SEXTA REUNION
SOCIEDAD CHILENA DE NEUROCIENCIA
VALDIVIA, CHILE.

Lugar: **Hotel Del Rio, Valdivia**
Fecha: **29 Septiembre al 1 de Octubre, 2010**

PROGRAMA

La Sociedad Chilena de Neurociencias se acerca a la Comunidad

- Charlas a colegios de la zona: Miércoles 29, 9:00-10:30
Conversando de temas de la Neurociencia de interés para los jóvenes en 10 colegios. Actividad a cargo de investigadores jóvenes de la SCN

- Conferencia a la Comunidad de Valdivia: Miércoles 29, 18:30-19:30.
Actividad organizada en conjunto con la Municipalidad de Valdivia

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I CONFERENCIAS PLENARIAS

Conferencia 1:
Miércoles 29, 18:30-19:30
"IL-6 signaling and actions in the central nervous system". Ian L Campbell (School of Molecular Bioscience, Universidad de Sydney, Australia).

Conferencia 2:
Jueves 30, 9:00-10:00
"Neural mechanisms of species-typical odorant responses in salamanders". Heather Eisthen, PhD. (Department of Zoology, Michigan State University, EEUU)

Conferencia 3:
Jueves 30, 18:30-19:30
"The vertebrate midbrain: Cells, circuits, and concepts". **Dr. Harald Luksch** (Chair of Zoology, Technichal University of Munich, Germany)

Conferencia 4:
Viernes 1, 11:30-12:30
"The Biology of Childhood Attention-Deficit Hyperactivity Disorder (ADHD)". **Dr. Robert D. Oades** (University Clinic for Child and Adolescent Psychiatry, Essen, Germany)

Conferencia 5:
Viernes 1, 12:30-13:30
"CP5 The MARCKS and MARCKSL1 proteins in brain and retinal development". Dr. Perry Blackshear (Polypeptide Hormone Action Group. NIH/NIEHS, USA)

Conferencia 6:
Viernes 1, 16:30-17:30
"Transmission of Neurodegenerative Diseases by Infectious Proteins". Dr. Claudio Soto (Mitchell Center for Alzheimer's disease and related Brain disorders, University of Texas, and Facultad de Medicina, U. de los Andes).

II SIMPOSIOS

Simposio 1. Miércoles 29, 11:30-13:30 Coordina: **Dr.Esteban Rodríguez**
"HYDROCEPHALUS AND ABNORMAL NEUROGENESIS AS INSEPARABLE PHENOMENA: STUDIES IN ANIMAL MODELS AND HUMANS"
11:30-12:00 "The brain-specific transcription factor RFX4_v3 in the regulation of brain development and non-communicating hydrocephalus". P. Blackshear (NIH/NIEHS, USA)
12:00-12:30 "Role of membrane trafficking and cell adhesion in the onset of hydrocephalus and other neurodevelopmental disorders" F. Bátiz (Universidad Austral de Chile)
12:30-13:00 "Abnormal expression of cell-cell junction proteins in the neuroepithelium/ependyma of human hydrocephalic foetuses with spina bifida aperta: a common molecular pathway for hydrocephalus and abnormal neurogenesis". M. Guerra (Universidad Austral de Chile).
13:00-13:30 "Early diagnosis of a human foetus undergoing a progessive obliteration of Sylius aqueduct and hydrocephalus allowed early and successful postnatal surgery. A case report". F. Guerra (Universidad Austral de Chile)

Simposio 2: Miércoles 29, 11:30-13:30 Coordina: **Dra. Ursula Wyneken**
"REGULATION AND FUNCTION OF GLUTAMATERGIC RECEPTORS"
11:30-12:00: "Signaling mechanisms controlling AMPA receptor trafficking and clustering at the postsynaptic membrane interaction with CaMKII and RasGRF1"**.** José A. Esteban (Universidad Autónoma de Madrid).
12:00-12:30: "NR2B-NMDA receptors regulate dendritic branching via interaction with CaMKII and RasGRF1". Brigitte van Zundert (Universidad Andrés Bello).
12:30-13:00 "Role of NR2 subunits in synaptogenesis and synapse stabilization". Andres Barria (University of Washington, USA).
13:00-13:30 "Long term antidepressant treatment induces structural and functional changes at glutamatergic forebrain synapses". Ursula Wyneken (Universidad de los Andes).

Simposio 3: Jueves 30, 11:30-13:30 Coordinan: **Drs. Rodolfo Madrid y Juan Bacigalupo**
"CANALES TRP EN TRANSDUCCIÓN SENSORIAL".
11:30-12:00 "La dependencia de temperatura y voltaje en canales termo TRP vista a través de modelos alostéricos". Ramón Latorre (Universidad de Valparaíso).
12:00-12:30 " Dinámica de membrana de canales TRPM8 y su captura en dominios de confinamiento transitorio". Sebastián Brauchi (Universidad Austral de Chile)
12:30-13:00 "Thermally activated TRP channels: the yin and yang of pain". Peter A. McNaughton. (University of Cambridge, UK).
13:00-13:30 " Contribución de canales los TRPM8 y TRPA1 a la sensibilidad térmica de los termorreceptores de frío corneales". Rodolfo Madrid (Universidad de Santiago de Chile).

Simposio 4: Jueves 30, 11:30-13:30 Coordina: **Gonzalo Marin**
" CIRCUITOS NEURONALES Y CONDUCTA VISUAL EN AVES "
11:30-12:00: "El núcleo Geniculado lateral y su rol oculomotor, un caso de modulación centrífuga". Jorge Mpodozis (Universidad de Chile).
12:00-12:30: "¿Cómo un estímulo visual repentino captura la atención?". Gonzalo Marin (Universidad de Chile).
12:30-13:00 "Optical imaging of tecto-isthmic network activity in the chick brain slice". Harald Luksch (Technical University Munich).
13:00-13:30 "Modelación neuronal en sistemas sensoriales". Juan Carlos Letelier (Universidad de Chile).

Simposio 5: Jueves 30, 16:30-18:30 Coordina: **Dr. Bernardo Castellano**
"THE ROLE OF MICROGLIAL CELLS IN THE NEUROIMMUNE RESPONSE"
16:30-17:00 "The immune and immune accessory role of activated and phagocytic microglia following facial axotomy" Gena Raivich (University College London, UK.).
17:00-17:30 "Regulation of microglial activation: role of IL-15". Diego Gómez-Nicola (Instituto Cajal, España).
17:30-18:00 "Microglial cells regulate the adaptative immune response by modulating the lymphocytic function in acute EAE, a model of multiple sclerosis" Beatriz Almolda. (Universidad Autónoma, España).
18:00-18:30 "Immunomodulation as a therapeutic approach for Alzheimers: the role of TGFbeta". Rommy von Bernhardi. (Pontificia Universidad Católica de Chile).

Simposio 6: Jueves 30, 16:30-18:30 Coordina: **Dr. Jorge Mpodozis**
"NEUROETHOLOGY AND EVOLUTION OF THE VOMERONASAL SYSTEM"
16:30-17:10: "Why do aquatical animals have a vomeronasal system?**".** Heather Eisthen (Michigan State University, USA).
17:10-17:50: "Ecological contingencies and anatomical trends in the vomeronasal system of Caviomorph rodents". Jorge Mpodozis (Universidad de Chile).
17:50-18:30 "Evolution of sexual dimorphisms and multiple events of sensory loss in the vomeronasal system of eutherians". Rodrigo Suárez (Universidad de Chile)

Simposio 7: Viernes 1, 9:00-11:00 Coordina: **Dr. Claudio Hetz**
" NÚCLEO MILENIO NEMO: "NEURAL MORPHOGENESIS: FROM SHAPE TO FUNCTION"
9:00-9:30: "Shaping an asymmetric brain nucleus in the embryonic zebrafish"**.** Miguel Concha (Universidad de Chile).
9:30-10:00: "Regulation of axonal growth and guidance by *hindsight* (*pebbled*) in the *Drosophila* visual system". Jimena Sierralta (Universidad de Chile).
10:00-10:30 "Organelle Stress and Protein Misfolding Disorders affecting the Nervous System". Claudio Hetz (Universidad de Chile).
10:30-11:00 "In vivo approaches to the study of the role of transcriptional regulators in the development of the brain cortex". Manuel Kukuljan (Universidad de Chile).

Simposio 8: Viernes 1, 9:00-11:00 Coordina: **Dr**. **Alejandro Múnera**
"PLASTICIDAD EN LA CORTEZA MOTORA PRIMARIA DE LAS VIBRISAS"
9:00-9:40: "Plasticidad a corto y largo plazo de las entradas sinápticas a la corteza motora primaria de las vibrisas"**.** Alejandro Múnera (Universidad del Rosario, Bogotá, Colombia).
9:40-10:20: "Modificaciones en la actividad electrofisiológica y en la morfología de células piramidales de la corteza motora tras la lesión del nervio facial contralateral". Julieta Troncoso (Univ Nacional de Colombia, Sede Bogotá, Colombia).
10:20-11:00 "Cambios en la expresión de marcadores neuronales y gliales en la corteza sensoriomotora asociados a la lesión
periférica del nervio facial". Efraín Buriticá Ramírez (Universidad del Valle, Colombia).

<u>Simposio 9</u>: Viernes 1, 14:30-16:30 Coordina: **MS Zulma Dueñas**
" EFECTOS DEL ESTRÉS SOBRE EL COMPORTAMIENTO EN MODELOS ANIMALES"
14:30-15:10: "Efectos del estés sobre la expresión de crisis en modelos animales de epilepsia"**.** Andrea Milena García (Universidad de la Sabana-Colombia).
15:10-15:50: "Efectos del estrés agudo en el aprendizaje espacial y la expresión de quinasas y fosfatasas. Marisol Lamprea (Univ Nacional de Colombia-Colombia).
15:50-16:30 "Efecto del estrés por separación maternal temprana en la memoria, comportamiento defensivo y expresión de receptors GABA-A". Zulma Dueñas (Univ Nacional de Colombia-Colombia).

<u>Simposio 10</u>: Viernes 1, 14:30-16:30 Coordina: **Dr. Adrián Ocampo**
" STATE CONTROL MECHANISMS IN THE HYPOTHALAMUS AND THE ASCENDING ACTIVATING SYSTEM"
14:30-15:00: "What activates the ascending activating system nuclei?" Fernando Torrealba (Pontificia Universidad Católica de Chile).
15:00-15:30: "The infralimbic cortex-tuberomammillary nucleus axis and its central role in motivated arousal". María Eugenia Riveros (Pontificia Universidad Católica de Chile).
15:30-16:00 "Melanin concentrating hormone (MCH): an hypothalamic peptide that promotes sleep". Pablo Torterolo (Universidad de la República, Uruguay).
16:00-16:30 "The hypothalamus and the chronobiology of REM sleep in the rat". Adrián Ocampo (Universidad de Chile).

III SESIONES COMUNICACIONES LIBRES

1.	**Miércoles**	**29**	16:30-17:30
2.	**Jueves**	**30**	10:00-11:00

IV SESIONES PANELES

1.	**Miércoles**	**29**	14:30-16:30
2.	**Jueves**	**30**	14:30-16:30

Comité Organizador VI Reunión Anual SCN
Pedro Maldonado (Universidad de Chile)
Jaime Eugenín (Universidad de Santiago)
Fernando Torrealba (Pontificia Universidad Católica de Chile)

Comité Científico VI Reunión Anual SCN
Rommy von Bernhardi (Pontificia Universidad Católica de Chile)
Alejandra Alvarez (Pontificia Universidad Católica de Chile)
Alan Neely (Universidad de Valparaíso)
Francisco Nualart (Universidad de Concepción)
Jaime Eugenín (Universidad de Santiago)
Fernando Torrealba (Pontificia Universidad Católica de Chile)
Pedro Maldonado (Universidad de Chile)

RESUMEN PROGRAMA
V CONGRESO ANUAL DE LA SOCIEDAD CHILENA DE NEUROCIENCIA
Hotel Del Rio, Valdivia 29 Septiembre al 1 de Octubre, 2010

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RESÚMENES / ABSTRACTS

I CONFERENCIAS PLENARIAS

Conferencia 1: Miércoles 29, 18:30-19:30

CP.1. IL-6 signaling and actions in the central nervous system.

