than saline-treated ones, as the D-serine group choosed significantly more often the correct arm (p = 0,0023). To check whether D-serine induced effects could be related to exploratory behavior, additional open field testing was performed. Acute D-serine administration did not alter exploratory behavior in mice (p = 0,4857).

Conclusions: Our data demonstrate that acute intraperitoneal administration of D-serine leads to spatial learning improvement in mice. The results reinforce that D-serine can be usefull as therapeutic agent in diseases involving cognitive decline.

Palavras-Chave: D-serine, learning, memory, T-maze

Apoio Financeiro: FAPERJ

El receptor adrenérgico alfa-1 participa en la acción del antipsicótico atípico Clozapina en el bloqueo del síndrome conductual inducido por el antagonista NMDA, dizocilpina.

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Clozapina (Clz) es el antipsicótico atípico prototípico. Genera su acción terapéutica con una ocupación de receptores D2 menor al umbral terapéutico para el antipsicótico típico Haloperidol, explicando la ausencia de sintomatología extrapiramidal en su tratamiento. Esta diferencia estaría dada por la acción de Clz sobre otros receptores. Clz posee mayor afinidad por el receptor 5-HT_{2A} que por D2 [1], y posee alta afinidad por el receptor adrenérgico $\alpha 1$ (R- $\alpha 1$) [2], el bloqueo del cual podría contribuir al perfil atípico de Clz. Existe poca información disponible sobre el papel del R- $\alpha 1$ en el mecanismo de acción de Clz.

El trabajo se basa en un estudio comparativo de los efectos antagonistas de la administración sistémica de Clz a dosis equimolares y equipotentes con el antagonista selectivo $\alpha 1$ Prazosin (Prz), utilizando el modelo farmacológico del antagonista del receptor N-metil-D-aspartato (NMDA), dizocilpina (MK-801). Mediante el modelo de *open field* se registraron las conductas inducidas por MK-801 (0.2 mg/kg) y su bloqueo por Clz (1 mg/kg) ó Prz (0.3 y 1 mg/kg).

MK-801 indujo un aumento significativo en la locomoción (190% del control), una disminución significativa de *rearings* y signos de ataxia. Clz disminuyó parcial y significativamente la hiperlocomoción inducida por MK-801 (28% del MK-801) y los signos de ataxia, sin bloquear la disminución de *rearings* [3]. Prz 1 (dosis equimolar a Clz) disminuyó significativamente la hiperlocomoción y los signos de ataxia inducidos por MK-801 (74%) sin alterar la disminución de los *rearings*. Prz 0.3 (dosis equipotente a Clz) bloqueó parcial y significativamente la hiperlocomoción (27%), la disminución de los *rearings* y los signos de ataxia.

Clz 1 y Prz 0.3, ocupando un 40% de R- $\alpha 1$ [2], mostraron un perfil antagonista similar sobre las respuestas inducidas por MK-801. El 100% de ocupación de R- $\alpha 1$ (Prz 1) genera un antagonismo mayor al observado con Clz. Estos resultados demuestran que el R- $\alpha 1$ posee un papel fundamental en la acción antipsicótica de Clz, aportando información al estudio de la atipicidad del fármaco.

1. *Neuropsychopharmacol.* 21:S106,1999

2. *Brain Res. Prot.* 8:191,2001

3. *Int. J. Neuropsychopharmacol.* 11:519,2008

Palavras-Chave: receptor adrenérgico alfa-1, Clozapina, Prazosin

Apoio Financeiro: Fondo Clemente Estable 10 029

H.07.041

The Interactive process between serotonergic and glutamatergic system in the dorsal periaqueductal gray in behavioral performance of inhibitory avoidance task of rat.

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Objectives: Behavioral studies have shown that the dorsal periaqueductal gray matter (dPAG) is critical for selecting, organizing and commanding the appropriate behavioral and neuro-vegetative defensive reaction. This structure is known to be *involved* in the modulation of defensive behaviors and dPAG lesion studies have shown its participation in the acquisition and the expression of *learned defensive* responses **(1)**. Step-down inhibitory avoidance task (SD) consists in an associative learning between an initially neutral stimulus and a potentially threatening stimulus such as footstock. After a training session the conditioned behavior (inhibitory avoidance response-IAR) is displayed **(2)**. The present survey is outlined to study the role played by the dPAG in the acquisition and consolidation of fear conditioning to a SD task.

Methods: For this purpose, male Wistar rats had received microinjections (0.3 µl) through to guide cannulas at the dPAG: PBS, NMDA (25 and 100 pmol), prior or immediately after the training and pre treatment with receptor 5-HT_{1A} agonist 8-OH-DPAT (2 and 8 nmol), and antagonist WAY100135 (2 and 5 nmol) 10 min, prior the test in SD.

Results: The results had shown that the microinjections of the excitatory amino acid, NMDA agonist in dPAG impair the acquisition and consolidation of the IAR. 8-OH-DPAT or WAY 100135, alone, were not capable to interfere with the IAR acquisition. The Pretreatment with 8-OH-DPAT in the dPAG prevents the damage of acquisition but not the consolidation of fear conditioned by a SD task for NMDA injection.

Conclusions: Thus the results suggested that serotonergic modulation of aversive behaviors elicited specific chemical stimulation of the dPAG can be mediated by 5-HT_{1A} receptors. The present study suggests that dPAG is a fundamental structure in the expression of defensive behavior showed toward threaten stimulus, and also is involved in the process of learning defensive response.

Reference: 1-Behavio. Neurosc., 107(6):1093-1098,1993. 2- Neurobi. of Lear and Memory, 68:285-316,1997. **Palavras-Chave:** Periaqueductal gray matter; serotonin, inhibitory avoidance task

Apoio Financeiro: CNPq, FAPESP, UFSC.

Neonatal morphine exposure alters the behaviour in young and adult rats

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Objectives: The use of opioid analgesia has increased in the neonatal intensive care as a consequence of advances in the understanding, identification and treatment of pain in children. However, there is still a lack of knowledge on their specific effects during long-term administration in these patients. The aim of this study was to investigate whether repeated morphine exposure during early life alters the behavioural responses in short- and long-term. **Methods:** male Wistar rats aged 8-days old at the beginning of the experiment were used. The rats were divided into two groups: saline-control (C: n=26) and morphine-treated (M: n = 36). Each animal received saline or morphine (5 µg s.c. in the mid-scapular area) at P8, once a day for seven days. The behavioural procedures were performed at P15, P16, P30 and P60. The behavioural responses were evaluated by open field cage and were measured: locomotion (the number of line crossings), rearing (standing upright on the hind legs), latency to leave of the first quadrant and grooming. **Results:** at P15 and P60, no differences were observed between groups for all behaviour analysed (Student's *t* test, $P > 0.05$ for all). At P16 the morphine groups showed significantly increase in the time of grooming (C=19.7±2.06; M=22.8±2.5; Student's *t* Test, $P < 0.05$). At P30 this group showed differences in the time of grooming (C=16.4±2.6; M=7±1.4; Student's *t* Test, $P < 0.05$) and in the number of crossings (C=75.8±4.9; M= 97.9±5.8) and rearings (C=34.3±2.4; M=47.3±3.8; Student's *t* Test, $P < 0.05$ for both behaviours analyzed). **Conclusions:** We suggest that morphine exposure during early life could leave short- and long-term alterations in the opioid system, such as behavioural sensitization. The repeated exposure to abuse drugs such as morphine could causes sensitization to their stimulant properties in rats. Although we observed different behaviours, such as higher grooming at P16, and higher rearing and crossing at P30, it is probable that these animals develop behavioural sensitization according to age. These findings indicate the need for the evaluation of the clinical consequences of long-term opioid administration in early life.

Palavras-Chave: opioid analgesia; behavior; behavioural sensitization

Apoio Financeiro: CNPq, FAPERGS and PROPESQ/UFRGS

Investigation of the potential adverse effects induced by the putative antipsychotic alstonine.

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Introduction: Managing schizophrenia symptoms has never been a trivial matter, and one major problem is that the main reasons for discontinuing antipsychotic treatment have been the side effects induced by antipsychotics. Despite advantages obtained with newer agents, while classic ones induce extrapyramidal side effects and hyperprolactinaemia, atypicals lead to diabetes, hyperlipidemia, and weight gain. Alstonine (AL) is the major component of a plant-based treatment given to mentally ill patients in Nigeria. In rodents, AL shows a clear antipsychotic profile, closer to atypical than classical agents. **Objective:** The aim of this study was to examine if AL induces the hormonal and metabolic changes common to classic and/or atypical antipsychotics. **Methods:** *Glycemia:* Groups of mice (CF1 adult albino male mice, N=5-9) were treated ip with saline, clozapine (2.0 and 10.0mg/kg), and AL (0.5 and 1.0mg/kg); glucose measures (Accu-Chek Active® Roche) were taken immediately before the treatments and after 3h. *Body weight:* Mice (N=8-10) were treated ip for 6 days with saline, haloperidol (0.25mg/kg), clozapine (2.0mg/kg) or AL (0.5 and 1.0mg/kg); body weight was measured daily. *Prolactin (PRL) levels:* Groups of mice (N=6-8) were treated ip with saline, haloperidol (0.25mg/kg), clozapine (2.0mg/kg) or AL (1.0mg/kg), and 30min latter blood samples were collected; plasma PRL levels were measured by radioimmunoassay. **Results:** The fasting-induced hypoglycemia was absent with both clozapine and AL treatments. No differences in body weight gain were observed among the groups. Haloperidol markedly increased ($F_{3,23}=12.78$, $p<0.01$) PRL levels, whereas clozapine and AL did not. **Discussion:** Although longer treatment periods than those here used need to be analyzed, AL did not alter mice weight gain or plasma prolactin levels with the doses found to be effective in relevant behavioral mice models to detect and characterize antipsychotic-like properties. Since glycemia was altered, a thorough investigation is needed to better assess the possible influence of AL in glucose metabolism. **Palavras-Chave:** alstonine, antipsychotic, side effects, glycemia, body weight, prolactin

ESTUDO DOS EFEITOS DA ADMINISTRAÇÃO AGUDA DE CARVACROL NO SISTEMA NERVOSO CENTRAL EM CAMUNDONGOS.

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O carvacrol, 2-metil-5-(1-metil-etil)fenol, um monoterpene com PM= 150.22 g/mol, possui um gosto e odor pungente característico (Kimura et al., 2006; Dietz & Traud, 1978), estando nos óleos essenciais de diversas espécies, como de *Origanum onites* (*Phytotherapy Research* 10: 342-344, 1996). O objetivo do presente trabalho foi avaliar os efeitos do carvacrol em três modelos comportamentais em animais: o teste do nado forçado, descrito por Porsolt et al (1977), o teste da suspensão da cauda, descrito por Steru et al. (1985) e o teste do campo aberto, que verifica a atividade locomotora, *rearing* e *grooming*, descrito por Archer (1973) para investigar sua possível ação antidepressiva. Carvacrol foi emulsificado em 0,2% Tween 80 (Sigma-USA) e dissolvido em água destilada. Camundongos Swiss, machos (25-30g) receberam carvacrol nas doses de 25 e 50mg/kg, administrado por via oral uma hora antes dos experimentos. A imipramina (10 e 30mg/kg i.p.) foi utilizada como controle positivo no teste do nado forçado e no teste da suspensão da cauda. Os resultados são apresentados como média ± Erro padrão da média, a análise estatística realizada através do teste de ANOVA seguida de Student Newman Keuls *post hoc* e os dados foram considerados significativos a partir de $p < 0,05$. Os resultados mostraram uma redução do tempo de imobilidade no teste do nado forçado (Controle: $33,46 \pm 3,196$; Carvacrol 25: $6,924 \pm 0,7246$; Carvacrol 50: $6,846 \pm 1,356$; imipramina 10: $16,08 \pm 1,603$) e no teste da suspensão da cauda (Controle $69,28 \pm 4,575$; Carvacrol 25: $36,21 \pm 4,538$; Carvacrol 50: $36,64 \pm 4,786$; imipramina 30: $17,57 \pm 1,948$). No teste do campo aberto, não foi observado nenhuma alteração em todos os parâmetros estudados após tratamento com carvacrol nas duas doses administradas comparado ao grupo controle. A análise dos resultados obtidos, leva a sugerir que o carvacrol apresenta uma possível atividade antidepressiva no Sistema Nervoso Central. Experimentos posteriores serão realizados a fim de confirmar esta atividade, bem como investigar os possíveis mecanismos que possam estar envolvidos neste efeito.

Palavras-Chave: Carvacrol; antidepressivo; nado forçado; suspensão da cauda; campo aberto

Apoio Financeiro: CNPq

Atividade antinociceptiva do peptídeo PoTx 6.1 isolado da peçonha da vespa *Polybia occidentalis* (Hymenoptera: Vespidae) em ratos Wistar

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OBJETIVOS: Identificar e verificar a atividade antinociceptiva do peptídeo PoTx 6.1 (1300 Da) isolado da peçonha de *P. occidentalis* (1) em ratos Wistar submetidos aos modelos de “Hot Plate” e “Tail Flick” .

MATERIAL E MÉTODOS: Foram utilizados ratos machos Wistar (220-250g) anestesiados com tiopental sódico (50 mg/kg, i.p.) para implante de uma cânula guia no ventrículo lateral encefálico (AP: 1,0mm, L: 1,6mm e V: 3,5mm), por meio de estereotaxia. Após 7 dias de recuperação, os ratos foram submetidos aos modelos: “Hot Plate” e “Tail Flick” em diferentes períodos. Administrou-se nos ratos (n=6) PoTx 6.1 (1 e 2 µg), salina, morfina, naloxone e morfina + naloxone para os grupos controle. Os tempos máximos de latência dos ratos nos modelos foram quantificados e analisados utilizando ANOVA de medidas repetidas e o pós-teste de Tukey.

RESULTADOS: No “Hot Plate” o grupo de ratos tratados com a dose de 2 µg da PoTx 6.1 aos 60 min apresentaram um índice de analgesia (I.A.) significativamente maior em relação ao grupo controle, morfina + naloxone e 1 µg PoTx ($F_{(4,24)}=73,75$; $P<0,001$). Para os ratos que foram submetidos ao “Tail Flick”, o I.A. da dose de 1 µg da PoTx e morfina foi significativo aos 30 e 60 min quando comparado ao grupo morfina+naloxone. ($F_{(4,24)}=7,246$, $p<0,01$; $F_{(4,24)}=4,802$, $p<0,05$, para os tempos de 30 e 60 min respectivamente).

CONCLUSÕES: A resposta antinociceptiva do peptídeo PoTx 6.1 varia dependendo do modelo de antinocicepção térmica testado, do seu tempo de ação e da dose do composto administrada. Uma possível explicação para tais resultados, de acordo com LEV et al (2), seria uma diferenciada ação em nível espinhal (“Tail Flick”) e supraespinhal (“Hot Plate”).

REFERÊNCIAS: 1- British Journal of Pharmacology 151: 860, 2007; 2- Journal of Pharmacological and Toxicological Methods 34: 23, 1995.

Palavras-Chave: Antinocicepção, Peçonha, Vespa, Hot Plate, Tail Flick

Apoio Financeiro: CAPES

H.07.046

Diferenças na estereotipia induzida por apomorfina ou por cocaína em ratos cronicamente tratados com cocaína.

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Introdução: O uso crônico de cocaína pode variar de acordo com o padrão de adição apresentado. Modelos animais usualmente empregados não consideram esses padrões variáveis, dependentes de aspectos sociais e econômicos, que modificam o uso da droga no decorrer do tempo. A plasticidade dopaminérgica parece ser de fundamental importância para os sintomas apresentados pelos dependentes de cocaína.

Objetivos: No presente estudo, foi empregado um protocolo variável e aleatório de tratamento crônico com cocaína procurando mimetizar as condições de uso da droga entre usuários crônicos. Neste modelo objetivou-se estudar as respostas do sistema dopaminérgico através do estudo do comportamento estereotipado e avaliar a sensibilidade do mesmo.

Métodos: Vinte ratos Wistar machos pesando em média 170g ao início do tratamento receberam durante 90 dias de 0 a 15mg/kg de cocaína determinado por gerador de números aleatórios. Outros 20 animais foram submetidos ao mesmo tratamento com salina. Após o tratamento, todos os animais foram divididos em dois grupos e submetidos a 0,6mg/kg de apomorfina ou 10,0 mg/kg de cocaína. Imediatamente após, eles foram observados durante 10 s a cada 10 min durante 60 min. Foram aplicados os escores de Settler para estereotipia. Os dados foram expressos como média $\pm$ erro padrão. Os resultados foram comparados pelo teste U de Mann-Whitney.

Resultados: A estereotipia induzida por apomorfina foi significativamente menor no grupo tratado cronicamente com cocaína quando comparado ao grupo controle aos 10 ($3,70 \pm 0,33$ vs $4,75 \pm 0,31$; $p=0,034$), aos 40 ($4,08 \pm 0,26$ vs $5,12 \pm 0,35$; $p=0,043$) e aos 50 min ($2,85 \pm 0,25$ vs $3,75 \pm 0,16$; $p=0,037$). Já, o comportamento estereotipado induzido pela cocaína foi significativamente maior no grupo tratado com cocaína quando comparado com o grupo controle aos 30 min ($3,30 \pm 0,30$ vs $2,11 \pm 0,30$; $p=0,04$).

Conclusão: Nossos resultados sugerem que o protocolo de tratamento empregado determinou simultaneamente uma diminuição da sensibilidade do sistema dopaminérgico a agonistas e sensibilização à cocaína.

Palavras-Chave: cocaína, sensibilização, anfetamina, apomorfina, dopamina, estereotipia

Apoio Financeiro: FCMSCSP

H.07.047

Avaliação da interferência do ciclo circadiano sobre os comportamentos de camundongos avaliados no labirinto em cruz elevado.

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Introdução: Há muitas evidências de diferenças fisiológicas e comportamentais que ocorrem ao longo do ciclo circadiano em mamíferos. Para evitar interferências desta ordem, é comum a realização dos procedimentos experimentais sempre no mesmo período do dia (manhã ou tarde), mesmo sem a constatação prévia de que ocorre interferência circadiana no comportamento particular que é avaliado. Esta estratégia termina por limitar o número de grupos experimentais avaliado em cada sessão experimental, resultando na repetição de grupos controle e no conseqüente aumento do número total de animais utilizados.

Objetivo: Avaliar a ocorrência de diferenças nos principais parâmetros de ansiedade avaliados no labirinto em cruz elevado (LCE), modelo clássico de ansiedade em roedores, entre animais submetidos ao procedimento em diferentes períodos do dia.

Material e métodos: Grupos independentes de camundongos *swiss*, machos com cerca de 45 dias de idade (n=9-10) foram submetidos ao labirinto em cruz elevado no período da manhã (M - início às 10 h) e no período da tarde (T - início às 16 h). Foram registrados os parâmetros de motilidade: número total de transições entre os compartimentos do labirinto (braços abertos, fechados, plataforma central) e ansiedade generalizada: tempo total (seg) de permanência nos braços abertos. Os experimentos foram filmados em VHS e os comportamentos quantificados com a utilização do *software* Etholog v2.2.5©. Os resultados, apresentados como [mediana(Q1-Q3)], foram comparados pelo Teste de Mann-Whitney, com diferenças consideradas significantes quando $p \leq 0,05$.

Resultados: Não foram observadas diferenças significantes no número de entradas nos compartimentos do LCE apresentado pelos grupos M [24(22-33)] e T [21(14-26)]; ou no tempo de permanência nos braços abertos [4(2-8)] e [4(0-12)] para os grupos M e T, respectivamente.

Conclusão: Esses resultados sugerem que neste procedimento experimental, em camundongos, a variação circadiana não exerce interferência que impossibilite a execução de seções experimentais durante todo o período diurno. É necessário, ainda, a avaliação da interferência do ciclo circadiano sobre a ação de drogas ansiolíticas padrão.

Palavras-Chave: ciclo circadiano, labirinto em cruz elevado, camundongos

Apoio Financeiro: CAPES, FAPESP

O 7-NITROINDAZOL ATENUOU A SEVERIDADE DO ESTADO DEPRESSIVO EM DOIS MODELOS DE DESESPERO COMPORTAMENTAL EM CAMUNDONGOS PRÉ-TRATADOS COM RESERPINA

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Objetivo: testar a hipótese de que o 7-nitroindazol (7-NI) pode induzir efeitos do tipo antidepressivo nos testes da natação forçada (FST) e da suspensão da cauda (TST) em camundongos pré-tratados com reserpina

Métodos e Resultados: grupos diferentes de camundongos das linhagens Swiss e C57BL/6 machos (25-30g) foram submetidos aos testes da FST (1) e TST (2,3). Reserpina (ip) foi administrada 24 horas antes do experimento. Todos os animais foram testados 30 minutos (min) após a administração do 7NI ou seu veículo (ip). No teste da FST, os animais foram colocados dentro do cilindro com água e o tempo de imobilidade nos 4 últimos min de um total de 6 min, foi medido. No TST, os animais foram suspensos por suas caudas com fita adesiva e o tempo total de imobilidade em 6 min foi medido.

Tab 1. Efeito da administração aguda de 7-NI (25mg/kg, i.p) e imipramina (25mg/kg) na média do tempo de imobilidade em camundongos Swiss and C57BL/6 normais e pré-tratados com reserpina (N = 10-12) no FST. Os valores estão expressos como média ± S.E.M. ANOVA de uma via seguida de Newman-Keuls. Diferenças entre as linhagens Swiss e C57BL/6 estão representadas por [#]p < 0.001. ^ap < 0.01 comparado com o grupo Vei - Vei da respectiva linhagem. *p < 0.05 comparado ao grupo controle da mesma linhagem.

Drogas	TEMPO (s) (Swiss)	TEMPO (s) (C57BL/6)
Veículo – Veículo	139,70 ± 10,51	47,09 ± 6,50 [#]
Veículo – Imipramina	123,30 ± 10,82	47,00 ± 12,17
Veículo – 7NI	124,4 ± 12,06	32,44 ± 6,06
Reserpina – Veículo	182,8 ± 6,39 ^a	98,55 ± 8,58 ^{a #}
Reserpina – Imipramina	180,6 ± 8,17	28,60 ± 4,56*
Reserpina – 7NI	137,1 ± 8,15*	96,82 ± 10,25

Fig 2. Efeito da administração aguda de 7-NI (25mg/kg, i.p) e imipramina (25mg/kg) na media do tempo de imobilidade em camundongos Swiss and C57BL/6 normais e pré-tratados com reserpina (N = 10-12) no TST. Os valores estão expressos como média ± S.E.M. ANOVA de uma via seguida de Newman-Keuls. ^ap < 0.01 comparado com o grupo Vei - Vei da respectiva linhagem. *p < 0.05 comparado ao grupo controle da mesma linhagem. ^bp < 0.01 indica uma diferença significativa entre os grupos Res - Imi e Res - 7NI para cada linhagem.

Drogas	TEMPO (s) (Swiss)	TEMPO (s) (C57BL/6)
Veículo – Veículo	149,20 ± 8,47	187,04 ± 5,21
Veículo – Imipramina	46,00 ± 14,46*	97,83 ± 21,31*
Veículo – 7NI	93,5 ± 9,18*	130,00 ± 12,02*
Reserpina – Veículo	260,5 ± 8,78 ^a	239,6 ± 10,98
Reserpina – Imipramina	80,71 ± 27,36*	112,4 ± 18,35*
Reserpina – 7NI	167,8 ± 15,35* ^b	180,30 ± 18,54* ^b

Tanto no TST, quanto no FST houve um aumento do tempo de imobilidade dos animais reserpinizados em relação ao controle. O inibidor da óxido-nítrico sintase (NOSn), 7NI foi capaz de reverter este efeito nas duas linhagens, nos dois modelos, exceto na linhagem C57BL/6 (FST) (4,5), sugerindo uma maior sensibilidade, diferente entre estes animais.

Conclusões: A inibição da síntese de óxido nítrico pode estar envolvida na reversão da depressão induzida pela reserpina nos modelos da FST e TST. Pode-se, também, questionar se o prejuízo na produção de óxido nítrico estaria envolvido na fisiopatologia de transtornos depressivos associados à depleção de dopamina.