Ian L Campbell. *School of Molecular Bioscience, The University of Sydney, NSW 2006, Australia*

IL-6 is a highly pleiotropic cytokine implicated in the pathogenesis of many neurological disorders. To study the actions of IL-6 in the CNS we generated transgenic mice with IL-6 production engineered to the astrocytes. These so-called GFAP-IL6 mice exhibit wide-ranging alterations in the brain that include proliferative angiopathy, loss of BBB integrity, gliosis, acute phase protein production and progressive neurodegeneration. There is limited understanding of how IL-6 communicates with different cells in the CNS. Astroglia lack membrane bound IL-6Ra but respond to IL-6 plus soluble IL-6Ra via trans-signaling. Microglia respond to IL-6 by both classical- (IL-6/IL-6Ra) and trans-signaling. We found that IL-6 actions in the brain involve specific molecular and cellular responses that are dependent on trans-signaling. The importance of trans-signaling in mediating many of the CNS effects of IL-6 is highlighted by the finding that inhibiting trans-signaling reduces the severity of neuroinflammatory disease in GFAP-IL6 mice. IL-6 receptor signaling is mediated via gp130 which activates the JAK/STAT1/STAT3 and SHP2/MAPK pathways. Downstream of gp130, the signaling intensity and kinetics of activation of the JAK/STAT and SHP2/MAPK pathways in response to IL-6 trans-signaling is similar in astroglia and microglia. While gp130-JAK/STAT signaling drives increased expression of many STAT1 and STAT3-dependent genes, few genes are regulated in response to gp130-SHP2/MAPK signaling whose primary function is negative regulation of the gp130-JAK/STAT pathway via SOCS3. In summary, the genetic dissection of IL-6 actions and communication in the brain has provided new insights into key mechanistic processes that underlie the neuropathogenic actions of this cytokine.

Conferencia 2: Jueves 30, 9:00-10:00

CP.2. Neural mechanisms of species-typical odorant responses in salamanders.

Mecanismos neuronales de respuestas a odorantes especie-específicas en salamandras.

Eisthen H Department of Zoology, Michigan State University

Sense organs detect and process information from the external environment, but do not simply transduce all available stimuli; instead, they are often tuned to detect stimuli that are be important for a given species or individual. I will describe two examples of such tuning in the vertebrate olfactory epithelium. First, the terminal nerve appears to have arisen in jawed vertebrates and serves a neuromodulatory function. Our data demonstrate that the two major peptides present in the terminal nerve, gonadotropin releasing hormone and neuropeptide Y, modulate odorant responses and voltage-gated ion channels in the olfactory epithelium of axolotls (*Ambystoma mexicanum*), a type of aquatic salamander. The effects of these peptides differ with the sex of the animal, its nutritional and reproductive state, and the behavioral significance of the odorant. Thus, the terminal nerve appears to function to tune responses in the olfactory epithelium with respect to the animal's behavioral context or physiological state. I will also describe new work in my lab in which we are examining responses to tetrodotoxin (TTX), a potent blocker of voltage-gated sodium channels, that is produced in the skin of rough-skinned newts (*Taricha granulosa*). Our data demonstrate that newts are attracted to TTX and that it elicits robust odorant responses from their olfactory epithelium. Interestingly, our data suggest that TTX is transduced by an unusual mechanism that differs from that used by other odorants, and that newts may have coopted a sodium channel for use as a TTX receptor.

Supported by grants from the US National Institutes of Health (DC05366) and US National Science Foundation (IBN 9982934; IOS 0817785) as well as fellowships from the Marine Biological Laboratory.

Conferencia 3: Jueves 30, 18:30-19:30

CP.3. The vertebrate midbrain: Cells, Circuits, Concepts

Harald Luksch, Dept. of Animal Sciences, Technische Universität München

The vertebrate dorsal midbrain (superior colliculus in mammals, optic tectum in all other vertebrate classes) is a central interface between sensory stimuli and behavioral motor patterns. The tectum consists of multiple laminae with specific cell types and connectivity with many similarities between vertebrate classes. However, a homologization of the various layers across vertebrates is difficult.

The tectum receives a strong retinal projection that forms a map of visual space in the upper layers. This map acts as a master coordinate system for other sensory afferents (auditory, somatosensory etc.), leading to a multimodal representation of the sensory environment. While the size of the optic tectum is variable among vertebrates, the sensorymotor functions of the tectum appear to be conserved for all vertebrate classes.

With a high degree of structural order, identifiable cell types and known input and output connectivity, the analysis of the tectum with a combined experimental-computational approach can provide a mechanistic understanding of sensory computation. By integration of projections with other visual and non-visual nuclei in the brain, the basic neuronal computations can be gradually extended to generate more complex functions. Recent advances have been made in the analysis of feedback loops formed between the optic tectum and a group of nuclei in the isthmic area in several avian species. The function of these circuits is considered to be a bottom-up attentional system that identifies salient objects in the multimodal parameter space and allows for both orienting movements as well as fast motor responses in, for example, escape behaviour.

Conferencia 4: Viernes 1, 11:30-12:30

CP.4. The Biology of Childhood Attention-Deficit Hyperactivity Disorder (ADHD)

Robert D. Oades, Clinic for Child and Adolescent Psychiatry, University of Duisburg-Essen, Essen, Germany

ADHD of the combined type in children, with a worldwide prevalence of 4-10%, describes restlessness, impulsivity and attentional difficulties that cause a clinical impairment. For many this persists in adolescence, and for a third of cases there is a marked residuum in adulthood. A heritability of >70% implies marked genetic and familial contributions to the aetiology. It is a developmental disorder often accompanied by comorbid problems that point to a heterogeneity underlying the diagnosis. Recent genome wide association studies show that no major single gene is involved, but there are numerous genes contributing very small effects. While these studies have implied developmentally relevant contributions from adhesion molecules (cadherin), ion channels (Na+-K+ exchanger) and neurotrophins (NPY), candidate gene investigations suggest a potential functional involvement of monoaminergic circuitry in neuromodulation using dopamine (DRD4, transporter), noradrenaline (alpha-2, DBH) and serotonin (TPH, transporter). While subtypes of ADHD emphasize dimensions of inattention and impulsivity, a strong overall candidate endophenotype is one of the variability of response. This may involve dysfunction of the tripartite synapse within fronto-striatal-thalamic circuitry. Neurophysiological and neuroimaging studies demonstrate a frequent, early right-lateralized impairment in communication between and selective processing within the cortices. In many cases this is supported by anatomical work that suggests a 2-3 year delay in maturation. These features may also explain difficulties children with ADHD experience with motivation and self-control. Indeed, they also illustrate the potential interface for environmental influences. The nature of the interaction of biological and environmental features in the expression of ADHD requires close attention.

Conferencia 5: Viernes 1, 12:30-13:30

CP.5. The MARCKS and MARCKSL1 proteins in brain and retinal development

Perry J. Blackshear and Deborah J. Stumpo
National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA, 27709

The myristoylated alanine rich C-kinase substrate (MARCKS) and the MARCKS-like protein 1 (MARCKSL1) are the only known mammalian members of a small family of high-affinity substrates for protein kinase C (PKC). Both proteins are heat- and acid-stable, myristoylated, bind to calmodulin and the cytoplasmic face of the plasma membrane in a PKC-dependent manner, and are expressed at high levels in the developing central nervous system. We have shown that MARCKS-deficiency in mice results in 100% perinatal lethality, with high frequencies of exencephaly, omphalocele, retinal ectopia, agenesis of the corpus callosum and other forebrain commissures, abnormal cortical and retinal laminations, and neuronal ectopia. In contrast, about 34% of MARCKSL1-deficient mice survived to adulthood, although high percentages of knockout mice exhibited exencephaly and retinal ectopia. Surviving adult knockout mice did not have neuronal ectopia or abnormal forebrain commissures. MARCKS and MARCKSL1 may share common roles during some aspects of development (e.g., neurulation) while exerting different roles in other developmental processes (e.g., forebrain commissure formation). We have interbred these two lines to generate mice with various allelic combinations. Results from these studies indicate that the double knockout is lethal early in embryo development, with complete lack of neural tube closure along its entire length (chraniorachischisis). Among the various allelic combinations, there was a greater frequency of exencephaly in the tri-allelic knockout embryos than would be predicted based on frequencies seen in the single knockout and the single hemizygous embryos, implying some degree of gene interaction or epistasis in the phenotype of failed neurulation.

Conferencia 6: Viernes 1, 16:30-17:30

CP.6. Transmission of Neurodegenerative Diseases by Infectious Proteins.

Claudio Soto. Mitchell Center for Alzheimer's disease and related Brain disorders, Department of Neurology, University of Texas Houston Medical School, and Facultad de Medicina, Universidad de los Andes, Santiago, Chile.

Misfolded protein aggregates are implicated in a variety of diseases known as protein misfolding disorders (PMDs), which include some highly prevalent and insidious illnesses such as Alzheimer's, Parkinson's disease, diabetes type 2 and more than 20 other human maladies. These diseases share as a hallmark event the misfolding of a protein to form ß-sheet rich aggregates that deposit in diverse organs inducing cell death and tissue damage. Among PMDs, prion disorders are unique in that the pathology can be transmitted by an infectious process involving a heretical agent known as prion. Prions are infectious proteins capable to transmit biological information by propagation of protein misfolding and aggregation. The hypothesis that prions are composed exclusively by a protein with the unprecedented ability to behave like a micro-organism was controversial during decades, but recent studies have settled all doubts.

The process of misfolding and aggregation follows a seeding-nucleation mechanism in which small oligomers act as seeds to trigger an uncontrolled aggregation process. The seeding mechanism appears to be the basis by which misfolded prion protein propagates prion disease in an infectious manner. We have been able to create infectious prion protein in the test tube and produce a variety of novel diseases. So far, prion disease is the only member of the group of PMDs that has an infectious origin. However, the inherent infectious nature of misfolded aggregates propagating by a seeding mechanism suggests that other diseases of the group may be transmissible in the same manner as prion disorders. In this presentation, we will show evidences that the pathology of other PMDs, including Alzheimer's disease and diabetes type 2 can be also experimentally transmitted in vivo. These findings may change our understanding of these diseases and provide novel strategies for treatment.

II SIMPOSIOS

Simposio 1. Miércoles 29, 11:30-13:30 Coordina: Dr.Esteban Rodríguez

S1.1 The brain-specific transcription factor RFX4_v3 in the regulation of brain development and non-communicating hydrocephalus

Perry J. Blackshear, Donghui Zhang and Darryl C. Zeldin
National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA 27709

The regulatory factor X4 variant transcript 3 (*RFX4_v3*) is a novel isoform of the winged helix transcription factor RFX4. The *RFX4_v3* transcript variant is the only RFX4 isoform significantly expressed in the brain, and its expression is restricted to brain. Insertional mutagenesis in mice demonstrated that *Rfx4_v3* is crucial for brain development. Specifically, mice that were apparently null for this allele died in utero with failure of brain midline structure development; mice that were hemizygous for this allele developed post-natal non-communicating hydrocephalus that was associated with apparent lack of development of the subcommissural organ. More recently, we have investigated the regulatory mechanisms that govern the neuronal-specific expression of the *RFX4_v3* mRNA. Transient transfection assays demonstrated that a -489 to +378 bp fragment (relative to the transcription start site, TSS) was more active as a promoter in neural cell lines compared to non-neural cell lines. There are several predicted RFX protein binding sites in the *RFX4_v3* proximal promoter. Co-transfected RFX4_v3 activated its own promoter in neural cell lines, and mutations of the predicted RFX protein binding site at -130 abolished the RFX4_v3 activation. Activation of the *RFX4_v3* promoter by its own protein could be potentiated by co-expression of GPS2, and this potentiation effect seemed to be specific to neural cells. Gel shift experiments demonstrated that the RFX4_v3 protein could form a complex with the identified RFX site at -130 bp. These results suggest that RFX4_v3 is a key factor in the neural-specific expression of its own gene.