Referências: 1- Psychopharmacology 94:147, 1988; 2- Behavioural Brain Research 144:87-93, 2003; 3- Neuroscience and Behavioral Reviews 29:571, 2005; 4- Psychopharmacology 155:315, 2001; 5- Psychopharmacology 166:373, 2003

Palavras-Chave: Depressão, 7-nitroindazol, Óxido nítrico

Apoio Financeiro: CNPq

Neonatal morphine exposure alters the nociception and inflammatory responses in rats

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Objectives: The neonatal nervous system is both structurally and functionally immature and significant changes in opioid mechanisms occur before and after birth. Studies have shown that exposure to drugs in early life can have long-lasting implications on the developing nervous system. Others have shown that opioid exposure could lead changes in pain circuits. The formalin test, utilized in this study, produces persistent pain in animals by supraspinal structures activate. Here, we investigate whether repeated morphine exposure during early life alters the neurogenic and inflammatory pain in short- and long-term.

Methods: male Wistar rats were divided into two groups: saline (C: n=26) and morphine (M: n = 36). Each animal received saline or morphine (5 µg s.c. in the midi-scapular area) once a day at P8 until P14. The formalin test was performed at P16, P30 and P60 in the cage of observation. The animals was injected s.c. into the plantar surface with 0.17 µL/kg of a 2% formalin solution and the nociceptive response was recorded for a period of thirty minutes. The summation of time (seconds) spent in licking, biting and flicking of the formalin-injected hindpaw was recorded in two phases: phase I (0-5 minutes) and phase II (15-30 minutes). **Results:** At P16, the groups did not showed differences for both phases (Student's *t* test, *P*>0.05). At P30, the morphine group showed higher response in phase II (C= 446.1±46.37; M= 650.29±54.62; Student's *t* test, *P*<0.05) and at P60 showed higher response during phase I (C= 116.77±17.68; M= 201.75 ± 12.66) and phase II (C= 339.1±85.1; M= 554.8±61.5; Student's *t* test, *P*< 0.05 for both phases). **Conclusions:** Studies showed that chronic exposure to opioids induces a sensitization in spinal cord neurons that can be manifested as neuronal hyperactivity during opiate withdrawal. Consistent with these results, we suggest that the repeated morphine exposure during early life promotes changes in excitatory systems. Thus, this study challenge the view that early exposure to opiate results in the subsequent development of altered nociceptive responses, which may be expressed until adulthood.

Palavras-Chave: morphine, nociception, formalin

Apoio Financeiro: CNPq, FAPERGS and PROPESQ/UFRGS

H.07.050

Avaliação da atividade antidepressiva do Escitalopram em ratos submetidos ao teste do nado forçado

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Objetivos: O objetivo do presente estudo foi avaliar o efeito do escitalopram, após administração por 14 ou 21 dias, em ratos submetidos ao teste do nado forçado. O inibidor altamente seletivo de recaptação de serotonina, escitalopram, tem apresentado efeito antidepressivo mais potente e de início mais rápido que outros, incluindo o citalopram (1). No teste do labirinto em T elevado, o escitalopram apresentou efeito ansiolítico após a administração aguda ou crônica (21 dias) e efeito panicolítico após administração crônica (2).

Metodologia: Ratos Wistar machos pesando inicialmente 230-240g foram tratados por gavagem com escitalopram (8 e 12 mg/kg), imipramina (15 mg/kg; controle positivo) ou salina por 14 ou 21 dias consecutivos e foram submetidos ao teste do nado forçado, para observação do tempo de imobilidade. A avaliação da atividade locomotora, medida através do número de cruzamentos foi realizada como análise complementar utilizando o teste do campo aberto. Os resultados foram analisados através de ANOVA de um fator seguido pelo teste de Dunnett, utilizando o programa GraphPad Prism versão 4.0.

Resultados: O tratamento por 14 dias, mostrou redução significativa no tempo de imobilidade apenas para os ratos que receberam imipramina ($91,4 \pm 13,1$; $p < 0,01$), comparados à salina ($199,1 \pm 14,1$). O tratamento por 21 dias mostrou redução no tempo de imobilidade para o escitalopram 12mg/kg ($148,7 \pm 20,25$; $p < 0,05$) e para a imipramina ($74,0 \pm 8,0$; $p < 0,01$), quando comparados à salina ($206,3 \pm 10,4$). O número de cruzamentos no campo-aberto não foi alterado em nenhum dos tratamentos.

Conclusão: O escitalopram não reduziu o tempo de imobilidade após tratamento por 14 dias, como a imipramina, mostrando perfil de atividade antidepressiva apenas após 21 dias de tratamento no teste do nado forçado.

Referências: 1- J Affect Disord 87:161-167, 2005; 2- J Psychopharmacol 22(2): 132-137, 2008.

Palavras-Chave: escitalopram, teste do nado forçado, atividade antidepressiva

Apoio Financeiro: Capes/CNPq

H.07.051

Interactions between chronic olanzapine treatment and cafeteria diet in rats: metabolic and cognitive effects.

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Objectives: Olanzapine is an antipsychotic associated with significant metabolic adverse effects. Conversely, olanzapine seem to improve cognitive deficits. High palatable diets may affect metabolism and brain function. Here, we evaluate possible interactions between chronic treatment with olanzapine and cafeteria diet on metabolic parameters as well as on cognitive function.

Methods: Wistar rats were separated in 4 groups (n=10 each): standard diet vehicle (SD); standard diet olanzapine (SDO); cafeteria diet vehicle (CD); cafeteria diet olanzapine (CDO). All groups were subjected to four months of treatment.

Results: Cafeteria diet groups increased body weight ($p<0.05$) and fat pads ($p<0.05$) when compared to all groups. Cafeteria groups presented increased TAG level when compared to standard diet groups. Total cholesterol and HDL cholesterol levels were higher in CDO group when compared to all other groups ($p<0.05$). CDO group showed higher fasting glucose and longer lasting glucose increases compared to all groups ($p<0.05$). On Morris water maze and inhibitory avoidance tasks CDO altered the behavioral effects of the medication alone (SDO group).

Conclusion: Altogether, these results may have clinical relevance, suggesting that cafeteria diet may not only potentiate the metabolic effects of olanzapine, but may also impair its effects on brain and behavior.

References: 1 - J. Psychopharmacol. 21, 405-413, 2007, 2 - Obesity and Metabolism. 7, 478-487, 2005. 3 - Neuropsychopharmacology. 30,1511-20, 2005.

Palavras-Chave: Atypical antipsychotics, Olanzapine, cafeteria diet, metabolic syndrome,

Diminuição na atividade motora de ratos tratados cronicamente com cocaína.

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Introdução: Cocaína é uma droga com grande capacidade de ativar mecanismos de dependência. No uso humano, apresenta padrões variáveis de consumo, o que não é considerado em modelos animais na exploração deste fenômeno.

Objetivo: No presente estudo, foi empregado um protocolo variável e aleatório de tratamento crônico com cocaína para avaliar a atividade motora.

Material e Métodos: Vinte ratos Wistar machos pesando em média 170g ao início do tratamento receberam durante 90 dias de 0 a 15mg/kg de cocaína determinado por gerador de números aleatórios. Outros 20 animais foram submetidos ao mesmo tratamento com salina. Ao término do tratamento os animais foram submetidos à medida de atividade motora em actômetro durante 5 min. Além dos parâmetros obtidos pela interrupção dos feixes fotoelétricos do aparelho (locomção e levantar), foram medidos também o tempo de grooming e o tempo de parado a cada 1 min. Após o actômetro, os animais foram treinados em aparelho de esquiva passiva e testados 24h após. Os dados foram expressos como média±erro padrão e comparados com teste t'student.

Resultados: Os animais tratados cronicamente com cocaína apresentaram diminuição dos parâmetros de locomoção no 1º min (217,7±12,3 vs 257,2±15,8, p=0,03) e no 2º min (152,2±13,6 vs 196,6±13,2, p=0,02); no levantar no 2º min (49,9±5,9 vs 73,1±7,1, p=0,02), 3º min (26,7±4,2 vs 48,4±6,4, p=0,01), 4º min (21,3±4,6 vs 36,3±6,7, p=0,03) e no tempo total (181,7±14,4 vs 257,6±24, p=0,02), além de aumentarem o tempo de treino na esquiva passiva (51,9±14,8 vs 19,2±2,7, p=0,03) quando comparados com o grupo controle.

Conclusão: Esses dados sugerem que a atividade motora desses animais está diminuída. Essa diminuição pode estar relacionada a alterações plásticas nos sistemas catecolaminérgicos, em especial o dopaminérgico já que o animal responde menos aos estímulos de exploração e aversivos de ambientes novos.

Palavras-Chave: atividade motora, dopamina, cocaína, esquiva passiva

Apoio Financeiro: FCMSCSP

Validação farmacológica de um novo teste integrado de ansiedade

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Objetivo: O teste comportamental integrado consiste na junção de três testes comportamentais amplamente utilizados, o campo aberto (CA), o labirinto em cruz elevado (LCE) e a caixa branca/preta (CBP), o que permite que o mesmo animal seja avaliado ao mesmo tempo nestes diferentes ambientes. Neste estudo, tentamos validar farmacologicamente este novo teste integrado após administração de drogas ansiolíticas e ansiogênicas. **Métodos:** Ratos Wistar machos (13-16/grupo) foram tratados intraperitonealmente com metilcelulose 0,5% ou clordiazepóxido (0.5, 5 e 10 mg/kg; CDZ) 30 min antes do teste (experimento 1); com salina (NaCl 0,9%) ou midazolam (0.187, 0.375 e 0.750 mg/kg; MDZ) 30 min antes do teste (experimento 2); com salina ou pentilenotetrazol (10 e 20 mg/kg; PTZ) 5 min antes do teste (experimento 3). Os animais foram colocados no centro do CA, onde poderiam explorar livremente os três aparatos durante 15 minutos. Foram registrados os comportamentos clássicos de ansiedade e locomoção nos três aparatos simultaneamente. **Resultados:** No experimento 1, a ANOVA de uma via revelou efeitos significativos do CDZ a 5 e 10 mg/kg, respectivamente, aumentando a % de entradas ($p < 0.01$), (16.0 ± 2.0), (18.2 ± 3.0) e de tempo gasto nos braços abertos ($p < 0.05$), (35.8 ± 4.9), (37.9 ± 6.9) e diminuindo o número de entradas nos braços fechados do LCE somente na dose de 10 mg/kg ($p < 0.01$), (23.3 ± 2.5). No experimento 2, MDZ a 0.75 mg/kg aumentou a % de entradas ($p < 0.01$), (20.7 ± 3.4) e de tempo gasto nos braços abertos ($p < 0.05$), (38.0 ± 6.0) e diminuiu o número de entradas nos braços fechados ($p < 0.01$), (24.8 ± 3.2) do LCE. No experimento 3, PTZ a 10 mg/kg diminuiu a locomoção central do CA ($p < 0.05$), (4.1 ± 0.9), a % de entradas ($p < 0.001$), (1.9 ± 1.0) e de tempo gasto nos braços abertos ($p < 0.01$), (3.5 ± 2.3) do LCE e locomoção do compartimento branco da CBP ($p < 0.05$), (4.5 ± 2.1). **Conclusão:** Estes resultados sugerem que a integração física dos aparatos pode contribuir para o estudo da ansiedade, por promover um método rápido, abrangente e confiável de avaliação de comportamentos relacionados à emocionalidade. **Palavras-Chave:** ansiedade, labirinto em cruz elevado, campo aberto, caixa branca e preta, rato

Apoio Financeiro: CAPES

Efeitos comportamentais do agonista do receptor 5-HT_{1a} (8-OH-DPAT) em um modelo genético de ansiedade/emocionalidade, as linhagens de ratos LEWIS e SHR

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As linhagens isogênicas de ratos Lewis (LEW) e SHR (*spontaneously hypertensive rats*), que diferem em testes como o campo aberto (CA) e o labirinto em cruz elevado (LCE), constituem um importante modelo genético para o estudo de comportamentos relacionados à ansiedade/emocionalidade. Recentemente, o receptor 5-HT_{1a} vem sendo foco de grande atenção devido a sua participação em transtornos relacionados à ansiedade e a depressão. Então, nós avaliamos o efeito do agonista do receptor 5-HT_{1a} (8-OH-DPAT) sobre o comportamento dos animais LEW e SHR nos testes do CA e do LCE. Foram utilizados 60 machos (10/linhagem/tratamento) e 47 fêmeas (7-9/linhagem/tratamento) divididos em três grupos [salina (0.9%), 0,3mg/kg (A) ou 1,0mg/kg (B) de 8-OH-DPAT injetados via intraperitoneal]. Trinta minutos após o tratamento os animais foram submetidos ao CA e em seguida ao LCE. Os dados foram analisados pela ANOVA de 2-vias, separadamente por sexo, e em casos de interação o teste post hoc Newman-Keuls foi utilizado. No teste do CA, em machos, ocorreu uma interação para a medida locomoção central onde ambas as doses do agonista diminuíram a locomoção somente na linhagem SHR [salina 21,3±2,6; (A) 10,2±1,5 e (B) 15,0±2,9; p<0,05]. Em fêmeas, na medida locomoção central ocorreu um efeito geral de linhagem (LEW 4,7±0,9 e SHR 24,2±2,7; p<0,001) e tratamento onde as doses do agonista diminuíram a locomoção [salina 18,1±3,4; (A) 10,4±2,9 e (B) 13,5±3,9; p<0,05]. Na locomoção periférica ocorreu uma interação onde as SHR tratadas com a menor dose do agonista fizeram maior locomoção do que todos os outros grupos [salina 90,1±4,2; (A) 183,9±25,7 e (B) 99,0±18,3; p<0,001]. No teste do LCE, em machos, ocorreu uma interação na % de entrada nos braços abertos onde a dose mais alta do agonista aumentou a % somente nos ratos LEW [salina 17,6±3,1; (A) 37,5±10,8 e (B) 44,6±7,2; p<0,05]. Em fêmeas, ocorreu um efeito significativo de linhagem no tempo nos braços abertos [LEW 42,8±7,3 e SHR 88,2±11,5; p<0,01]. A sensibilidade diferente ao 8-OH-DPAT entre os machos LEW e SHR sugere que o receptor 5-HT_{1a} esteja participando na expressão diferencial do comportamento destas duas linhagens. **Palavras-Chave:** campo aberto, labirinto em cruz elevado, serotonina, farmacologia, ansiedade

Apoio Financeiro: CNPq

DESNUTRIÇÃO FETAL PROGRAMA A AÇÃO HIPOFÁGICA DA SEROTONINA.

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Objetivo: Existe importante relação entre o ambiente fetal e o controle do comportamento alimentar na vida adulta (1). O presente estudo objetiva avaliar a ingestão alimentar e a sequência comportamental de saciedade (SCS) em resposta a inibidor de recaptção da serotonina em ratos que foram desnutridos durante a vida fetal.

Métodos: Ratos da linhagem Wistar foram submetidos a dieta hipoproteica (D, caseína 8%) ou normoproteica (C, caseína 17%) durante o período de gestação. Aos 35 dias de vida, esses animais foram submetidos à administração aguda de fluoxetina (DF, CF, 10mg/Kg p.c.) ou salina (DS, CS, NaCl 0,9%). Foi realizada a análise da SCS após privação alimentar de cinco horas, com registro da duração (s) dos comportamentos de alimentação (CA), limpeza (CL), descanso (CD) e atividade (A), bem como a ingestão alimentar (g) durante 40 minutos (min). O declínio na duração do CA e o aumento do CD (min) indicam o ponto de aparecimento da saciedade (2).

Resultados No grupo D houve retardo ($p < 0.05$) no aparecimento da saciedade (30 min) comparado ao grupo C (27 min). Os grupos DF e CF apresentaram adiantamento ($p < 0.05$) no aparecimento deste ponto (CF= 25; DF= 20) comparado aos grupos CS e DS (CS= 27.5; DS= 35), com adiantamento mais intenso para os desnutridos. A ingestão alimentar foi maior ($p < 0.05$) nos grupos DS (2.62 ± 0.1) e DF (0.9 ± 0.1) comparado aos CS (1.6 ± 0.2) e CF (0.7 ± 0.1) respectivamente.

Conclusões: Os resultados deste estudo indicam a existência de uma programação fetal dos mecanismos de desencadeamento da saciedade em resposta ao ambiente nutricional. Nossos dados indicam alterações na ação hipofágica da serotonina em desnutridos.

Referências: 1- Eur. J. Neurosci. 1400-8, 2008 Mar; 2- Pharmacol Biochem Behav. 159-68, 1998.

Palavras-Chave: programação fetal, serotonina, comportamento alimentar

Apoio Financeiro: CNPq

EFEITOS DE 1,4-CINEOL EM MODELOS EXPERIMENTAIS DE SUSPENSÃO DA CAUDA, NADO FORÇADO E TEMPO DE SONO INDUZIDO POR PENTOBARBITAL EM CAMUNDONGOS.

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O objetivo do presente trabalho foi avaliar o efeito sedativo do 1,4-cineol no modelo de tempo de sono induzido por pentobarbital bem como seus efeitos em modelos comportamentais de depressão. O 1,4-cineol (eucaliptol) foi administrado nas doses de 100, 200 e 400 mg/kg, v.o., em camundongos Swiss (machos, 25-30 g, n =8). Os controles (C) receberam água destilada. Diazepam (1 mg/kg, i.p.) e imipramina (10 e 30 mg/kg, i.p.) foram utilizados como drogas padrão. Os resultados são apresentados como MÉDIA±ERRO PADRÃO com o n° de animais em parênteses.

Os resultados mostraram que no tempo de sono, o 1,4-cineol apresentou uma redução significativa na latência de sono de 11,6(100=183,9±6,68,n=14), 25,2(200=155,7±6,37,n=16) e 24,7 % (400=156,7±6,16,n=16) semelhante ao diazepam (182,6±8,66,n=9) quando comparados ao C (208,1±11,57,n=16). O 1,4-cineol apresentou uma tendência a aumentar a duração do sono (100=71±11,98,n=14; 200=71,6±9,81,n=14; 400=80,6±8,97,n=16) enquanto que o diazepam aumentou significativamente este parâmetro em 63,9 % (79,5±5,68,n=9) comparando com o C (48,5 ±8,07,n=15). No teste de suspensão da cauda o 1,4-cineol (400 mg/kg) causou um aumento significativo no tempo de imobilidade de 33,3 % (178,6±10,41,n=13) enquanto que a imipramina (30 mg/kg), droga antidepressiva, causou uma redução significativa de 80,4 % (26,2±4,60,n=10) comparados ao C (134,0±5,01,n=17). No nado forçado, o 1,4-cineol (200 e 400 mg/kg) causou um aumento significativo no tempo de imobilidade de 28,7 e 30,7 % (200=157,4±6,86,n=14; 400=159,8±6,47,n=12) enquanto que a imipramina (10 mg/kg) causou uma redução significativa de 85,1 % (18,2±2,70,n=8) comparados ao C (122,3±5,51,n=16).

Os resultados sugerem uma possível atividade sedativa e depressora central de 1,4-cineol nos modelos estudados. Entretanto, outros modelos experimentais serão executados para confirmar estes efeitos.

Palavras-Chave: 1,4-cineol; tempo de sono induzido por pentobarbital; modelos comportamentais de depressão.

Apoio Financeiro: CNPq

PARTICIPAÇÃO DO SISTEMA ENDOCANABINÓIDE NO APRENDIZADO É MEDIADA POR GLICOCORTICÓIDES.

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Introdução: A liberação de glicocorticóides em decorrência de um estímulo estressor gera uma enorme gama de alterações bioquímicas e fisiológicas, dentre elas está a liberação de endocanabinóides. Os receptores CB1 estão expressos em grande quantidade em todo o encéfalo, especialmente em regiões relacionadas com o aprendizado e memória, como o hipocampo, estrutura chave para a formação de memórias aversivas.

Objetivo: Os objetivos desse trabalho são verificar: (1) a participação do sistema endocanabinóide (SCB) na formação de memórias aversivas; (2) a participação do SCB na formação de memórias com componente menos aversivo; (3) se o SCB é recrutado somente em situações de estresse; (4) se a administração exógena de glicocorticóides recruta a participação desse sistema.

Métodos: Para tanto 83 ratos Wistar machos foram canulados bilateralmente em hipocampo dorsal; treinados e testados na tarefa de condicionamento aversivo ao contexto (CAC), no seguinte desenho experimental: (a) Choque Forte (0,7mA); (b) Choque Fraco (0,3mA); (c) Choque Fraco (0,3mA) com estresse agudo por nado forçado pré-treino; (d) injeção i.p de dexametasona (0,01 mg/Kg – dose facilitatória previamente estabelecida em nosso laboratório) ou seu veículo (etanol 2,5 % em salina). Todos os animais receberam 0,5ul (em cada cânula) de AM251(5.5 ug/lado) ou seu veículo(DMSO 8%) - injeção intra-hipocampal imediatamente pós-treino.

Resultados e Discussão: Nossos resultados mostram um efeito amnésico do AM251 nos experimentos (a) e (c) ($P=0,02$, *t-Student* ; $P= 0,026$, *one-way ANOVA* respectivamente) entretanto não houve efeito amnésico da droga no experimento choque fraco sem estresse (b) ($P= 0,751$, *t-Student*). No experimento (d) o AM251 foi amnésico no grupo que recebeu dexametasona ($P=0,01$, *one-way ANOVA*). Estes resultados sugerem que o sistema endocanabinóide pode estar modulando positivamente a formação da memória somente quando há ativação do sistema via glicocorticóides. Visto que o antagonista canabinóide AM251 só produz efeito amnésico na presença de um componente estressor forte (liberação de glicocorticóides) ou quando há administração de glicocorticóide exógeno.

Palavras-Chave: sistema endocanabinóide, Memória, Canabinóides, estresse, AM251, hipocampo, CB1, rato, dexametasona ,glicocorticóides.

Apoio Financeiro: CNPq, CAPES, FAPERGS, IFS

Akt levels modulated by typical or atypical antipsychotics in rat brains

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Introduction: Akt, also known as protein kinase B (PKB), is a protein involved in many cell functions such as the cell survival signaling pathway (1). When activated, Akt inhibits glycogen synthase kinase 3 beta (GSK-3b). It has been shown that Akt levels are decreased on the prefrontal cortex (PFC) of schizophrenics. Recently, it has been demonstrated that D2 dopamine receptors, the main target of typical antipsychotics, inhibit the phosphorylation of Akt (2). Also, it is well known that serotonin receptors, which are targets of atypical antipsychotics, modulate Akt/GSK-3b signaling.

Objectives: The main aim of this study was to evaluate the effects of chronic treatment with typical and atypical antipsychotics on Akt expression on rat PFC and hippocampus.

Methods: Male Wistar adult rats were treated with typical (Haloperidol - 1.5 mg/kg) and atypical (Clozapine - 25 mg/kg, Olanzapine - 2.5, 5 or 10 mg/kg or Aripiprazole - 2, 10 or 20 mg/kg) antipsychotics in a 1.0 ml/kg volume for 28 days. Prefrontal cortex and hippocampus extracts were prepared with lysis buffer and samples were submitted to electrophoresis and transferred to a nitrocellulose membrane. Membranes were incubated with antibody anti-Akt (Cell signaling 9272) and analyzed using the enhanced chemiluminescent (ECL) detection. The results were analyzed using the ImageJ software that quantificated the pixels of the images. The numerical results were then analyzed using the Sigma Plot software.

Results: Data evidenced decreased levels of Akt expression in PFC and hippocampus of rats treated with Ari. Also, levels of Akt in hippocampus were dose-dependent downregulated in both PFC and hippocampus of rats treated with Olz. There were no alterations in Akt expression in PFC and hippocampus of rats treated with Hal and Clz.

Conclusion: Our results suggest that atypical antipsychotics Ari and Olz may modulate Akt expression.