S1.2 Role of membrane trafficking and cell adhesion in the onset of hydrocephalus and other neurodevelopmental disorders.

Luis Federico Batiz, César González, Alexander Ortloff, Cristian Oliver, Gabriela Toro, Camilo Muñoz, Guillermo Márquez, Sara Rodríguez, Esteban M. Rodríguez.
Intituto de Anatomía, Histología y Patología. Facultad de Medicina. Universidad Austral de Chile. Valdivia, Chile. federicobatiz@uach.cl

Soluble N-ethylmaleimide-sensitive fusion (NSF) attachment protein-alpha (α-SNAP) is involved in several intracellular SNARE (SNAP receptor)-mediated membrane fusion events. Naturally occurring hyh (hydrocephalus with hop gait) missense mutation of the gene encoding for α-SNAP (Napa) causes an autosomal recessive disorder characterized by a disruption of the polarized organization of radial glial cells (RGCs) and ependymal lining, hydrocephalus and brain maldevelopment. What is the link between the loss of function of a ubiquitous protein such as α-SNAP and the neuropathological phenotype of hyh mouse? Is α-SNAP-mediated membrane trafficking required for maintenance of N-cadherin-based adherens junctions of RGCs and ependymal cells during brain development? Recently, we have shown that α-SNAP and N-cadherin are preferentially expressed at the ventricular lining in wild type mice. Interestingly, α-SNAP expression follows a temporal pattern that matches that of ventricular lining disruption in mutants. Loss of adherens junctions and changes in the subcellular distribution of N-cadherin have been observed at the ventricular surface of mutant animals prior to its disruption. Moreover, neuropathological features previously related to defective cell-cell adhesion at the ventricular surface (non-polarized cells protruding into the ventricular lumen, the presence of rosette-like structures) are clearly distinguished in hyh mutant brains. NPC cultures (neurospheres) derived from hyh mutant mice are dramatically disorganized when treated with EGTA or N-cadherin blocking peptide. Aggregation properties are also diminished in NPCs derived from hyh mice when

compared to WT NPCs. Taking together, our results suggest that a defective α-SNAP function leads to an abnormal cell-cell adhesion at the ventricular surface which, in turn, causes the disruption of the ventricular lining and the neuropathological phenotype of hyh mice. Supported by FONDECYT 1070241 (EMR) and 11070393 (LFB)

S1.3 Abnormal expression of cell-cell junction proteins in the neuroepithelium/ependyma (ventricular zone) of human hydrocephalic foetuses with spina bifida aperta: a common molecular pathway for hydrocephalus and abnormal neurogenesis.

Guerra M[1], Sival D[2], Jiménez AJ[3], Domínguez Pinos MD[2], den Dunnen WFA[2], Bátiz LF[1], Pérez-Fígares JM[2], Rodríguez EM[1]. *monserratguerra@uach.cl*

[1]Instituto de Histología y Patología, Facultad de Medicina, Universidad Austral de Chile, Valdivia; [2]Pediatric Neurology, University Medical Center, University of Groningen, Holland; [3]Departamento de Biología Celular, Facultad de Ciencias, Universidad de Málaga, España.

Background. Spina bifida aperta (SBA) is associated with cerebral pathology, such as hydrocephalus, heterotopias and cortical abnormalities. We hypothesize that these abnormalities are associated to, or triggered by a disruption of the neuroepithelium/ependyma (ventricular zone) caused by cell junction pathology. To test this hypothesis we investigated the expression of junction proteins in the VZ of these patients.

Methodology. Antibodies against N-cadherin, connexin 43 and ependymal markers (βIV-tubulin, caveolin 1) were used for double-fluorescence analyses of the VZ and subventricular zone of control and SBA foetuses, 16-40 weeks gestational ages.

Results. The areas of VZ disruption in SA were associated with abnormal expression of N-cadherin and connexin 43, and the formation of subependymal rosettes, closely resembling the findings in mutant animals where the VZ loss is due to abnormal transport of junction proteins to the plasma membrane. In the telencephalon, the abnormal expression of N-cadherin and Cx43 in the VZ is associated to: (i) disruption of the VZ; (ii) loss of radial glia/stem cells; (iii) displacement of neuroblasts into CSF; (iv) formation of periventricular rosettes; (v) paraventricular heterotopias.

Conclusions. 1. In SBA patients the VZ pathology (cell-junction pathology?) precedes and probably triggers hydrocephalus and abnormal cortical development. 2. The abnormal expression of junction proteins in SA would lead to hydrocephalus through two mechanisms: (i) abnormal CSF flow due to impaired ciliar beating; (ii) stenosis of SA following ependymal denudation. 3. Hydrocephalus and abnormal neurogenesis should be regarded as two inseparable phenomena, with a common history: disruption or the VZ.

Supported by Fondecyt 1070241 (EMR).

S1.4 Early diagnosis of a human foetus undergoing a progressive obliteration of Sylvius aqueduct and hydrocephalus allowed early and successful postnatal surgery. A case report

F. Guerra, R.I. Muñoz, E. Ortega, S. Rodriguez, K. Vio, L.F. Bátiz, M. Guerra and E.M. Rodríguez. Institutos de Ginecologia y Obstetricia, Neurociencias Clínicas, Histología y Patología, Facultad de Medicina. Universidad Austral de Chile.

Background. We have shown in mutant mice the loss of the ependyma of Sylvius aqueduct (SA) starting in development leads to a communicating hydrocephalus; shortly after birth ependymal denudation is completed and leads to SA obliteration and severe hydrocephalus. Thus, communicating and non-communicating hydrocephalus are two sequential phases of a single pathological phenomenon. We have hypothesized that a similar phenomenon occurs in human cases with foetal onset hydrocephalus. We here report on the first case of a human foetal communicating hydrocephalus becoming a non-communicating hydrocephalus after birth. Clinical case. A communicating hydrocephalus, with no other foetal anomalies, was detected in a 28-week-old foetus by use of ultrasound. At 33 week GA the diagnosis was confirmed by MRI and *stenosis of SA* was evident. At 38 week GA the infant was delivered by caesarean section. At PN2 a computerized axial tomography showed

a large expansion of the lateral ventricles. At PN5 a new MRI revealed SA *obliteration*. At PN14 an endoscopic thirdventriculostomy was performed and CSF was collected. Direct vision showed *complete occlusion* of SA. One year after surgery, the child displays a minor neurological impairment.
A CSF sample was obtained at surgery. Immunoblotting using an antibody specific for the secretion of the subcommissural organ (SCO) showed a compound of 180kDa, which is not present in the CSF of non hydrocephalic children. Conclusions. 1. This case was closely followed by a team of basic and clinic investigators allowing an early diagnosis and confirmation of a predicted evolution. This, in turn, prompted neurosurgeons to perform surgery at the right moment. The overall outcome has been a child with a minor neurological impairment. 2. The change in SCO secretion supports the hypothesis that abnormalities of the SCO are associated with the development of congenital hydrocephalus.

Simposio 2: Miércoles 29, 11:30-13:30 Coordina: Dra. Ursula Wyneken

S2.1 "Signaling mechanisms controlling AMPA receptor trafficking and clustering at the postsynaptic membrane interaction with CaMKII and RasGRF1".
José A. Esteban (Universidad Autónoma de Madrid).

Synaptic connections in the brain are continuously remodeled in response to neuronal activity. This process, known as synaptic plasticity, is widely accepted as the cellular process underlying learning and memory. We now know that an important contributor to synaptic plasticity in the hippocampus and other brain regions is the regulated addition and removal of glutamate receptors at excitatory synapses. In particular, AMPA-type glutamate receptors can be transported in and out of the postsynaptic membrane in a regulated manner, resulting in long-lasting changes in synaptic strength.

Our research group has pioneered the studies on the cellular mechanisms that mediate the transport and sorting of neurotransmitter receptors at synaptic terminals. Thus, we have identified a network of endosomal compartments and motor proteins that relay neurotransmitter receptors from the dendritic shaft towards the synaptic membrane for their final insertion at the synapse (Gerges et al., **J Neurosci** 24:4758-4766, 2004; Brown et al., **Neuron** 45:81-94, 2005; Gerges et al., **EMBOJ** 25:1623-1634, 2006; Brown et al., **J Neurosci** 27:13311-13315, 2007; Correia et al., **Nat Neurosci** 11:457-466, 2008).
In this symposium, we will discuss some of out recent results, concerning the role of phosphoinositides in the organization of synaptic signaling and in the clustering of neurotransmitter receptors at the postsynaptic membrane.

S2.2 NR2B-NMDA receptors regulate dendritic branching via interaction with CaMKII and RasGRF1.
Brigitte van Zundert (Universidad Andrés Bello).

NMDA receptors (NMDARs) are known to regulate axonal refinement and dendritic branching. However, since NMDARs are abundantly present as tri-heteromers (e.g. NR1/NR2A/NR2B) during development, the precise role of the individual subunits NR2A and NR2B in these processes has not been elucidated. Ventral spinal cord neurons (VSCNs) provide a unique opportunity to address this problem, because the expression of both NR2A and NR2B (but not NR1) is down-regulated in culture. At 4 DIV, exogenous NR2A or NR2B was introduced into these naturally ´NR2 null neurons´ to form synaptic NR1NR2A-receptors and NR1NR2B-receptor, respectively. Analysis of the dendritic architecture demonstrated that introduction of NR2B, but not NR2A, dramatically increased the number of secondary and tertiary dendritic branches of 11 DIV VSCNs. The NR2B subunit role in regulating branch formation was corroborated *in vivo* by virus-driven NR2B-GFP expression in hypoglossal motoneurons, and *in vitro* by knocking-down NR2B in hippocampal cultures. NR2B-expressing neurons displayed an increase in frequency of miniature AMPA-receptor synaptic currents, indicating that these newly formed branches were able to form functional synapses. Using previous described mutants, we also found that disruption of the interaction between NR2B and either CaMKII or RasGRF1 impaired dendritic branch

formation, whereas branch number was remarkable increased by a gain in the NR2A-CaMKII binding. We conclude that the association between NR1NR2B-receptors and CaMKII and RasGRF1 is required for dendritic branch formation. The limited interaction between the NR2A subunit and signaling proteins such as CaMKII may contribute to the restricted dendritic arbor development in the adult CNS. Part of this work has been recently published (Sepulveda et al., J. Neurophysiology, 2010).

S2. 3 Role of NR2 subunits in synaptogenesis and synapse stabilization.

Andres Barria (Univ of Washington, USA).

NMDA-Rs exhibit unique properties like slow deactivation kinetics, high calcium permeability, and voltage-dependent magnesium blockade. These properties allow the receptor to play a critical role during maturation of functional neuronal circuits, synaptic plasticity, and pathology. The NMDA-R is a tetrameric complex and an obligate heteromultimer composed of glycine-binding NR1 subunits and one or more of the glutamate-binding NR2 subunits (A-D). The predominant NR2 subunits in mammalian hippocampus, NR2A and NR2B, exist mostly as di-heteromeric NR1/NR2A or NR1/NR2B complexes.