References:

1 - Genes & Dev. 13:2905, 1999

2 – Cell. 122:261, 2005

Palavras-Chave: Akt, Schizophrenia, antipsychotics

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Tryptophan administration induces oxidative stress in brain cortex of rats

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Objectives: Is possible that free radicals generation may be associated to brain damage in hypertryptophanemia and other neurodegenerative diseases. In the present work, we determined the thiobarbituric acid-reactive substances (TBA-RS), 2',7'-dihydrodichlorofluorescein oxidation (DCFH), the antioxidant enzyme catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in cerebral cortex from rats treated with tryptophan (Trp) and pretreated with the well known antioxidants taurine or α -tocopherol (vitamin E) plus ascorbic acid (vitamin C). **Methods and Results:** Male Wistar young rats received three subcutaneous injections of Trp (2 mmol/g of body weight) in one day at 3 h intervals or the same volume of saline (30 ml/g body weight) of 0.85% NaCl. The animals were killed 1 h after the last injection by decapitation and the brain was rapidly removed for the determination of the parameters of oxidative stress. First, we investigated the effect of Trp administration on some oxidative stress parameters in brain cortex. Trp administration significantly increased TBA-RS ($t_{(12)} = 2.29$; $p < 0.05$), DCFH oxidation ($t_{(12)} = 4.43$; $p < 0.001$), GSH content ($t_{(12)} = 2.29$; $p < 0.05$), and reduced catalase activity ($t_{(12)} = 6.19$; $p < 0.001$). Next, we investigated the effects of pre-treatment with taurine (1,6 mmol / g of body weight) and/or vitamins C plus E (40 mg/g and 100 mg/g body weight, respectively) with Trp on the parameters of oxidative stress. Trp by taurine interaction was significant for TBA-RS ($F(1,16) = 25.57$; $p < 0.001$), DCFH oxidation ($F(1,24) = 3.94$; $p < 0.05$), GSH content ($F(1,20) = 16.09$; $p < 0.001$), and CAT activity ($F(1,16) = 8.32$; $p < 0.01$). Trp by vitamins interaction were significant for TBA-RS ($F(1,20) = 4.14$; $p < 0.05$), GSH content ($F(1,20) = 16.09$; $p < 0.001$), and CAT activity ($F(3,24) = 6.96$; $p < 0.01$), but not for DCFH oxidation ($F(1,16) = 0.38$; $p > 0.54$). **Conclusion:** Our results show that Trp administration causes oxidative stress, an imbalance between free radical content and the capacity of the tested scavenging systems, in the cerebral cortex. Oxidative stress may be induced by Trp and/or some of its metabolites.

Palavras-Chave: Tryptophan; Oxidative Stress; Antioxidant Defenses

Apoio Financeiro: CNPq-Brazil, FAPERGS, RS-Brazil, PRONEX-CNPq /FAPERGS –Brazil, IBN-Net, FINEP

Neuroprotective effects of FrPbAII isolated from the venom of the spider *Parawixia bistriata* (Araneae: Araneidae) in Wistar rats submitted to the pilocarpine-induced *Status Epilepticus*.

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Objective

This work was aimed at the investigation of the neuroprotective effects of FrPbAII [1] on hippocampal cells of rats submitted to pilocarpine(pilo)-induced *Status Epilepticus* (SE).

Material and Methods

Male Wistar rats (230-250g; n=5, each group) were surgically implanted in the right ventricle with a guide cannulae. After recovery, animals were injected with Pilo (2.4 mg; 1 μ L) in order to induce a SE, which was characterized by seizures of class 5 from Racine scale [2]. 3 hours after seizure onset, a single injection of sodium thiopental (35 mg/kg, i.p.) was administered in order to attenuate SE, and one hour later, treatment was initiated. Groups of animals received saline (SAL; 0.9%), nipecotic acid (NIP; 92.9 μ mol); riluzole (RIL; 10 μ mol) or FrPbAII (0.43 and 0.86 μ mol). 24 hours later, animals were sacrificed, cardially perfused with saline and paraformadheyde, brains were removed and placed in fresh fixative for further histological treatment. Brains slices stained with cresyl violet were analyzed and images of CA1, CA2 and DG of hippocampal formation were captured for neuronal density estimates (nr of cells/mm²). We used Ambergrombie correction to normalize data and One way ANOVA followed by Student Newman-Keuls as statistical tests.

Results

Histopathological analysis revealed pyramidal layer disorganization, gliosis and extensive cell loss in the hippocampal formation of untreated animals (SAL). Sections of animals treated with FrPbAII (0.43 μ mol), RIL and NIP presented density of viable neurons significantly higher than SAL animals in CA1 [$F_{(5,283)}=269.9$; $p<0.0001$], CA3 [$F_{(5,283)}=224.9$; $p<0.0001$] and DG [$F_{(5,283)}=1160$; $p<0.0001$]. Rats treated with FrPbAII in the dose of 0.86 μ mol presented the highest neuronal densities in brain slices.

Conclusions

A relevant dose dependent neuroprotective effect of FrPbAII was observed after a long lasting SE, which was more potent than riluzole and nipecotic acid. These findings indicate that FrPbAII may prevent neuronal damage and thus function as a powerful tool in neuroscience research.

References

[1.]Mol Pharmacol 69:1998; 2006; [2.]Eletroencephal Clin Physiol 32: 381;1972. **Palavras-Chave:** Parawixia bistriata, FrPbAII, epilepsy, neuroprotection, GABA

Apoio Financeiro: CAPES, FAPESP

LONG-TERM EFFECT OF MORPHINE ADMINISTRATION IN YOUNG RATS ON THE ANALGESIC OPIOID RESPONSE IN ADULT LIFE.

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Objectives: Neonates, infants and children are often exposed to pain from invasive procedures during intensive care and during the post-operative period. Opioid anesthesia and post-operative opioid analgesia have been used in infants and result in clinical benefits. The objectives of this study were to verify the effect of repeated 5µg-morphine administration (subcutaneous), once a day for seven days, at P8 until P14 on the analgesic response. To verify the long-term effect of morphine on the analgesic response, the animals were submitted a second exposure of 5mg/kg (intraperitoneal) of morphine at P80 until P86.

Methods: male Wistar rats aged 8-days old at the beginning of the experiment were used. The rats were divided into two groups: saline-control (n=9) and morphine-treated (n=7). Each animal received saline or morphine (5 µg s.c. in the midi-scapular area) at P8, once a day for seven days. For the long-term effect experiment, animals of both groups were subdivided at P80 into control saline/saline (n=7), control morphine/saline (n=10), saline/morphine (n=10) and morphine/morphine (n=11) and received saline or morphine (5 mg/kg, i.p.) at P80 once a day for seven days. Nociception was assessed, for both experiments, with the tail-flick apparatus (TFL) before (basal), immediately 30, 60, 90 and 120 minutes on first and seventh day of which treatment. **Results:** At P14, the analgesic effect was observed until 90 minutes (30 min: C=4.5± 0.74; M=9.5±1.43; 60 min: C=4.8±0.5; M=10; 90 min: C=4.8±0.28; M=8.6±1.9; repeated measures ANOVA, *P*<0.05). At P86, after seven days of daily morphine administration the morphine analgesic effect was not observed in either of the groups (Bonferroni test, *P*>0.05).

Conclusions: Ours results showed that tolerance to repeated injections of morphine in pups is less pronounced than in adults. It may be suggested that the opioid analgesia in P14 animals occurs because the opioid system is under the maturation process during the treatment period. These findings may have important implications for the human neonate, suggesting a possible explanation for the differences in morphine requirements observed in the youngest patients.

Palavras-Chave: analgesia; morphine; tolerance; young rats

Apoio Financeiro: CNPq, FAPERGS and PROPESQ/UFRGS

Behavioral changes and adenosine receptors density modifications in different brain structures of adult mice treated with Methylphenidate (Ritalin®)

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Adenosine (ADO) is a Central Nervous System (CNS) neuromodulator, which regulates synaptic transmission and neuroexcitability through its A₁ and A_{2A} receptors. Methylphenidate (MPH, Ritalin®) is a drug prescribed for attention deficit hyperactivity disorder (ADHD), being used by teenagers and adults to increase cognitive performance. A number of neurotransmission systems are related to MPH action mechanism, especially dopaminergic system, but little is known about the impact of MPH treatment on adenosinergic system. OBJECTIVE: to verify whether adult mice treated with MPH present behavioral changes and modifications on ADO receptors density. METHODS: Adult mice were treated for fifteen days with MPH (5mg/kg, i.p., one dose daily). Afterwards, they were submitted to elevated plus maze, to evaluate anxiety behavior, and also to inhibitory avoidance and object recognition task, both of which evaluate learning and memory process. Brain structures (hippocampus and frontal cortex) were then dissected and housed in 5% SDS for A₁ and A_{2A} immunocontent analysis by Western blot. RESULTS: There was no difference neither on long-term memory nor on recognition memory, comparing treated and control groups. However, MPH increased the number of rearings compared to control group (14.33±1.34 vs 6.07±1.64, respectively, p=0,0037) and also increased the number of entries on the open arms (4.93±0.47 vs 3.64±0.45, respectively, p=0,047) in elevated plus maze. A₁ receptor immunocontent of frontal cortex of mice treated with MPH did not differ from that of control group, whereas this immunocontent was increased in the hippocampus of treated mice compared to control ones (14.57±1.17 vs 10.11±0.81, respectively, p=0,047). CONCLUSION: Mice treated with MPH for 15 days did not present any alterations on learning and memory process, while it seems to increase exploratory activity, the latter shown in elevated plus maze. Given the important role of CNS neuromodulators, the increase of A₁ receptor immunocontent in the hippocampus presented on this work suggest the involvement of ADO in the effects of MPH on CNS.

Palavras-Chave: methylphenidate; adenosine receptors; elevated plus maze; inhibitory avoidance; object recognition task; western blot; hippocampus.

Apoio Financeiro: CNPq, FAPERGS, BIC/UFRGS, PROPESQ, CAPES, LOreal Grants, IBN-Net.

Fatores genéticos e ambientais envolvidos com o consumo de etanol e seus possíveis correlatos comportamentais nas linhagens de ratos Lewis e SHR

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Estudos clínicos sugerem que existam diversas comorbidades presentes conjuntamente com o alcoolismo. As linhagens de ratos Lewis (LEW) e SHR diferem em relação a diversos modelos de ansiedade e de consumo de etanol. Assim, neste estudo nós avaliamos alguns fatores comportamentais e farmacológicos que podem estar envolvidos com o consumo de etanol nestes animais. Desta maneira, 44 ratos LEW e SHR (11/linhagem/sexo), isolados em gaiolas individuais com 8-9 semanas de idade, foram submetidos aos testes comportamentais da busca pela novidade (BN); campo aberto (CA); labirinto em cruz elevado (LCE); armazenamento de comida (AC); consumo de sacarina, quinino e etanol; e perda do reflexo postural (PRP) em resposta ao etanol, com coleta de sangue na recuperação do reflexo. Os ratos LEW e SHR diferiram: na BN no tempo gasto no compartimento familiar (SHR 352,8±23,6; LEW 274,9±26,7; p<0,05), no CA na locomoção (SHR 15,4±2,2; LEW 6,6±1,1; p<0,001) e tempo gasto (SHR 35,1±3,9; LEW 10,3±1,2; p<0,001) no centro, no LCE no número de entradas nos braços abertos (LEW 3,9±0,6; SHR 1,8±0,4; p<0,001) e fechados (LEW 10,5±0,6; SHR 7,8±0,5; p<0,001), no AC no armazenamento (SHR 7,9±0,6; LEW 5,8±0,7; p<0,05) e ingestão de ração (SHR 3,1±0,2; LEW 2,5±0,2; p<0,05), no consumo de sacarina (SHR 149,6±6,9; LEW 57±3,5; p<0,001), quinino (SHR 31,7±3,4; LEW 19±2,3; p<0,01), etanol forçado (SHR 39,7±4,8; LEW 26,9±3,8; p<0,05) e etanol 10% em livre escolha (SHR 8,4±1,1; LEW 4,7±0,9; p<0,01). No PRP, fêmeas recuperaram o reflexo mais rápido (F 17197,6±634,9; M 30982,2±2530,9; p<0,001) e com maior concentração de etanol no sangue do que machos (F 5,0±0,3; M 3,5±0,3; p<0,01), mas não ocorreram diferenças entre as linhagens. Ratos LEW pareceram mais “ansiosos” que ratos SHR no CA, mas não no LCE, como seria esperado. No teste do AC, ratos LEW pareceram mais impulsivos que ratos SHR. Os resultados inesperados nos testes BN e LCE sugerem que o isolamento social pode ter influenciado o comportamento destas linhagens. O alto consumo de etanol em ratos SHR pode estar associado a maior preferência por ingestão de líquidos inéditos.

Palavras-Chave: rato, linhagens, consumo álcool, ansiedade

Apoio Financeiro: CNPq

I.01.001

Os Diversos Conceitos Sobre Mente e Cérebro Em Algumas Civilizações Antigas

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OBJETIVOS: O estudo das relações entre o cérebro e a mente não é recente. Desde a pré-história até os dias atuais, os mais variados questionamentos a respeito da localização das funções da mente humana foram feitos. De fato, diversas civilizações antigas desenvolveram teorias com diferentes graus de complexidade acerca desta questão. O presente estudo apresenta uma breve reflexão crítica acerca dos indícios que sugerem uma possível importância atribuída ao cérebro pelos hominídeos assim como no homem pré-histórico. Em seguida, apresentam-se as principais as idéias acerca do debate corpo-mente desenvolvidas pelas antigas civilizações egípcia, mesopotâmica, indiana e chinesa. **METODOLOGIA:** Uso de uma revisão sistemática da literatura e uma descrição das perspectivas encontradas nestas civilizações. **RESULTADOS:** Observa-se a existência, dentro de seu contexto histórico-cultural, um conhecimento próprio acerca da relação entre a mente e o corpo, onde há uma preocupação em se estabelecer um esboço de uma teoria da mente e de como esta mente interage com o seu corpo. Ao mesmo tempo, vemos as primeiras observações humanas sobre as funções do cérebro e sua importância para os processos mentais. Entretanto, o conhecimento anatômico e funcional do corpo apresentado por estas civilizações fica limitado devido à falta de maiores explorações e análises sistemáticas do corpo humano. Além disso, há uma associação entre o conhecimento “científico” e a prática religiosa, onde cada civilização, à sua maneira, procurou responder as questões mais fundamentais através de seu próprio pensamento. **CONCLUSÃO:** Conceitos sobre o corpo, a mente (ou alma), doença e saúde se entrelaçam com conceitos religiosos e culturais de cada povo. Ao mesmo tempo, é interessante observar que estes conjuntos de conhecimento surgem de forma independente. Apesar de uma relativa proximidade física entre o Egito, a Mesopotâmia, a Índia e a China e de uma constante troca entre eles, cada um apresenta seu escopo de conhecimento de forma própria.

Palavras-Chave: Relação Mente-Corpo; Civilizações Antigas; História da Neurociência

Apoio Financeiro: FAPERJ

AS CONTRIBUIÇÕES DE PIERRE PAUL BROCA PARA AS NEUROCIÊNCIAS

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Objetivo: Discutir as contribuições de Pierre Paul Broca para a história das neurociências. **Métodos:** Revisão da literatura — utilizando as bases de dados BVS (*Biblioteca Virtual em Saúde*), SCIELO (*Scientific Electronic Library Online*) e PUBMED (*U.S. National Library of Medicine*), bem como livros-texto da área de neurociências — e leitura / reflexão crítica sobre os textos obtidos. **Resultados:** O anatomista, antropólogo e cirurgião francês Pierre Paul Broca foi pioneiro nos estudos sobre as localizações funcionais do córtex cerebral. Iniciou seu trabalho em 1861, correlacionando o fenômeno clínico da afasia de expressão com o achado patológico de lesão da porção posterior do giro frontal inferior (1). Esta região posteriormente foi denominada de área de Broca, sendo essa nomenclatura até hoje utilizada. As investigações do cientista desencadearam o interesse de outros estudiosos que passaram a relacionar as demais regiões do cérebro com as respectivas funções, servindo de base para a consolidação das neurociências como área do saber dedicada à elucidação dos processos neurais (2). **Conclusões:** Um dos principais intuitos das neurociências é o de compreender como o cérebro produz a admirável individualidade da atividade humana e, sendo a linguagem o veículo de comunicação entre os humanos, que permite a interação interpessoal, exteriorização de sentimentos, descrição de sensações, o empenho de Broca foi primordial para o cumprimento da principal tarefa da neurociência, que é a de fornecer explicações do comportamento com base na atividade cerebral. **Referências:** 1- Arq Neuro-Psiquiatr 58:1149, 2000; 2- Rev Bras Edu Méd, 31:131-132, 2007.

Palavras-Chave: história, neurociência, Broca.

I.02.001

Importância da associação teoria-prática e da colaboração de monitores para a aprendizagem em Neurofisiologia.

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Objetivo: Avaliar a aceitação e eficiência de um curso de Neurofisiologia baseado em aulas teóricas associadas com aulas práticas e apoiado por alunos de pós-graduação.

Metodologia: Foram sujeitos 25 alunos do 2º semestre do curso de Fisioterapia da Universidade de São Paulo no ano de 2007. Todas as 14 aulas de Neurofisiologia consistiram de uma parte teórica ministrada pelo docente, seguida de uma parte prática, conduzida pelos monitores do Programa de Aperfeiçoamento de Ensino (PAE). Na parte teórica foram utilizados recursos áudio-visuais e apresentações de textos curtos pelos alunos. Na parte prática foram incluídas simulações em computador, representações teatrais, e experimentos em que os próprios alunos eram testados. Ao final da parte prática os alunos respondiam algumas questões relacionadas com as observações que haviam feito. Ao final do curso foi aplicado aos alunos um questionário com 14 questões objetivas. Duas delas com resposta direta, nove com respostas entre pouco a muito variando de 1 a 6, e três com alternativas.

Resultados: A nota final média dos alunos para as 2 provas dissertativas e para os relatórios de aulas práticas foi 7,2 ($\pm$ 0,2, epm). Cem por cento dos alunos consideraram o aprendizado de neurofisiologia importante para sua formação profissional. Sessenta e cinco por cento afirmou ser desnecessário a utilização da experimentação animal, 73% concordou haver coerência das aulas práticas com as teóricas, 62% aprovou o tempo dedicado às aulas práticas, 92% observou relação entre as questões das provas e as das aulas práticas, e 96% afirmou que as aulas práticas auxiliaram na realização das provas. Apenas 4% considerou pequena a contribuição das aulas práticas para seu aprendizado. Noventa e seis por cento considerou valiosa a colaboração dos monitores no curso. Em média os alunos atribuíram a nota 7,4 para o seu aproveitamento nas aulas práticas. Controle da atividade muscular, sensibilidade proprioceptiva e ciclo vigília-sono foram as aulas práticas respectivamente mais coerente com a aula teórica, mais interessante e mais difícil de discutir.

Conclusão: A metodologia utilizada mostrou-se eficiente para a aprendizagem de Neurofisiologia. Ela foi muito bem aceita pelos alunos, que em sua maioria relataram maior facilidade no aprendizado do conteúdo do curso. Além disso, os alunos consideraram útil a presença de monitores nas aulas práticas para dinamizar as discussões relativas aos experimentos realizados.

Palavras-Chave: Neurofisiologia, aprendizagem

Apoio Financeiro: Reitoria da Universidade de São Paulo, CAPES, CNPq e FAPESP.

I.02.002

NEPENC: RECURSOS PARADIDÁTICOS INCLUSIVOS EM NEUROPSICOLOGIA COGNITIVA

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TEMA: Produção de recursos paradidáticos inclusivos para o ensino e a divulgação da neuropsicologia cognitiva, ramo multidisciplinar da psicologia que se dedica a compreender como a atividade neural transformando-se em abstratas representações mentais dá origem e desenvolve funções cognitivas tão complexas e elaboradas como as que caracterizam a mente humana (pensamento, linguagem, sono, sonhos, motricidade voluntária, memórias, afetos, aprendizagem).

OBJETIVOS: O Núcleo de Estudos e Pesquisas em Neuropsicologia Cognitiva (NEPENC) do CCH da UENF tem como principais objetivos 1- a criação de um método de ensino multisensorial que possibilite aos alunos, de variadas formações acadêmicas e diferentes condições cognitivas, adquirirem de forma rápida e eficaz os conhecimentos neuropsicológicos indispensáveis a todos aqueles que atuam ou pretendem atuar na área das neurociências e 2- estimular a divulgação desta metodologia.

MÉTODOS: Fundamentado na força do trabalho coletivo e no espaço participativo de ação, o NEPENC criou diferentes espaços interacionais ou oficinas nas quais suas ações são desenvolvidas (Oficina de Objetos Anatômicos; Exposições de Objetos Anatômicos (exposições temáticas didáticas); Oficina de Apresentações Temáticas Áudio-Visuais; Oficina de Textos Inéditos (cartazes e apostilas) e de Traduções; Oficina de Folhetos em Literatura de Cordel; Ciclo de Debates). Através destas ações, Os participantes transformam abstratos e complexos saberes neuropsicológicos em atraentes fáceis objetos de estudo.

CONCLUSÃO: A metodologia aqui proposta tem otimizado a compreensão e a expressão de conhecimentos através da função internalizadora e recontextualizadora da cognição, possibilitando que indivíduos leigos e especialistas no tema, íntegros ou privados sensoriais, captem e recriem em suas mentes, as muitas vezes, imperceptíveis e complexas estruturas e funções neuropsicológicas cognitivas.

Palavras-Chave: Representação mental, Paradidático, Inclusão

O ENSINO DE NEUROCIÊNCIAS COMO FERRAMENTA DE CAPACITAÇÃO DOCENTE PARA PRÁTICA PEDAGÓGICA DE QUALIDADE.

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Cursos de docência contemplam didática geral e práticas, mas os conhecimentos resultantes de pesquisas em neurociências não aparecem em licenciaturas ou mestrados e doutorados, que dão acesso à docência superior. Como aprendizagem é assunto do cérebro, entende-se que professor completo é quem conhece tais regras e as usa em favor da aprendizagem do aluno. Para preencher tal lacuna, incluiu-se a disciplina Cérebro, Cognição e Aprendizagem ao currículo de Docência em Educação Superior, da Pós-graduação Newton Paiva, BH/MG. Entre os objetivos da inovação curricular estão oferecer aos futuros docentes oportunidade de (re)conhecer os processos mentais no ser humano, estabelecer relações entre processos mentais e aprendizagem educacional, aplicar conhecimentos neurocientíficos às atividades de aula e conhecer razões neuropsicológicas para problemas escolares e de aprendizagem, para orientar adequadamente cada aprendiz. A metodologia enfatizou o protagonismo do futuro docente, co-responsável pelas aulas, apresentando o estado-da-arte de temas, tais como sensação, percepção, atenção, memória, utilização de múltiplas inteligências e estilos pessoais em atividades pedagógicas, assim como a caracterização de dificuldades escolares, transtornos e distúrbios de aprendizagem e como ajudar indivíduos com desenvolvimento acadêmico comprometido por tais condições. As aulas foram espaços de discussões fundamentadas em leituras prévias e observações de campo orientadas por perspectiva interdisciplinar, com conteúdos integrando eixos temáticos e atividades complementares. Os resultados das avaliações da disciplina, feitas pelos alunos, confirmou que o ensino de neurociências é um fator motivacional na formação de professores e que a inclusão da disciplina Cérebro, Cognição e Aprendizagem representou valor significativo também no desenvolvimento pessoal desses profissionais. Conclui-se que ensino de neurociências em cursos de formação de docentes fortalece as competências para os papéis essenciais que deve representar o Professor-Educador, que precisa do ensino das neurociências para ser melhor mediador do conhecimento e criador de situações mais propícias para a aprendizagem.

Palavras-Chave: Neurociências. Ensino. Capacitação Docente. Qualidade Pedagógica. Aprendizagem.

I.02.004

Produção e divulgação de material para-didático em neuropsicologia cognitiva

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O presente trabalho se caracteriza por trazer a indissociabilidade entre o ensino, a pesquisa e a extensão, na medida em que se alimenta da interação do conhecimento acadêmico/científico/filosófico com o conhecimento comunitário em suas diferenças cognitivas, afetivas, sociais, políticas e culturais.

A proposta é transformar saberes múltiplos e complexos de neuropsicologia cognitiva em material paradidático de atraente e fácil compreensão. Tem como públicos alvos tanto profissionais quanto leigos, das mais diversas faixas etárias, sensorialmente íntegros ou não (cegos e surdos); possibilitando assim a inclusão de todos.