The NR2B subunit is expressed prenatally and is required for normal neuronal pattern formation and for viability of the animal. In contrast, NR2A subunit increases in expression and synaptic incorporation progressively with age. This switch in subunit composition of synaptic NMDA-Rs from NR2B to NR2A-containing receptors is governed by sensory experience, synaptic activity, and learning.

We have described that synaptic incorporation of NMDA-Rs is regulated by NR2 subunits with different rules controlling NR2A and NR2B containing receptors. In addition, we have studied the physiological consequences of synaptic expression of different NR2 subunits.

In this symposium we will discuss new experiments that seek understanding of the mechanisms controlling the switch in NR2 subunit composition, as well as the role of these subunits in synaptogenesis and synaptic stabilization.

S2.4 Long term antidepressant treatment induces structural and functional changes at glutamatergic forebrain synapses.

Ursula Wyneken (Universidad de los Andes).

Major depressive disorder, characterized primarily by decreased mood, but also by cognitive, autonomic, endocrine and sleep/wake abnormalities, is treated primarily with antidepressant drugs such as fluoxetine (flx). Although the neuronal mechanisms involved in the therapeutic action of antidepressants are largely unknown, there is general agreement that neuroadaptive mechanisms, occuring with a slow time course (weeks to months), underlie mood improvement. These plastic changes affect glutamatergic synaptic connections in the brain and may regulate neuronal processing and network activity. Glutamatergic neurotransmission occurs between axon terminals and dendritic spines, that contain ionotropic (NMDA, AMPA and kainate subtypes) and metabotropic glutamate receptors. We have examined structural plasticity and glutamate receptor content in several forebrain areas, including the hippocampus, following long-term flx administration at therapeutically relevant doses. We have identified forebrain areas in which a selective change in glutamate receptor stoichiometry is associated to the presence of larger spines. Although these structural and molecular adaptations favored enhanced basal neurotransmission, plasticity at these synapses (such as long-term potentiation and long-term depression) was impaired, probably due to abnormal synaptic delivery of AMPA receptor GluR1 subunits. In behavioral studies, decreased depressive-like behaviors following flx were accompanied by impaired hippocampal-dependent learning, suggesting that in a normal (non depressed) brain, long-term antidepressant treatment may have deleterious effects on cognitive functions. The understanding of the mechanisms involved in region-specific plastic changes at glutamatergic forebrain synapses that underlie mood

improvement would help to identify new targets for better and faster acting antidepressant therapies.

Simposio 3: Jueves 30, 11:30-13:30 Coordinan: Drs. Rodolfo Madrid y Juan Bacigalupo

S3.1 TRANSPLANTABLE SITES CONFER TEMPERATURE AND PIP_2 SENSITIVITY TO THERMO TRANSIENT RECEPTOR POTENTIAL (TRP) CHANNELS

Ramón Latorre, David Báez and Natalia Raddatz.
Centro Interdisciplinario de Neurociencias, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile.

TRPV1 and TRPM8 are two thermoTRP channels able to sense both voltage and temperature. Although progress has been made in understanding the temperature-dependent properties of these channels the mechanisms by which temperature and voltage activate these channels are far from clear. Thus, a key question in understanding thermoTRP channel function is how do the protein domains in sensing stimuli (sensors) and opening the pore (gates) communicate. Taking advantage of our finding that the C-terminal domain of thermoTRPs is modular and determines the channel phenotype regarding temperature and sensitivity and PIP_2 binding, I have investigated its role in thermosensation and PIP_2 binding using wild type, deletion mutants and chimeric channels, and C-terminal fusion proteins of TRPM8 and TRPV1. The results indicate that thermoTRP channel-forming proteins are modular and certain structures confer temperature-dependent and PIP_2 activation, whereas others confer voltage-dependent regulation.
Supported by Grant FONDECYT 1070049

S3.2 TRPM8 NEAR-MEMBRANE DYNAMICS AND CHANNEL STABILIZATION AFTER STIMULATION

Sebastián Brauchi.
Instituto de Fisiologia, Facultad de Medicina, Universidad Austral de Chile, Campus Isla Teja, Valdivia 511-0566, Chile.

TRPM8 is a non-selective cation channel expressed on a subset of peripheral neurones, and is the molecular machine that allow us to detect cold signals from our surroundings. Some members of the TRP channel family changes their cellular distribution in response to stimulation. Here, we will describe membrane/near-membrane dynamics of TRPM8-GFP containing particles in both, HEK-293T and F-11 transfected cells. 2D and 3D trajectories together with the velocity of individual protein containing vesicles were obtained by Total Internal Reflection of Fluorescence Microscopy (TIRFM) and single particle tracking (SPT). Trajectories were analyzed by plotting the mean-square displacement against time. Four characteristic types of motion were observed: (a) stationary; (b) simple Brownian diffusion; (c) directed diffusion; and (d) confined diffusion, in which particles undergoing Brownian diffusion are confined within a limited area. Our data suggests that TRPM8, when inserted into the plasma membrane, is confined into small domains of about 3 μm in diameter, in which receptor molecules resides in the time scale of 2-8 s. In the absence of stimuli TRPM8 vesicles constantly move along a network that cover the plasma membrane, periodically stopping, most often just briefly. Stimulation halted this hop-diffusion probably by stabilizing TRPM8 channels, as a result, release from plasma membrane became significantly slower. This slower release of TRPM8 determined the overall increase of available receptors.
Supported by Grant FONDECYT 11070190; Pew Programs in Biomedical Sciences; SeedingLabs.

S3.3 MODULATION OF TRP CHANNELS: IMPACT ON PAIN SENSATION

Peter A. McNaughton.
Department of Pharmacology, University of Cambridge, UK.

One of the distinguishing characteristics of the sensation of pain is that it is increased by inflammatory mediators such as prostaglandin E2, bradykinin and nerve growth factor (NGF). At least three different signalling mechanisms mediate the actions of inflammatory mediators on the heat-sensitive ion channel, TRPV1. Bradykinin and PGE2 enhance the probability that TRPV1 channels will be activated by a heat stimulus, and they act by promoting phosphorylation of TRPV1 by protein kinases C and A, respectively. NGF by contrast increases the expression of TRPV1 channels in the neuronal cell membrane by promoting trafficking from a subcellular vesicle store. Phosphorylation of TRPV1 by PKC and PKA depends critically on a scaffolding protein, AKAP79, which binds PKA and PKC into a signalling complex together with TRPV1. AKAP79 is therefore a final common element in heat hyperalgesia, on which the effects of multiple proinflammatory mediators converge. In more recent work we have examined the effect of inflammatory mediators on other members of the TRP family. TRPV4 is activated by pleasant warm temperatures, and it is potentiated by phosphorylation by PKA and PKC, in a similar manner to TRPV1. TRPM8, which is activated by cool temperatures and menthol, is by contrast inhibited by inflammatory mediators, apparently by a quite different mechanism. Because of their central roles in pain sensation, the modulation of TRP channels may prove an attractive target for the development of novel analgesics.

S3.4 CONTRIBUTION OF TRPM8 AND TRPA1 CHANNELS TO THERMAL SENSITIVITY OF COLD THERMORECEPTORS OF THE MOUSE CORNEA

Rodolfo Madrid[1], Andrés Parra[2], Félix Viana[2] and Carlos Belmonte[2].
[1]Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Chile. [2]Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-CSIC, San Juan de Alicante, Spain.

TRPM8 and TRPA1 ion channels are believed to be respectively involved in transduction of mild and strong temperature decreases, but their contribution to physiological cold detection in peripheral thermosensitive receptor endings is still under dispute. We recorded for the first time nerve terminal impulse activity (NTI) in cold-sensitive terminals (CST) of the mouse cornea *in vitro*, characterizing their firing responses to cold and studying the contribution of these thermo-TRP channels to their thermal sensitivity. Corneal CST of mice exhibited a low, often regular spontaneous activity (4.0 ± 0.4 impulses/s) at 34ºC, which increased with cooling (threshold: 32.2 ± 0.2ºC) and transiently silenced by rewarming. 96% of these CST increased their spontaneous activity and response to cold in the presence of menthol (50 µM). Only in 9% of the corneal CST, the TRPA1 activator mustard oil (100 µM) enhanced ongoing firing activity. Corneal endings of TRPM8$^{-/-}$ mice discharged at very low frequency (0.6 ± 0.2 impulses/s) and were insensitive to cooling or menthol application. In TRPA1$^{-/-}$ mice, spontaneous activity and responses to cooling were similar to wild-type animals but increases in ongoing activity and in responsiveness to cooling produced by menthol were significantly reduced. TRPA1$^{-/-}$ mice were insensitive to mustard oil. Our results indicate that the mice cornea is exclusively innervated by a population of cold receptors in which TRPM8 channels are essential for the generation of spontaneous and cold-evoked NTI activity, and that TRPA1 channels seem to play a minor role in the detection of temperature reductions.

Supported by Grants BFU2008-04425 and CONSOLIDER-INGENIO 2010 CSD2007-00023 (C.B.); BFU2007-61855 (F.V.); R.M. thanks the support of Grant FONDECYT 1100983.

Simposio 4: Jueves 30, 11:30-13:30 Coordina: Gonzalo Marin

S4.1 The avian ventral lateral geniculate nucleus and its oculomotor role: Behavioral consequences of a centrifugal cholinergic gate of primary retinal synapses.

Tapia Sebastian, Madrid, N, Marin,G, Letelier J.C. Mpodozis, J (presenting author) Depto. Biologia, Facultad de Ciencias, Universidad de Chile
The ventral nucleus of the lateral geniculate (GLv) is a prominent retinal and tectal target in all amniotes. In birds, the retinal affrences are formed by glutamatergic axons expressing Beta2 nicotinic receptors, while the tectal afferences originates from visually active ChAT immunopositive neurons located in tectal layer 10. Tectal and retinal afferences terminate homotopically inside the GLv neuropil, while GLv efferences innervate several hypothalamic, pretectal and tegmental targets, to finally end upon the medial pontine nucleus. (PM), a pre-oculomotor cerebellar structure.
Previous studies in brain slices have shown that the application of acetylcholine enhances the glutamatergic retino-geniculate synaptic activity. We then hypothesized that tectal fibers acts presynaptically as cholinergic gates upon glutamatergic retinal terminals, and that the concurrency of tectal and retinal activity upon a given GLv locus triggers or modulate gaze shifts. In this study we investigated "*in vivo*" the dynamics of the retino-tecto-GLv interactions and its presumptive oculomotor consequences. We found that the microinjection of lidocaine (4%) in a given tectal locus reduces greatly and transiently the visually elicited activity in the homotopic GLv locus. The microinjection in the GLv of the beta2 blocker erisodine (6 μM) also produces a significant transient reduction of the visual activity in the affected locus. On the other hand, the microstimulation (100-300 μamp, 300Hz) of specific visual loci in the GLv elicit clear eye movements whose direction and amplitude depends on the position of the stimulated locus. These findings illustrate for the first time an oculomotor role for the retino-GLv pathway.
Founded by Fondecyt 1080094 to JM

S4.2 How a salient visual stimulus may capture attention: Synchrony and global responses in the avian visual system.
Gonzalo Marín.Laboratorio de Neurobiología, Facultad de Ciencias, Universidad de Chile
When a salient object in the visual field captures the attention of the perceiver, the neural representation of the object is enhanced at the expense of competing stimuli. How neural activity evoked by a salient stimulus evolves to take precedence over the neural activity of other stimuli is a matter of intense investigation. In the optic tectum of anaesthetized pigeons (TeO), we have described the operation of spatially-specific, synchronized feedback signals from the isthmic region that seem to promote the transformation of a pre-attentional afferent volley into a global attentional state. This idea is based on three well-established observations. First, retinal afferent volleys arriving to the superficial layers of the optic tectum do not proceed to the nucleus rotundus (caudal pulvinar in mammals) in the thalamus unless they are boosted by bursting feedback signals from the isthmic region. Second, these feedback signals are spatially organized and the position in the tectal visual map which they impinge upon is controlled by long-range competitive interactions, such that one or few visual afferent volleys may be transmitted upstream at any given time. And finally, the bursting feedback from the isthmi seems to imprint a bursting rhythm on the transmitted inferences that, on the one hand, may constitute the actual mechanism of their successful propagation and, on the other, seems to synchronize their targets at several stages in the tecto-fugal pathway. This cross-regional synchronization among engaged areas is perhaps the landmark of a focused attentional state directed to the salient stimulus.Fondecyt (to GM) 1080220

S4.3 Optical imaging of tecto-isthmic network activity in the chick brain slice
Harald Luksch, Dept. of Animal Sciences, Technische Universität München.
The network that consists of the avian optic tectum and the various nuclei of the isthmic area has been thoroughly analysed on both anatomical and physiological terms over the last years. Through a combination of approaches and model systems ranging from pigeons to owls and from slice work to in-vivo recordings, a picture has emerged that attributes a fundamental role in bottom-up attentional processing to this network. This function is not restricted to visual computation, but (taking into account the tectal role as a multisensory

spatial center) deals predominantly with spatial coordinates to identify potential targets through a saliency-based process.
Recent electrophysiological as well as modelling studies have suggested that a variety of mechanisms is implemented in the tecto-isthmic network, ranging from stimulus competition and attentional switching to inhibition of return and novelty preference. Most of these functions are however mechanisms that comprise the entire network and are hard to evaluate from single-cell recordings, but require the analysis of the network's spatio-temporal activation pattern. To this end, we have taken to optical imaging of voltage-dependent dyes in a slice preparation where the tecto-isthmic network is largely intact. By mimicking sensory input with patterned electrical stimulation, we can record the network activation upon various stimulation protocols and evaluate whether the current models of tecto-isthmic circuitry are valid.

S4.4 Action potential propagation in complex geometries: Experiments and simulations.

Juan Carlos Letelier[1], Bryan Raynaert[1], Oscar Hernández[2]
[1] Departamento de Biología, Facultad de Ciencias Universidad de Chile.
[2] Facultad de Medicina, Universidad del Norte, Barranquilla, Colombia
The Hodgkin-Huxley model (HH) is an essential part of the neurophysiologist's toolbox, but it is used mostly for qualitative assessments. In effect it is not simple to work with non-linear partial differential equations. But since the early 1990´s a family of high quality, robust and fast neural simulation programs have been introduced. These simulation tools opened new avenues of research and now experimental results can be checked against neural models. In this talk we will show how NEURON (www.neuron.yale.edu) can be used to simulate the propagation of action potential in a complex geometry: the so called paintbrush axon found in the avian optic tectum. Our simulation used an 800+ segment model of such axons. We will show how the very unique profile of the extracellular responses generated by such axons can be explained by the propagation of action potentials in such unique morphology. In addition we will model the effect of Kv3.1 conductance on the propagation of action potentials. We hope to stimulate the interest of neurophysiologists in the field on realistic neural simulations.

Simposio 5: Jueves 30, 16:30-18:30 Coordina: Dr. Bernardo Castellano

S5.1 IMMUNE ACCESSORY FUNCTIONS OF ACTIVATED AND PHAGOCYTIC MICROGLIA IN THE INJURED CNS: THE FACIAL AXOTOMY MODEL

G. Raivich
Perinatal Brain Repair Centre, Department of Obstetrics and Gynaecology, Department of Anatomy, University College London, UK (g.raivich@ucl.ac.uk)
Neural injury triggers wide-ranging inflammatory changes in neighbouring glia (astrocytes, microglia), and the recruitment of leukocytes; activated and phagocytic microglia play a leading role in these diverse effects. Microglial activation itself shows a generally tri-phasic process, with early activation within hours to neuronal injury (A), followed by homing and proliferation onto the damaged but surviving neurons (B) and removal of dying neurons and their debris (C).
Early microglial activation takes place under strict neuronal control, depending on neuronal expression of early regeneration-associated transcription factors (Jun, STAT3), neuronal cytokines such as IL6, but also permissive glial factors such as MCSF and TGF beta1. Deletion of these early cytokines will block the TGFb1-dependent proliferation, MCSF-dependent homing and the IL6-dependent microglial swelling (engorgement) that coincides with first expression of antigen presenting machinery (MHC1, B7.2). The latter 2 cytokines are also critical for T-cell recruitment into the axotomized facial motor nucleus, in the case of MCSF, the receptors are only found on microglia pointing to a circumscribed microglial role in posttraumating immune surveillance.

Neuronal cell death ushers a 3rd phase of microglial activation, with phagocytic microglia transforming into professional antigen presenting cells, expressing very high levels of MHC and costimulatory factors and creating a scaffold, to which numerous invading T-cells attach. These responses are not cytokine dependent, but they are associated with TNFalpha and IL1beta-dependent bystander activation of neighboring, non-phagocytic microglia which may play a role in suppressing immune response to non-phagocytic self-antigens.

S5.2 REGULATION OF MICROGLIAL ACTIVATION: ROLE OF IL-15

D. Gomez-Nicola
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IL-15, a pro-inflammatory cytokine involved in regulating the response of T and B cells, also appears to play a key role regulating early inflammatory events in the nervous system. We have shown that IL-15 is expressed *in-vivo* after microglial and astroglial activation with LPS, and that its activity blockade is an effective strategy to control the development of the gliotic response. Similarly, and using a neuropathic pain experimental model, we observed that IL-15 controls macrophage and T-cell recruitment in the damaged peripheral nerve, leading its blocking strategies (i.e. gangliosides or blocking antibodies) to a reduction of the pro-inflammatory scene.
Moreover, using a cell culture model of inflammation with LPS and IFNg we evaluated the role of IL-15 in the initial events of the proinflammatory response of microglial cells. Our results indicate that blockade of IL-15 bioactivity, both with specific blocking antibodies or with the ganglioside neurostatin, inhibited the activation of the NFkB, p38 and ERK1/2 pathways. The major consequence of these inhibitory effects, analyzed using cytokine antibody arrays, was a severe decrease in the production of the chemokines, cytokines and growth factors, essential for the development of microglial and astroglial reactivity.
In conclusion, the IL-15 system activation seems a necessary step for the development of glial reactivity and the regulation of the physiology of glial and neuronal cells. Modulating IL-15 activity opens the possibility of developing new strategies to control gliosis and, therefore, CNS injury repair.

S5.3 MICROGLIAL CELLS REGULATE THE ADAPTIVE IMMUNE RESPONSE IN ACUTE EAE BY MODULATING THE LYMPHOCYTIC FUNCTION

B. Almolda[1]; M. Costa[2]; M. Montoya[3]; B. González[1]; B. Castellano[1]
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Acute experimental autoimmune encephalomyelitis (EAE) induced in Lewis rat by myelin basic protein (MBP) immunization is a well-established model of multiple sclerosis characterized by a unique peak of disability with inflammation but no demyelination. A wide number of reports have described microglial reactivity and T-cell infiltration after EAE, however major part of studies are focused on the peak, and aspects taking place during the inductive and recovery phases are poorly understood. The aim of our study was to perform a detailed analysis of microglial activation and their relationship with different infiltrated T-cell populations along the evolution of the disease. MBP-injected rats were sacrificed at inductive, peak and recovery phases, attending exclusively to their clinical-score. Spinal cords were analysed by flow-cytometry and immunohistochemistry. During the inductive phase and peak, in parallel to high infiltration of CD3+ T-cells (Th1 phenotype), microglia became reactive and a subpopulation displayed an immature dendritic-cell-like phenotype (CD1+/MHC-class I+/MHC-class II+/B7.1-/B7.2-) which may induce an apoptotic/anergic signal to infiltrated lymphocytes. During the recovery phase, there was an important reduction in Th1 cells, concomitant with a progressive increase in Th17 and T-reg populations. Microglia remained highly activated and a subpopulation around blood vessels displayed a CD1+/MHC-class I+/MHC-class II+/B7.1-/B7.2+ phenotype suggesting that they can act as antigen presenting cells. In conclusion, our results indicate that microglial cells may regulate the dynamics of lymphocyte subpopulations and may be involved in leading

the resolution of the pathological process and the induction of the subsequent tolerance which is characteristic of this EAE-model.
Supported by: the Fundació Marató TV3, Fundación Alicia Koplowitz and BFU2008-04407/BFI.

S5.4 IMMUNOMODULATION AS A THERAPEUTIC APPROACH FOR ALZHEIMERS: THE ROLE OF TGFβ

R. von Bernhardi
Depto. Neurología, Facultad de Medicina, Pontificia Universidad Católica de Chile.

Microglia are the main immune effectors of the brain. However, besides protective functions, microglia undergo cytotoxic activation, and appear to mediate damage in Alzheimer's disease. Astrocytes and Neurons modulate microglial cytotoxic activation through secretion of transforming growth factor β (TGFβ). On the other hand, TGFβ is increased in the brain of aging personas and Alzheimer disease (AD) patients, but the expression of Smad3, its main effector pathway, is reduced; leading us to think that changes on TGFβ-dependent signal transduction pathways could lead to changes on the activation pattern of microglia. We assessed TGFβ Smad3 signaling pathway in WT and APP/PS1 animals during aging and after being subjected to acute and chronic inflammatory stimuli, determining the participation of TGFβ Smad3 pathway on microglial cell activation, phagocytosis receptor pattern expression and uptake of Aβ. We found that whereas Smad3 pathway is induced after acute inflammatory activation, it shows no change or is reduced in mice exposed to chronic stimulus. Chronic inflammatory stimuli also increase hippocampal TGFβ, and decrease in phospho-Smad3 levels, differences that are more robust in female APP/PS1 mice. In association with inhibition of Smad3, hippocampal Aβ plaques are increased in transgenic mice exposed to chronic inflammatory conditions. In culture, TGFβ increased Aβ uptake by microglia, effect that depend on the activation of Smad3. Our results suggest that TGFβ-Smad3 pathway is relevant for microglial cells regulation. Changes on Smad pathway by aging and inflammatory conditions appears to be relevant for the pathogenesis of AD.
Supported by: Fondecyt 1090353 and NIH R03 TW008019 to RvB

Simposio 6: Jueves 30, 16:30-18:30 Coordina: Dr. Jorge Mpodozis

S6.1 Why do aquatic animals have a vomeronasal system?

¿Por qué los animales acuáticos tienen un sistema vomeronasal?

Eisthen H Department of Zoology, Michigan State University

The vomeronasal system is an olfactory subsystem that is present in tetrapods, the group of vertebrates that includes amphibians, reptiles, and mammals. Its function is unclear. Some researchers have suggested that the vomeronasal system is specialized for detecting pheromones, chemicals that are released by an animal and which have specific behavioral or physiological effects on members of the same species. Nevertheless, the vomeronasal system detects odorants that are not pheromones, and some pheromonal effects are mediated by the main olfactory system. Another hypothesis is that the vomeronasal system arose in terrestrial vertebrates to detect large, non-volatile molecules, whereas the main olfactory system detects small, relative volatile molecules. This idea is consistent with the anatomy and physiology of the sense organs in some reptiles and mammals, but appears to be contradicted by the presence of vomeronasal and olfactory organs in amphibians that live in water, where volatility is irrelevant. Further, recent data indicate that separate olfactory and vomeronasal receptor neurons are intermingled in the olfactory epithelium of teleost fishes and probably lampreys. We are conducting experiments to try to understand how and why these two populations of neurons came to form separate sense organs and neural pathways in tetrapods. I will present a developmental model that may explain how the two systems became separated. In addition, I will discuss recent data from our lab in which we are examining odorant responses from the vomeronasal and olfactory organs in axolotls (*Ambystoma mexicanum*), a type of aquatic salamander.