A metodologia utilizada neste projeto é a metodologia participativa. Fundamentando-se então no trabalho coletivo, a pesquisa lançou mão de diferentes espaços interacionais e de oficinas para desenvolver as suas ações. Através delas um processo participativo de troca se instaura entre o espaço acadêmico e o comunitário. Logo, são firmadas parcerias com instituições educacionais, de saúde e outras de apoio comunitário.

Um dos objetos produzidos na oficina de objetos anatômicos, por exemplo, foi o “Bebê Sensorial”. Com uma estrutura tátil-visual baseada no “Homúnculo de Penfield”, permite melhor compreensão do processo maturacional e a entrada em ação das diferentes modalidades sensoriais durante o desenvolvimento cognitivo do ser humano. Este estudo revela-se importante tendo em vista que ao se adiantar ou retardar, artificialmente, o aparecimento de uma determinada modalidade sensorial, o desenvolvimento natural das modalidades subseqüentes é prejudicado.

Observa-se após a apresentação deste, ou de tantos outros objetos, o despertar da “curiosidade investigativa” no público alvo, levando assim a busca de novos conhecimentos. É perceptível ainda a divulgação dos conhecimentos adquiridos por eles em conversas informais com as pessoas de seu convívio social.

Palavras-Chave: ensino; neuropsicologia cognitiva; material paradidático; modalidade sensorial

Apoio Financeiro: PIBIC-UENF

Real-life stories in neuroscience education: evidence based neuroanatomy through clinical cases.

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Although written and web-based text plays a central role in education, many people are visual learners and are able to assimilate information better by observing images. The present video in neuroscience education is dedicated to teach evidence-based neuroanatomy through clinical cases to demonstrate that in addition to usual lectures and technical material, social and environmental context of real life patient's increases motivation to learn and stimulate compassion in medical education. The video sequence is dedicated to learn neuroanatomy of the motor pathways and illustrate brain plasticity after an accidental brain damage induced by trauma. KJC is a young Amazonian boy that was walking under Brazil nuts tree (*Bertholletia excelsa*, large tree, reaching 30–45 [metres](#)) when a large capsule 10–15 centimeters diameter resembling a [coconut endocarp](#) in size and weighing up to 2 [kilograms](#) hit his head. Three dimensional reconstruction of the fractured head bones after serial tomography, magnetic resonance images of the damaged area, animations and video images of the triplegia in different time windows, were documented side by side with social, affective and environmental aspects of KJC's life. Prior to filming, a story-board was developed to outline the scenes and filming sequence. A 6-minute video was produced for presentation at the Neurosciences Latin American international conference. The video will be subsequently placed on an institutional intranet web site and uploaded to opened access websites, where it could be reviewed by students and medical teachers. Evaluation of this approach is presently in course, but preliminary data reveals that increased student motivation may be the main reason to explain higher interest in neuroscience learning.

Apoio Financeiro: Research Funds: CNPq, CAPES, FINEP/IBN-net, UFPA.

I.02.006

Microelectrode brain implant surgery 3D incursion

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Stereopsis is an important component of binocular visual function because it really enables performance of tasks requiring accurate depth perception, such as performing microelectrode implant surgery. Developing a 3D incursion material enable students to experience some circumstances formerly possible only in actual practice.

Two video-cameras recorder (GZ-MG21U) were aligned allowing a red-cyan anaglyph could be color computed (red component is the green channel of the left stereo pair and the green and blue components are the green channel of the right stereo pair) to record a microelectrode surgery implantation in a rat motor cortex. All procedures were approved by the local Ethical Committee and performed in accordance with NIH guidelines. A Wistar rat, 3 months old, 300g, was used to implant a microelectrode of 4 by 8 Teflon coated tungsten sharp microwire electrodes (50mm diameter separated by 300µm) in the left motor cortex (1-3mm rostral to bregma, 2-3 mm lateral to bregma, 1.8-2.0 mm depth for layer V of M1). Rat was anesthetized with Ketamine (100mg/kg) and Xylaxine (5mg/kg) following induction with Halothane.

The 3D incursion movie allowed students experience more than the surgery procedure, but they could be aware with depth details of micro scale during surgery. The perception of differences among physical rules, present in this micro scale world involved in a microelectrode brain penetration, supports students in the next understandings steps: brain layers and neuron cell potential, neuron spikes and motor action, thus, from the micro brain function to the macro mind action.

Palavras-Chave: anaglyph, stereopsis, microelectrode, neurosurgery, brain

Apoio Financeiro: Alberto Santos Dumont for Research Support, Sírio Libanês Hospital, FINEP, CNPq, DUKE

Students' misconceptions on synapse and neurotransmission

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Introduction: Students' understanding of many physiological phenomena is often seriously flawed. That is, students have faulty mental models of many of the things we ask them to learn. Such conceptual difficulties are often referred to as misconceptions. The problem with misconceptions is that they are often quite persistent, and they seriously interfere with the students' ability to learn physiology (1). We detected that graduate students from two distinct groups refers to the synapse as a transmission of the nervous impulse, from one neuron to the next. The aim of the present work was to quantify the occurrence of this misconception among students in some neuroscience post graduation courses.

Procedures: The detection was made asking post graduate students to comment on a serious conceptual flaw in an excerpt from. The excerpt was presented to students enrolled in two post postgraduate courses, namely Physiology Teaching (**n=10**) and Attention & Memory (**n=34**).

Results: Of 34 students enrolled in the Attention & Memory course, only five identified the flaw in the excerpt. The rest (**29**) declared they considered the text as correct or had no serious objections. Of 10 students enrolled in the Physiology Teaching course only two students detected the flaw in the excerpt. The rest (**8**) referred to the text as correct or had no serious objection to the misconception that there is no transmission of the potential at chemical synapses.

Discussion: Possible origins of this misconception are discussed and the result is consistent with problems even detected with membrane potential concept (2). Traditionally established terms, such as synaptic transmission, and language in textbooks are potential sources for misconceptions.

References: 1 - Advan Physiol Educ 26:5-6, 2002; 2 - Advan Physiol Educ 26:69-71, 2002.

Palavras-Chave: Misconceptions, Synapse, Neurotransmission, Conceptual Change

Apoio Financeiro: CNPq

AValiação DE GANHO DE CONHECIMENTO NUM CURSO DE NEUROCIÊNCIAS

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Introdução: Este trabalho evidencia a importância de novas tecnologias na avaliação de ganho de conhecimento em atividades de cursos de Neurociências. O que foi mostrado pelo estudo realizado com o programa Stalex nos textos dos participantes do I Curso latino-americano de Neurociências: Fundamentos Morfofuncionais, Divulgação Científica e Inclusão sócio-Cultural realizado no Instituto de Ciências Biomédicas III da USP em janeiro de 2007, com carga horária de 90 horas. **Objetivo:** Avaliar os discursos escritos num curso de Neurociências identificando o ganho de conhecimento. **Metodologia:** A avaliação foi feita pela performance escrita das redações pré e pós-curso do tema: Curso Latino-Americano de Neurociências: Fundamentos Morfofuncionais, Divulgação Científica e Inclusão Sócio-Cultural, n=21. Para a análise lingüística dos textos foi utilizado o programa matemático-estatístico-computacional Stalex, que analisa o texto em suas variáveis lexical e discursiva. O grupo foi constituído, na sua maioria, por estudantes da área da saúde e quatro de humanas (Lingüística, Música História e Pedagogia). Os estudantes foram continuamente encorajados por meio de palestras, aulas teóricas e práticas a pesquisar e entender os princípios subjacentes das várias funções do Sistema Nervoso. **Resultados:** A avaliação efetuada pelo programa Stalex evidencia 50 % de ganho de conhecimento, pela variável lexical dos substantivos, principalmente nos textos pós-curso, o que indica aumento na frequência dos itens lexicais (palavras) utilizados anteriormente e surgimento de novos itens de valor semântico em clara indicação de aquisição de aprendizagem. **Conclusão:** Este estudo evidencia ganhos de conhecimento com a atividade desenvolvida e que a análise do léxico, apoiada ao programa Stalex é ferramenta útil para avaliar atividades didáticas, da mesma forma, permite distinguir as dificuldades de ganho.

Palavras-Chave: Ensino, Neurociências, aspectos lexicais

Apoio Financeiro: Master Note Informatica

Textbook phrasing as a potential source of misconceptions on synapse

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Introduction: Misconceptions are concepts held by people that are at odds with current scientific understanding. The problem with misconceptions is that they are persistent, and interfere with the students' progress in their learning of physiology [1]. We detected that two distinct groups of graduate students considered that at synapses the action potential is transmitted from one neuron to the next. The aim of the present work is to suggest one possible origin of this misconception, in the choice of terms in current textbooks of physiology and neurosciences.

Procedures: We selected books according to the follow criteria: (1) release date since year 1998, (2) availability to our students in the university libraries, (3) official recommendation of the textbook in at least one undergraduate physiology course. Our analysis aimed at: (1) identifying the section where the concept of synapse is explained, (2) analysing the definition of synapse, (3) comparing this concept with the current neuroscience literature.

Results: In general (1) the books do not approach the subject directly and do not provide an explicit definition, (2) often the word synapse does not specify if what is addressed is the anatomical structure or the processes that occur there, (3) the word transmission is used, which in our understanding is misleading, (4) books leave room to the interpretation that all synapses are basically the same, with differences only in detail (which neurotransmitter or receptor), (5) a one-way description is presented, going from pre to postsynaptic.

Conclusion: With this in view we conclude that textbooks may be hindering learning by maintaining traditionally established terms, such as synaptic transmission, in spite of the fact that this leads to wrong interpretations.

References: 1. Advan Physiol Educ 26:5-6, 2002.

Palavras-Chave: Misconceptions, Synapse, Textbook

Apoio Financeiro: CNPq

I.03.001

Neurônios-espelho e o conceito de imitação em Vigotski: aproximações entre neurociência e psicologia histórico-cultural

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Este trabalho objetiva articular a teoria vigotskiana a conceitos da neurociência, apontando sua atualidade. Vigotski buscou a construção de uma psicologia marxista, orientando-se metodologicamente pelo materialismo histórico-dialético. Criticou seu contemporâneo Pavlov, pois não acreditava em uma psicologia materialista limitada ao comportamento observável. Insistiu no papel central do indivíduo historicamente construído, singularizado a partir da progressiva e dialética internalização das relações sociais.

Propõe-se que é possível traçar um instigante paralelo entre sua teoria e algumas descobertas neurocientíficas. Particularmente, os NE, descobertos por Rizzolatti e colaboradores na década de 90. Esses neurônios, localizados na área pré-motora de macacos Rhesus, eram ativados tanto em situações de realização de movimentos quanto de observação. Estudos subseqüentes com humanos apontaram para a existência nestes de uma funcionalidade semelhante, envolvendo particularmente o aprendizado da linguagem.

Descobertas deste tipo são importantes na compreensão da aprendizagem, ressaltando o papel determinante da observação e da imitação para a construção simbólica humana. Tal importância foi devidamente enfatizada por Vigotski ao postular o papel da imitação na aprendizagem. A ação com ajuda ou a partir de modelos também está na base do conceito de zona de desenvolvimento proximal proposto pelo autor. Vigotski propõe que a criança, com ajuda da imitação e na atividade coletiva orientada pelo adulto, pode superar sua capacidade de compreensão independente. O desenvolvimento infantil é assim tomado em dois níveis dinamicamente articulados, o proximal e o real, tendo a imitação um papel importante na antecipação do processo de aprendizagem e subseqüente desenvolvimento.

Conclui-se que uma leitura material da psicologia humana não está necessariamente associada a um recorte epistemológico mecanicista. A PHC coloca-se como alternativa às abordagens cognitivistas e comportamentais no sentido de incorporar achados da neurociência à teoria do desenvolvimento e aprendizagem humanos.

Palavras-Chave: Psicologia histórico-cultural, neurônios-espelho

Apoio Financeiro: FEUSP

I.03.002

**PSICOLOGIA E NEUROCIÊNCIAS UMA ANÁLISE DE TEORIAS PSICOLÓGICAS
SEGUNDO PARÂMETROS PARA VALIDADE DE MODELOS EXPERIMENTAIS**

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Objetivos: a ciência psicológica caracteriza-se por ser composta de múltiplas teorias e sistemas que visam explicar o comportamento. Cada uma enfatiza determinados aspectos em detrimento de outros, invariavelmente falhando em parte de suas explicações. O objetivo deste trabalho é analisar as principais teorias e sistemas da Psicologia utilizando a metodologia de validade de modelos experimentais.