Supported by grants from the US National Science Foundation (IBN 9982934 and IOS 0817785).

S6.2 Ecological contingencies and anatomical trends in the vomeronasal system of Caviomorph rodents.

Contingencias ecológicas y patrones morfológicos en el sistema vomeronasal de roedores caviomorfos.

Jorge Mpodozis and Rodrigo Suárez. Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

Social and sexual interactions in tetrapods rely heavily on the communication by semiochemicals. In most mammals, the vomeronasal organ contains two populations of sensory neurons that project to glomeruli of either the rostral or caudal subdomains of the accessory olfactory bulb (rAOB and cAOB), respectively. The rAOB-projecting neurons express Gai2 protein and are activated by volatiles in inter-sexual (male-female) chemoinvestigations. The cAOB-projecting neurons, in turn, express Gao and are activated by non-volatiles, mostly in same-sex (mostly male-male) interactions. Most of the literature about the mammalian AOB comes from studies of muroids (rats, mice and hamsters). The AOB subdomains of muroids are indistinguishable in shape and size, forming a continuous lentiform structure whose boundary can only be depicted with histochemistry. We compared glomerular morphometry at the AOB of capybaras (Hydrochoerus hydrochaeris) and degus (Octodon degus), two Caviomorph rodents with remarkable contrasts in lifestyle: humid vs. arid environments, crepuscular vs. diurnal habits, oily vs. volatile scentmarking, and male-male vs. male-female chemosignalling, respectively. We found some traits shared only amongst Caviomorpha, such as the lateral vomeronasal innervation of the AOB and cell indentation between AOB subdomains, and traits proper to each species we attribute to particularities of lifestyle, such as a rostral-biased glomerular heterogeneity in degus vs a caudal-biased heterogeneity in capybaras.

We propose that these anatomical trends may reflect plastic traits related to aspects of natural history and that they could be also found in other species within Caviomorpha and other mammals

Supported by Fondecyt 1080094 to JM.

S6.3 Evolution of sexual dimorphisms and multiple events of sensory loss in the vomeronasal system of Eutherians.

La evolución del dimorfismo sexual esta asociada a múltiples eventos de perdida sensorial en el sistema vomeronasal en Eutherios.

Rodrigo Suárez, Pedro Fernández and Jorge Mpodozis. Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

The accessory olfactory bulb (AOB), the first synaptic loci of the vomeronasal system, is remarkably diverse across mammals. It ranges from very large in Caviomorph rodents, to very small in carnivores and ungulates, to its complete absence in Old World primates, some bats, and marine mammals. In opossums, rodents and rabbits, two populations of neurons send segregated projections to rostral and caudal domains of the AOB. These neurons express differentially Gai or Gao proteins and are activated in volatile/intersexual or nonvolatile/same sex contexts, respectively. Recent studies showed that the Gai-pathway was exclusively expressed throughout AOB glomeruli in shrews, goats, horses, dogs and monkeys, without Gao-expression. However, the conditions that may have allowed for the deterioration of the Gao pathway in the VNS remain unknown.

We noticed that species with a Gai-only AOB (Laurasiatheres and Primates) all show visually conspicuous sexual dimorphisms. We reasoned that the ability of detecting other males at distance (thus reducing the risk of aggression) would have allowed the deterioration of the Gao-pathway by disuse. To support our hypothesis, we studied G-protein expression in the AOB of species that independently evolved visually conspicuous sexual dimorphisms, and found that the California ground squirrel (Spermophilus beecheyii; Rodentia:Sciurognathi) and the rock hyrax (Procavia capensis; Afrotheria:Hyracoidea), both

diurnal species nested in dimorphic lineages, have also lost the Gao-pathway, with Gai-expressing glomeruli only, throughout the AOB. These results suggest that the deterioration of the Gao-pathway has occurred at least four times independently in Eutherian lineages that evolved sexual dimorphisms.
Supported by Fondecyt 1080094 to JM.

<u>Simposio 7</u>: Jueves 30, 16:30-18:30 Coordina: Dr.Claudio Hetz

S7.1 Shaping an asymmetric brain nucleus in the embryonic zebrafish.
Carmen Lemus, Karina Palma, Alicia Colombo, Lorena Armijo, Steffen Härtel and Miguel Concha. ICBM, Faculty of Medicine, Universidad de Chile, Santiago, Chile.
Despite our increasing understanding of the genetic and morphogenetic mechanisms giving rise to individual neurons, we know very little of how neuroepithelial cells reorganise during development to form supra-cellular structures such as brain nuclei. In this study, we developed and applied non-parametric mathematics imaging tools to *in vivo* 3-dimensional microscopic data sets to unravel the transformations of neuroepithelial cell morphology and topology underlying nucleus formation in the embryonic zebrafish brain. Precursors of the parapineal nucleus initially arrange as bilateral stripes of diencephalic roof plate cells, and during nucleus formation contract and re-align their axis of apico-basal elongation to assemble as a compact cluster in the shape of a three-dimensional rosette. Cellular changes leading to rosette formation emerge in a discrete temporal window and precede detachment of the parapineal nucleus from the roof plate and pineal complex, a process that also involves the concurrent formation of a cellular rosette within the pineal anlage, the protrusive activity of cells at the parapineal-pineal interphase, and the deposition of extracellular matrix protein laminin. Genetic analyses indicate that rosette formation and nucleus detachment both require the activity of Fgf signalling, and resemble the process of sensory organ morphogenesis in the peripheral nervous system. In addition, Fgf signalling is required for subsequent left-sided migration of the parapineal nucleus. Our results reveal an unexpected mechanism by which neuroepithelial cells segregate from the neural tube to form nuclei in the central nervous system, and demonstrate that neuroepithelial cells undergo events of supra-cellular organisation homologous to other embryonic epithelia.
Grant sponsors: HHMI, ICM P07-048-F, Fondecyt 1090242 & 1090246, EU FP6-2004-NEST-PATH EDCBNL.

S7.2 Regulation of axonal growth and guidance by *hindsight* (*pebbled*) in the *Drosophila* visual system.
Carlos Oliva and Jimena Sierralta. ICBM, F. of Medicine, Universidad de Chile.
Once a cell is committed to its fate, transcriptional programs are started that will guide the morphological changes that allow the cell to acquire its mature shape. For neurons this is a crucial step since it will permit the acquisition of a proper connectivity and position in the brain. The genes that are important for this process are not completely known. We have used a misexpression screen to discover genes involved in neuronal morphogenesis in the *Drosophila* adult nervous system using as a read out the complex morphology of the dorsal cluster neurons labeled by the atonal driver. We identified the gene *hindsight* (*pebbled, hnt*), which encodes a transcription factor know for its role in early development (with functions linked to morphogenetic processes such as the embryonic germ band retraction and the dorsal closure in embryos) and in the retinal development (affecting the cell morphology, planar cell polarity and epithelial integrity). We describe here an unreported function for HNT by showing that both loss and gain of function of HNT affects targeting, growth and guidance of the axons of the photoreceptors. Manipulating the time of the development of the overexpression, the number of cells over-expressing HNT or diminishing partially the expression we show that the axonal phenotype is independent of the defects reported in cell morphology and cell polarity of retinal cells. Based on genetic interaction experiments conducted we propose that HNT function in axonal development through the

regulation of genes involved in the dynamic of actin cytoskeleton upstream of the RhoGTPase pathway.
Financed by NEMO-ICM-P07-048-F an FONDECYT 1090272

S7.3 Organelle Stress and Protein Misfolding Disorders affecting the Nervous System

Claudio Hetz *FONDAP Center for Molecular Studies of the Cell CEMC, Institute of Biomedical Sciences, and NEMO, Faculty of Medicine, Universidad de Chile.*

The most common neurodegenerative diseases, such as Alzheimer's Disease, Parkinson's Disease, Amyotrophic lateral sclerosis, and Huntington's disease, affect millions of people worldwide, but there is neither preventive nor curative therapy for them. These diseases share a common neuropathology, primarily featuring the presence of abnormal protein inclusions containing specific misfolded proteins. Recent evidence indicates that alteration in organelle function is a common pathological feature of protein misfolding disorders. The endoplasmic reticulum (ER) is an essential compartment for protein folding, maturation, and secretion. Signs of ER stress have been extensively described in most experimental models of neurological disorders. ER stress is caused by functional disturbances, which result in the accumulation of unfolded/misfolded proteins at the ER lumen. To cope with ER stress, cells activate an integrated signaling response termed the Unfolded Protein Response (UPR), which aims to reestablish homeostasis through transcriptional upregulation of genes involved in protein folding, quality control and degradation pathways. In this talk we overview our efforts to assess the role of ER stress in protein misfolding disorders, and discuss possible strategies to target the UPR with therapeutic benefits.
This work was supported by the FONDECYT no. 1070444, FONDAP grant no. 15010006, Millennium Nucleus no. P07-048-F, High Q Foundation-CHDI, Michael J. Fox Foundation for Parkinson's Research, and ICGEB

S7.4 In vivo approaches to the study of the role of transcriptional regulators in the development of the brain cortex.

Patricio Fuentes, José Cánovas, F. Andrés Berndt, Yerko Fuentealba, Manuel Kukuljan. Faculty of Medicine, Universidad de Chile, Santiago, Chile and NEMO.

Our laboratory is interested in understanding the transcriptional programs underlying neural development, and in particular the basis for the acquisition of the vast array of cell types, morphological and connectivity patterns in the mammalian brain. We use in utero electroporation (IUEP) of cortical progenitors of the ventricular zone of the mouse brain in order to manipulate gene expression and evaluate its consequences in pyramidal neuron differentiation. We studied REST/NRSF, a transcriptional repressor that recruits chromattin remodelling machinery to a miriad of genes,which has been ascribed important roles in the repression of neural fate in nonneuronal cells, including neural progenitors as well as in the regulation of gene expression in neurons. These roles have been postulated on the basis of in vitro and in silico approaches, but a clear picture of the in vivo function of this system is lacking. IUEP of cortical progenitors with shRNA targeting REST/NRSF did not associate to significant perturbations of pyramidal neuron development. Nevertheless, the loss of function of its corepressor CoREST lead to a marked impairment of cell migration and acquisition of normal pyramidal neuron morphology, without a noticeable effect on cell identity or proliferation. The effects of CoREST loss of function were rescued by the expression of CoREST constructs lacking the characterized domain of interaction with REST/NRSF. CoREST forms complexes which comprise histone demethylase LSD1; CoREST moeties lacking the LSD1 interacting domain were not able to rescue de defects caused by CoREST loss of function. These results indicate that CoREST function, in association with LSD1, but not with REST/NRSF is required for the expression of the physiological developmental program of cortical pyramidal neurons.
ICM P-04-068-F, FONDECYT 1090281.

<u>Simposio 8</u>: Viernes 1, 11:30-13:30 Coordina: Dr.Alejandro Múnera

S8.1 "Short- and long-term plasticity of vibrissal primary motor cortex synaptic inputs"

Alejandro Múnera
Profesor Asociado, Departamento de Ciencias Fisiológicas, Facultad de Medicina,
Universidad Nacional de Colombia, Bogotá, Colombia

Diverse inputs evoke stereotypical responses in vibrissal primary motor cortex (CxM1v). CxM1v evoked responses are characterized by a brief □40 Hz oscillation; when elicited by electrical stimulation of the somatosensory pathway such oscillation is preceded by a short-latency population spike, but not when elicited either by auditory or contralateral CxM1v stimulation. Extracellular unit recordings show that CxM1v layer V pyramidal neurons firing probability is maximal during the rising phase of evoked gamma oscillations. Moreover, the increase in layer V pyramidal neuron firing rate is directly correlated with an increase in contralateral intrinsic whisker-pad musculature activity. These findings indicate that evoked gamma oscillations are related with the generation of motor commands by the CxM1v. Paired-pulse stimulation experiments show that evoked gamma oscillations facilitate the processing of incoming somatosensory inputs but temporarily prevents the generation of new motor commands, probably by eliciting protracted inhibitory activity. Theta rhythm-patterned 100-Hz tetanic stimulation in structures sending somatosensory-related inputs, but not in the contralateral CxM1v, induces long-term potentiation of the CxM1v evoked gamma oscillation. These findings indicate differences in the plastic properties of CxM1v inputs. Contingent presentation of a binaural tone preceding an electric shock delivered to the whisker-pad elicits the acquisition of conditioned vibrissal protraction. In parallel, there is a potentiation of tone-evoked potentials in the CxM1v, which amplitude is highly correlated with the amplitude of the conditioned vibrissal movements. This finding shows that CxM1v evoked responses have a high degree of learning-related plasticity which is associated with increased efficiency of cortical motor commands. Financed by Colciencias Grant Nº 110145221092.

S8.2 "Facial nerve lesion-induced changes in electrophysiological and morphological properties of contralateral vibrissal primary motor cortex pyramidal neurons"

Julieta Troncoso
Profesora Asociada, Departamento de Biología, Facultad de Ciencias, Universidad Nacional de Colombia, Bogotá, Colombia

Peripheral nerve injuries are associated with variable degree sensory and motor function losses. Most peripheral nerve lesions are reversible; however, in a significant amount of patients, permanent functional deficiencies remain as irreversible sequelae, which could be due to inadequate sensorimotor cortex reorganization. To test this, facial nerve lesion-induced morphological and electrophysiological changes in motor cortex pyramidal neurons were evaluated in adult rodents. Both, mandibular and buccal facial nerve branches, that innervate whisker-pad intrinsic musculature, were transected. Contralateral vibrissal primary motor cortex (CxM1v) pyramidal cells were evaluated 1, 3, and 5 weeks after nerve transection, and were compared with that of non-lesioned animals. Electrophysiological changes were evaluated using unitary extracellular recording in anesthetized rats, and structural changes were evaluated using the Golgi-Cox stain or two-photon confocal fluorescence imaging. The following facial nerve lesion-induced changes in electrophysiological properties of CxM1v layer V pyramidal neurons were found: transient decay on basal firing rhythmicity; increased basal firing frequency; transient enhancement of the maximal firing frequency elicited by somatosensory stimulation; and, shortening of the silence time between the initial burst and the rhythmic rebound in somatosensory evoked responses. Additionally, facial nerve injury induced the following morphologic

changes in CxM1v: global dendritic branch pruning of layer V pyramidal neurons; diminished spine number in apical and basal secondary dendrites of layer V pyramidal neurons; and a significant augmentation of microglial cell density surrounding pyramidal neurons. Taken together; our data indicate that facial nerve injury induces functional and morphological reorganization in contralateral CxM1v layer V cells. Financed by Colciencias Grant Nº 110145221092.

S8.3 "Changes in neuronal and glial plasticity markers expression in the sensorimotor cortex induced by peripheral facial nerve lesion"

Efraín Buriticá
Profesor Auxiliar, Departamento de Morfología, Facultad de Salud, Universidad del Valle, Cali, Colombia

Preliminary evidence suggests that peripheral facial nerve injuries are associated with sensorimotor cortex reorganization. The expression of neuronal and glial markers of plasticity was followed after reversible facial nerve lesions to evaluate the degree and extension of such nerve lesion-induced reorganization processes. The mandibular and buccal branches of the facial nerve were crush injured. Immunoreactivity to NeuN (neuronal nuclei antigen), GAP-43 (growth-associate protein 43), and GFAP (glial fibrillary acidic protein) in both hemispheres was evaluated 1, 7 , and 14 days after the lesion and was compared to that of control and sham-operated subjects. Patches of decreased NeuN immunoreactivity were found bilaterally in vibrissal primary motor cortex (CxM1v) as well as in primary somatosensory cortex (CxS1) both at 1 and 7 days after facial nerve lesion; such pattern was partially reverted 14 days after the lesion. Significant increased GAP-43 immunoreactivity were found bilaterally one day after the lesion in hippocampus, striatum, and sensorimotor cortex; these changes waned after seven days and recurred after 14 days. One day after lesion GFAP immunoreactivity increased bilaterally in hippocampus, subcortical white matter of sensorimotor cortex, CxS1 laminae V and VI, and CxM1v whole thickness; these changes waned progressively thereafter, remaining restricted to the lamina V of CxS1 and CxM1v. Taken together these findings demonstrate that unilateral reversible facial nerve lesions induce a widespread bilateral reorganization of sensorimotor cortices encompassing transient neuronal decay, axon sprouting, and astrocytic activation. Moreover, this reorganization process spreads beyond somatosensory cortex, reaching at least the hippocampus and striatum. Financed by Colciencias Grant Nº 110145221092.

<u>Simposio 9</u>: Viernes 1, 14:30-16:30 Coordina: MS Zulma Dueñas

S9.1 EFFECTS OF CHRONIC STRESS ON THE SEIZURES EXPRESSION IN ANIMAL MODELS OF EPILEPSY.

Milena García. Facultad de Psicología. Universidad de la Sabana. Sede Bogotá

Some reports of literature as much show an increase in the frequency and gravity of the epileptic seizures associated to stress in patients and in animal models. Nevertheless, other studies also exist in which east effect is not observed, or it even describes to a diminution in the intensity of the seizure. The aim of this work was to identify the possible effects of the exposition to experimental chronic stress in the behavioral expression of audiogenic seizures in Audiogenic Wistar Rats (WAR) or seizure induced by the intra-hippocampal injection of the nonselective agonist muscarinic cholinergic pilocarpine in Wistar animals, as well as in the expression of glucocorticoids receptors in the brain of both rat groups. For this, the animals were exposed to immobilization stress by one hour, twice to the day during 10 days, after was induced the audiogenic seizure to WAR animals or were implanted with a hippocampal left cannule (AP: -6.0; ML: +4.5; DV: -4.5). 48 hours later, the animal received intra-hippocampal injection of pilocarpine (2.4 mg/ul) for the induction of the status epilepticus, were recording the latency of the beginning of the crisis, as well as the indices of gravity of the same during 90 minutes. Later, their brains were removed and make an immunohistochemistry for glicocorticoids receptors type II. The preliminary results

showed that chronic stress by immobilization produces an increase in the latency of beginning of the status, without being observed differences in the indices of gravity of the seizures.

S9.2 EFFECTS OF ACUTE STRESS ON THE ACQUISITION, RECOVERY AND EXTINCTION OF SPATIAL MEMORY IN THE BARNES MAZE

Marisol Lamprea
Grupo de Neurofisología Comportamental Universidad Nacional de Colombia. Facultad de Ciencias Humanas Psicología Sede Bogotá

Stress and glucocorticoids produce different effects in memory tasks. In this study, we showed that acute stress applied before the training, recovery or extinction impairs differentially the spatial memory in the Barnes maze. When rats are exposed to one or four hours of behavioral restriction before training, they showed an increase in hole errors and latency to reach the goal hole during the recovery phase. When applied before recovery, stress produces a decrease in the persistence to explore the goal hole when the escape box is absent. Finally, stress applied before extinction (without the escape box), produces an increase in the latency to reach the goal hole during the eight extinction trials. These results suggest that stress impairs the consolidation of the spatial task and the long time recovery of spatial memory, probably affecting the normal function of the hippocampus and related structures. Financed by Colciencias Grant Nº 110140820494

S9.3 EFFECTS OF STRESS INDUCED BY EARLY MATERNAL SEPARATION ON MEMORY, DEFENSE BEHAVIOR AND GABA-A RECEPTORS EXPRESSION.

Zulma Dueñas. Facultad de Medicina. Grupo de Neurofisiología Comportamental. Universidad Nacional de Colombia – Sede Bogotá

It is has been demonstrated that disruption of mother-pup interaction during early life exert long-lasting effects on the development and behavioral responses. The use of animal models allows an experimental approach to identify the potential consequences and the mechanisms involved in the alterations in both behavior and the development of individuals who have experienced early stress caused by a rupture of that relationship. Changes in GABA A receptors (GABAAR) have been proposed as a potential mediator of behavioral responses to stress. Here we are showing that maternal separation affects the memory process during adulthood, defense behavior and GABAAR expression in different brain areas and those are gender-dependent effects. For behavioral studies we use a Barnes and elevate plus maze (EPM) labyrinths. GABAAR expression was detected with immunohistochemistry and levels of corticosterone were measured using ELISA. The results show that females generally gain faster target hole location and the maternal separation favors them, while males who are separated, show learning difficulties. Additionally, separated females recover the spatial task better after 24 hours and separated males have very poor recovery. In the EPM separated females have more open arm entries and spent more time there, deeper and less grooming, in comparison with females and with males of the experimental and control group. The inmunohistochemistry shows less inmunoreactivity for anti GABAAR in the amygdala, hippocampus and orbitfrontal frontal cortex from rats that were separated in comparison with the control group. Finally, non-separated females have higher plasma corticosterone levels than separated females and males.

<u>Simposio 10</u>: Viernes 1, 16:30-18:30 Coordina: Dr. Adrián Ocampo

S10.1 "What activates the ascending activating system nuclei?" Fernando Torrealba Dep. Fisiología, Facultad Cs Biológicas, P. Universidad Católica de Chile

The level of arousal in mammals is controlled by an ensemble of interconnected subcortical nuclei which modulate the neural activity of the dorsal thalamus and cerebral cortex directly, by way of widespread axon terminals that release excitatory aminergic or peptidergic transmitters, and indirectly through intralaminar and midline thalamic nuclei which send widespread glutamatergic axonal projections to lamina I of the cortex. The

activation of this ascending arousal system (AAS) promotes the transition from sleep to wakefulness, and the increase of alertness in awake animals in response to external or internal stimuli. Studies that combined specific lesions to some of the components of the AAS with the use of common behavioral paradigms have provided indications of specific contributions of AAS regions to the performance of rats to the behavioral task. The emerging idea is that the components of the AAS contribute to the efficient performance of the task, but that each of the studied components modulates performance in a different manner. While this view on the specificity of effects of the components of the AAS at their outputs is receiving growing attention, much less studies have been dedicated to explore the inputs that drive the activity of the AAS components and the behavioral instances in which those inputs become active. We will review the similarities and differences in inputs to some of the AAS nuclei and discuss how they may contribute to determine the functions of AAS nuclei. Funded by Núcleo Milenio # P06/008-F & CONICYT Anillo ACT-66

S10.2 "**The infralimbic cortex-tuberomammillary nucleus axis has a central role in motivatedarousal**."