Métodos: cada teoria será analisada segundo os parâmetros Valor de Face, Valor de Construto e Valor Preditivo.

Resultados: de uma maneira geral, as teorias da Psicologia apresentam um Valor de Face de moderado a baixo, uma vez que tentam descrever elementos complexos como o comportamento geral do indivíduo. Em termos de Valor de Construto, há grande variabilidade entre as teorias. Merecem destaque, com alto escore neste item, a Psicanálise e a Epistemologia Genética, por permitirem a explicação de comportamentos mais complexos do que as teorias das Neurociências costumam abordar. Por fim, quase a totalidade das teorias psicológicas tem reduzido Valor Preditivo, uma vez que se ocupam, principalmente, com a descrição do comportamento, e não com a sua predição. A exceção, com alto resultado neste parâmetro, é o Behaviorismo Radical.

Conclusões: a metodologia de validade de modelos experimentais revela-se útil para analisar as teorias da Psicologia, permitindo uma comparação destas com as propostas pelo paradigma neurocientífico.

Palavras-Chave: modelos experimentais, psicologia, teorias psicológicas, neurociências.

HYPERMNESIA AND ATYIAH-WARD SPACE-TIMES

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Main purpose: To set up a possible relationship between the phenomenon of hypermnnesia and space-times where 2 time-like directions are present.

Methods: Critical revision of the related literature, by consulting the PUBMED and LANL data bases.

Results: One may characterise hypermnnesia, from the psychopathological viewpoint, as that situation for which one's memories are overwhelmingly evoked and with more precision than it usually occurs, or, in a weaker form, when facts and peculiarities that are not normally present in one's mind are re-evoked. In this process, one recognises the participation of the amygdala in the neural circuits (1). There are also situations in which one describes an instantaneous evocation of facts of a whole life, a non-trivial event, which takes place in contexts of a highly emotional impact, such as in case of a sudden death. After the threaten is over, surviving individuals report the experience of remembering, in short times and in an absolutely clear way (as if one was watching a moving picture), the development of one's life, overtaking the edges of the ego and the conventional bounds of time and space. In such cases, the experience takes place as if one had access to a new time regime, unreachable in usual situations.

Conclusion: Based on the considerations presented above, it is possible to conceive conceptual parallelisms between hypermnnesia and space-time scenarios with more than a single time-like direction. Spaces of this type – referred to as Atiyah-Ward space-time (2) – have been considered in connection with a number of systems of interest in the study of fundamental interactions and, in a macroscopic scale, in association with the issue of gravitacional collapse. It is actually possible to take the viewpoint of modelling hypermnnesia by proposing that, under very special circumstances, the neural system behaves as if it were embedded in a space-time scenario where 2 space-like directions are present.

References: 1- Gazzaniga MS, *et al.* Neurociência cognitiva, 2006; 2- Siqueira-Batista R, *et al.* XXXI Encontro Nacional de Física da Matéria Condensada, 2008. **Palavras-Chave:** Hypermnnesia; Atyiah-ward Space- times

I.04.001

PERCEPÇÃO DO EDUCADOR SOBRE A NEUROCIÊNCIA APLICADA À EDUCAÇÃO

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Objetivos.

O educador utiliza estratégias pedagógicas no processo de ensino e aprendizagem. Embora atue nas transformações neurobiológicas que geram aprendizagem, desconhece como o cérebro funciona (Neurociências, 3:6, 2006). Os objetivos deste estudo são avaliar o nível e lacunas no conhecimento de neurociência e funcionamento do cérebro humano demonstrado pelo educador.

Métodos.

Foi elaborado um questionário com 10 itens para mensurar a percepção do educador relativo se o conhecimento do cérebro, contribui para realçar o ensino, ancorado em 7 pontos da escala Likert, onde 1=discordo totalmente à 7=concordo totalmente. Este foi respondido por 83 professores (74 fem., 9 masc.), idades entre 25-54 anos, versando sobre cérebro, período sensível e crítico, plasticidade neuronal, memória, emoção, sono & aprendizagem, método alternativo de ensino e visita à museu, zoológico, botânico. A amostra foi coletada em Curitiba, PR, de professores atuantes da pré-escola ao ensino médio.

Resultados.

87,4% da amostra concorda fortemente que melhor conhecimento sobre o cérebro contribui para o ensino, 70,0% discorda sobre “período crítico” embora 59,0% concorde que experiências no meio ambiente moldem o cérebro. 80,0% são favoráveis a plasticidade neuronal e a vantagem do ambiente “enriquecido”. Visita à Museus, Zoológicos e J. Botânicos aprimoram o desempenho escolar (79,4%) e o conhecimento do mecanismo da memória aperfeiçoaria o ensino (79,5%), envolvendo emoção (84,2%), salientando a importância do sono na aprendizagem (87,0%) e que métodos alternativos de ensino, como mapa conceitual & resolução de casos, favorecem a representação de conhecimento na estrutura cognitiva da mente com 57,8% de concordância.

Conclusão.

No cômputo geral, mais de 80,0% da amostra dos professores pesquisados acredita, que um melhor conhecimento sobre o funcionamento do cérebro, melhoraria as estratégias do ensino-aprendizagem, refletido a “neuro-alfabetização” do público em geral (The Neuroscientist, 8:98, 2002). Em suma, urge oferecer curso de extensão sobre neurociência aos educadores.

Palavras-Chave: Ensino, Educação, Neurociência, Cérebro

I.05.001

Neuroética do Imageamento Cerebral e seus impactos nos direitos humanos

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Objetivos: Analisar o impacto das técnicas de imageamento cerebral na proteção aos direitos humanos, notadamente o direito à privacidade e o direito à saúde. Método: Revisão da literatura a respeito das pesquisas sobre imageamento cerebral e análise do impacto dessas técnicas à luz dos direitos fundamentais positivados na Constituição Brasileira e na Declaração Universal de Bioética e Direitos Humanos e da teoria dos direitos humanos. Resultados: O recente desenvolvimento das técnicas de imageamento cerebral propiciou aos pesquisadores e aos clínicos instrumentos de análise e diagnóstico bem mais precisos. A utilização dessas técnicas, todavia, impacta consideravelmente nos direitos humanos dos pacientes e pesquisados, gerando uma série de novos problemas éticos para os comitês de ética de pesquisa e de ética médica. O alto custo dessas técnicas de imageamento cerebral e o nível de especialização profissional exigido para operacionalização dos equipamentos afeta diretamente a efetividade do direito à saúde, dificultando, para as populações mais pobres, o acesso a um diagnóstico preciso. Por sua vez, o avanço no conhecimento do funcionamento cerebral e a tendência a uma abordagem cada vez mais biologizante da mente (reducionismo biológico) tornam a pesquisa com imagens cerebrais uma forte ameaça à privacidade dos pesquisados. Conclusões: A efetivação do direito à saúde, com o oferecimento de ferramentas de diagnósticos mais precisas, necessita de uma ampla cooperação entre os países, buscando-se a concretização do direito ao desenvolvimento por meio da diminuição da tributação incidente sobre esses aparelhos de diagnóstico e da burocracia para importação de equipamentos, bem como melhor formação de recursos humanos aptos a utilizar a aparelhagem. Por sua vez, para proteção ao direito à privacidade, é preciso levar em consideração que toda informação coletada e armazenada revela dados particulares dos indivíduos, necessitando de expressa autorização para a realização da pesquisa, sob pena de caracterização de pressuposto para indenização.

Palavras-Chave: Neuroética; Direitos Humanos; Direito à saúde; Privacidade

Importância da Capacitação de Profissionais de Saúde no Atendimento ao Idoso em PSF's e Hospitais Públicos no Entorno distante do Distrito Federal

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Objetivos: Descrever as características demográficas e identificar demandas na capacitação de um grupo de profissionais de saúde em comunidades distantes do centro urbano do DF e estimar expectativas quanto ao auxílio de ensino à distância por meio de um site educativo (www.cmi.unb.br/gedarni) referente aos temas de doenças do envelhecimento e atendimento ao idoso.

Métodos: Selecionou-se um município do Estado de GO, com 20.000 habitantes, distante a 300 km do DF, para ação de capacitação presencial e virtual de profissionais de saúde em nível técnico e profissional, ligados ao atendimento do idoso. Aplicou-se, em 35 sujeitos, questionário contendo 9 itens relativos ao conhecimento da realidade no atendimento ao idoso, antes do curso. Verificaram-se dados quanto ao conhecimento acerca do atendimento ao idoso e doenças do envelhecimento, bem como o interesse no aprofundamento do tema.

Resultados: 48,6% dos entrevistados mencionaram dificuldade no atendimento ao idoso. 60% destes possuem formação de nível superior e 40% de nível técnico. 22,9% dos profissionais declararam desconhecer o termo demência, 25% destes atuam em nível técnico e 20% em nível superior. 60% nunca tiveram contato com portadores de demência; 53,3% dos profissionais de nível superior se inseriram nesse grupo. 97,1% dos entrevistados afirmaram que o esquecimento é uma ocorrência imprópria na velhice. 48% manifestaram interesse em saber mais sobre demência, sendo 46,7% de nível superior e 50% de nível técnico. Entretanto, 94,3% manifestaram interesse em utilizar site educativo para saber mais sobre envelhecimento e demência, sendo 100% de nível superior e 90% de nível técnico.

Conclusão: A queixa de dificuldade no atendimento ao idoso foi significativamente maior em profissionais de nível superior. Estes expressaram, em maior número, a falta de contato com pacientes com quadro demencial. O desconhecimento acerca das doenças do envelhecimento mostrou-se um aspecto importante a se considerar. Tais dados permitem sugerir, portanto, a capacitação de profissionais de saúde como uma ação otimizadora da qualidade do serviço público no atendimento ao idoso, em cidades distantes de grandes centros urbanos.

Palavras-Chave: Envelhecimento, Capacitação Profissional, Saúde Pública, Demência

Apoio Financeiro: FINEP - CNPq

I.05.003

Avaliação Neuropsicológica do Paciente Idoso: questões técnicas e éticas da utilização de testes psicológicos.

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Fundamentação: Buscando assegurar maior qualidade técnica e ética dos instrumentos e procedimentos de trabalho dos psicólogos no Brasil, o Conselho Federal de Psicologia (CFP), através da Resolução 002/2003, define e regulamenta o uso, elaboração e comercialização de testes psicológicos. De acordo com essa resolução, o CFP avalia os testes psicológicos, considerando sua base teórica, suas propriedades psicométricas, validade, fidedignidade e clareza no sistema de correção e interpretação dos dados, emitindo então parecer de favorável ou desfavorável ao uso na população brasileira. Os instrumentos estrangeiros estão sujeitos às mesmas exigências, devendo passar por tradução adequada e adaptação à população brasileira. A utilização de instrumentos não avaliados como favoráveis para uso pelo CFP é considerada falta ética, conforme Art. 16 da mesma Resolução.

Delineamento e métodos: Estudo realizado sobre testes disponível para uso, de acordo com a Resolução 002/ 2003, e sua adequação ao trabalho de avaliação neuropsicológica de pacientes idosos.

Resultado: Na presente data (05/2008), há um total de 98 instrumentos de avaliação liberados para uso pelo CFP. Apesar de, entre esses instrumentos, haver testes dirigidos para avaliação neuropsicológica, como o TIG-NV e o Teste Wiscosin, a população abrangida por eles não contempla amplamente a população idosa. O WAIS-III pode ser utilizado até a faixa etária de 89 anos. Os demais instrumentos que avaliam aptidões específicas, e que poderiam fazer parte desse tipo de avaliação, também apresentam, em geral, limites superiores de idade não maiores que 70 anos, sendo uma exceção o TEPIC-M, que abrange até 97 anos. Assim, notamos o reduzido número de instrumentos disponíveis para o trabalho de avaliação de idosos, principalmente em idades superiores a 80 anos, sendo que, para pacientes acima de 89 anos, são praticamente inexistentes.

Conclusão: Mostra-se necessária a construção e validação de testes específicos para uso com pacientes idosos e/ ou ampliação das amostras de padronização e normatização dos testes utilizados atualmente para que possam abranger também essa população.

Palavras-Chave: Neuropsicologia; Testagem Psicológica; Avaliação Psicológica