María Eugenia Riveros. Departamento de Fisiología, Facultad de Cencias Biológicas, Pontificia
Universidad de Chile

Goal directed or motivated behaviors have an appetitive and a consummatory phase. The appetitive phase is unimaginable without an elevated energetic state or "motivated arousal". This energized state depends on an increase in brain activity and facilitation of physiological processes that will increase the performance of that behavior. For this to occur it is necessary that the brain regulates its own activity in response to the appropriate stimuli. The ascending arousal nuclei are the subcortical structures that by a diffuse brain innervation are capable of modulating general brain activity and therefore to control arousal level. Two questions arise: Do all these nuclei act in block in every arousing-demanding situation? What cortical structures activate these nuclei when increased arousal is necessary to start a motivated behavior? We will show that histamine and the tuberomamillary nucleus (TMN) have a central role, being the first arousal nucleus to become active at the beginning of appetitive behavior and intact TMN is necessary for arousal to occur in response to an appetitive stimulus. The infralimbic cortex sends excitatory projections to TMN and its normal function is essential for the unfolding of motivated behavior. We believe that the axis conformed by infralimbic cortex-TMN will be activated when other areas of prefrontal executive cortex decide to engage in a motivated behavior and TMN activated by infralimbic excitatory input is essential to supply the arousal component of a motivated behavior giving the behavioral readiness to act in a motivational state. Funded by Núcleo Milenio # P06/008-F & CONICYT Anillo ACT-66

S10.3 Melanin concentrating hormone (MCH): a hypothalamic peptide that promotes sleep Pablo Torterolo. Departamento de Fisiología, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay. The melanin-concentrating hormone (MCH) is a cyclic 19-aminoacid neuropeptide, whose biological function is mediated by two receptors, MCHR-1 and MCHR-2. In rodents, the MCHR-2 gene was found to be a pseudo-gene, while this receptor is functional in humans, monkey and carnivores. MCH is synthesized by neurons in the lateral hypothalamus and incerto-hypothalamic area that project broadly throughout the brain. The MCHergic system has been classically involved in the control of feeding and energy homeostasis. However, recent findings suggest a role of MCH in sleep regulation. In my presentation, I will review our following experimental data: 1. Utilizing immunohistochemistry and retrograde tracing techniques, we demonstrated a moderate MCHergic innervation of the nucleus pontis oralis (NPO) of the cat, the executive area for REM sleep generation. In addition, we showed that microinjections of MCH within the NPO facilitate the generation of REM sleep. 2. We demonstrated a large MCHergic innervation of the dorsal raphe nucleus (DR), a critical region for the generation of

wakefulness and REM sleep. We found in rats that microinjections of MCH into the DR facilitate the generation of REM sleep, while MCH immunoneutralization within this nucleus decreases REM sleep. Furthermore, MCH decreased the firing rate of presumptive serotonergic neurons of the DR; the inhibition of these neurons is necessary for REM sleep generation. Overall, these results indicate that the MCHergic system promotes REM sleep.

S10.4"The hypothalamus and the chronobiology of REM sleep in the rat". Adrián Ocampo-Garcés. Programa de Fisiología y Biofísica,ICBM, Facultad de Medicina, Universidad de Chile Any description of REM sleep timing should integrate three main chronobiological processes: i. circadian processes; ii. REM sleep homeostatic (hourglass) processes; and iii. masking phenomena. Here we discuss current evidences supporting the involvement of several hypothalamic nuclei in each process. Solid evidences relate the circadian organization of REM sleep to a neural network that includes the main Light Entrainable Oscillator located in the suprachiasmatic nucleus of the hypothalamus. Direct and indirect axonal projections from the suprachiasmatic nucleus innervate a putative subordinate ciradian oscillator (dorsomedial hypothalamic nucleus) and sleep-wake state generators through circuits that include the orexinergic, MCHergic neurons of the lateral hypothalamus, and GABAergic-galaninergic neurons located in the pre-optic hypothalamus. REM sleep homeostasis is evidenced by an almost complete REM sleep recovery after total and selective REM sleep deprivation in the rat. Preoptic hypothalamic neurons has been described as keeping track of REM sleep debt, suggesting that they could operate as an homeostatic sensor for REM sleep. Masking (direct influence) of environment on REM sleep generators has been described. We present evidences that photic masking of REM sleep is enhanced after neurotoxic (saporin-orexin) lesions in the lateral hypothalamus. Because REM sleep occurrence depends od the inhibition of Non-REM sleep and wakefulness state generators, current models face mayor dificulties in describing how hypothalamic networks integrate multiple state requirements in a single-state command (Fondecyt 1061089).

COMUNICACIONES LIBRES
1. Miércoles 29 16:30-17:30

CL1.1 PARTICIPACIÓN DE GLUT3 EN LA MODULACIÓN DEL TRANSPORTE DE GLUCOSA NEURONAL POR ÁCIDO ASCÓRBICO. GLUT3 INVOLVEMENT IN NEURONAL GLUCOSE TRANSPORT MODULATION BY ASCORBIC ACID. Beltrán F A, Acuña A I, Miró M P, Angulo C, Concha I I and Castro M A. Instituto de Bioquímica, Universidad Austral de Chile. Intracellular ascorbic acid (AA) is able to modulate neuronal glucose utilization between resting and activity periods. We observed that glutamatergic activity induces AA depletion from astrocytes and other cerebral reservoirs. AA is further removed from the extracellular space by neurons through the sodium/vitamin C transporter 2 (SVCT2). According to the astrocyte-neuron lactate shuttle hypothesis (ANLSH) glucose consumption in neurons occurs under resting conditions, whereas active neurons display a preference for lactate. We have previously demonstrated that intracellular AA inhibits deoxyglucose transport in cortical and hippocampal neuron primary cultures and HEK293 cells, while this effect could not be observed in astrocytes. Since this observation was valid only for cells expressing glucose transporter 3 (GLUT3), we evaluated the importance of this transporter on the inhibitory effect of AA on deoxyglucose transport. We analyzed the expression and function of glucose transporters and AA transporters in rat cortical astrocyte primary cultures and the rat glioma cell line C6. Then, the effect of AA over deoxyglucose transport was tested on astrocytes and astrocytes expressing GLUT3-EGFP. Intracellular AA was able to inhibit deoxyglucose transport only in astrocytes expressing GLUT3-EGFP. In C6 cells, which natively express GLUT3, the same effect on deoxyglucose transport was observed. Finally, knocking down the native expression of GLUT3 in primary cultured neurons and C6 cells using siRNA was sufficient to abolish the AA-dependent inhibitory effect on glucose analogues uptake. Therefore, AA would function as a metabolic switch

inhibiting glucose utilization in neurons under glutamatergic synaptic activity through a mechanism that requires GLUT3. FONDECYT 11070065.

CL1.2 Daam1 es necesario para el desarrollo de neuropila y conectividad axonal en el circuito habénulo-interpeduncular de pez cebra Daam1 is required for development of neuropil and axonal projections in the zebrafish habenulo-interpeduncular circuit Colombo A., Palma K., Díaz A., Concha M.L. "Anatomy and Developmental Biology Program, Institute of Biomedical Sciences, University of Chile, Santiago, Chile; and Millennium Nucleus of Neural Morphogenesis."

The establishment of complex neuronal circuits during development depends on the interaction between morphogenetic programs and neuronal activity. Several studies have shown that regulatory proteins involved in remodeling of cytoskeletal components such as actin filaments and microtubules play a central role in the control of axonal and dendritic growth. In this work, we show that Daam1 (Disheveled-associated activator of morphogenesis-1), a formin homology protein involved in remodelling of the actin cytoskeleton, is expressed asymmetrically on the left side of the ephitalamic habenular nucleus. This expression pattern matches temporally and spatially with morphogenesis of asymmetric habenular neuropil, and with the formation of axonal projections from the habenulae to the interpeduncular nucleus. Injection of morpholino antisense oligonucleotides against daam1 induces a reduced and bilaterally symmetric pattern of habenular neuropil, and a defective development of the habenulo-interpeduncular tract, which becomes thinner and shows a disorganised axonal terminal morphology. These results indicate that Daam1 is required for normal development of neuropil and axonal projections in the zebrafish habenulo-interpeduncular circuit. Grant sponsors: HHMI, Fondecyt 11090324, CONICYT (PBCT ACT47), EU FP6-2004-NEST-PATH EDCBNL, ICM (P07-048-F), Wellcome Trust IRDA.

CL1.3 Expresión de MCTs e influjo/eflujo de lactato en células gliales hipotalámicasMCTs EXPRESSION AND LACTATE INFLUX/EFFLUX IN HYPOTHALAMIC GLIAL CELLS. Cortés-Campos C (1), Elizondo R (1), Nualart F (1), García M A (1) (1) Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción Metabolic interaction via lactate between glial cells and neurons has been proposed as the glucosensing mechanism used in the hypothalamus. We have postulate that glial cells also known as tanycytes produce lactate by glycolitic metabolism of glucose and they transfer it to neighboring arcuate nucleus neurons. Lactate stimulates ATP synthesis in these neurons and thus contributes to their activation. Tanycytes are the main glial cells present in the hypothalamus thus it is necessary to demonstrate the presence and function of monocarboxylate transporters (MCTs) in tanycytes and neurons. Therefore, we studied MCT1 and MCT4 expression and their distribution in hypothalamic tanycytes both in vivo and in vitro. Western blot, RT-PCR and Q-RT-PCR analyses demonstrate that MCT1 and MCT4 are expressed in the hypothalamus. Immunohistochemistry for MCT1 displays a high immunoreaction in beta-tanycytes, which contact proximal and medial arcuate nucleus neurons whereas MCT4 was detected in hypothalamic tanycytes, which contact distal arcuate nucleus neurons. Using a primary tanycyte culture, we demonstrate that MCT1 and MCT4 are functional during lactate's influx, and that these glial cells are able to release lactate though MCTs. Because both the expression of MCT1 and MCT4 has been linked to the efflux of lactate, we propose that tanycytes participate in glucose sensing mechanisms based on a metabolic interaction with the neurons of arcuate nucleus which are stimulated using the lactate released by MCT1 and MCT4-expressing tanycytes. This study was supported by FONDECYT 1100705 # and Grant CONICYT # 23070095

CL1.4 Efectos del tratamiento crónico con corticosterona sobre el condicionamiento al miedo y la extinción de memoria. Effects of Chronic Corticosterone Treatment on Fear Conditioning and Memory Extinction Dagnino-Subiabre A (1), Pérez MA (1), Gonzalez N (1), Cheng M (2), Sapolsky RM (2) (1) Laboratory of

Neurobiology and Behavior, Department of Biomedical Sciences, Faculty of Medicine, Universidad Católica del Norte, Coquimbo, Chile. (2) Departments of Biological Sciences and Neurology, Stanford University, Stanford, USA.

Stress is known to have different effects on the acoustic and visual emotional processing. Stress also leads to secretion of corticosterone (CORT), the stress hormone. The aim of this study was to determine whether chronic CORT administration may affect the auditory and visual fear conditioning and memory extinction. Male Sprague-Dawley rats received CORT (400 ?g/ml) in their drinking water during 10 consecutive days. CORT produced a significant decrease of the auditory fear conditioning (% freezing; CORT: 27.8±10.1, control: 80.6±8.6, $p < 0.01$) and impairment of the memory extinction (% freezing; CORT: 68.5±10.1, control: 30.6±9.4, $p < 0.01$), but did not affect the visual fear conditioning. In addition, we analyzed the CORT effects on the neuronal morphology of the inferior colliculus (IC, a key brain area for auditory processing) and superior colliculus (SC, related to visual processing) by Golgi stain. CORT decreased dendritic arborization of the IC neurons (branch points; CORT: 2.8±0.3, control: 5.1±1.1, $p < 0.001$) and did not affect the SC. Finally, using RT-PCR technique we analyzed the glucocorticoid receptor (GR) expression in both IC and SC. CORT down-regulated the GR expression in the IC (pixels; CORT: 14.1±6.5, control: 60.8±15.1, $p < 0.05$) and upregulated the GR expression in the SC (pixels; CORT: 76.5 ± 2.5, control: 3.6±3.5, $p < 0.001$). Our results suggest that CORT has more deleterious effects in the auditory system than visual system and may affect the fear processing in the brain. Comparable alterations could be produced in posttraumatic stress disorder and major depresión." This work was supported by "Anillo de Ciencia y Tecnología" (Nº ADI-09), FONDECYT (Nº 1100413), and INNOVA (Nº 06FC01IBC-71) grants (D-S, A).

CL1.5 Interacción Molecular entre proteínas anomalamente plegadas en Alzheimer y Priones. Molecular cross talk between misfolded proteins in Alzheimer's and Prion diseases. Estrada LD (1,3), Morales R (2), Alvarez A (1), and Soto C (2,3). "(1) Laboratorio de Señalización Celular, Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile. (2) Protein Misfolding Disorders Laboratory, Department of Neurology, Mitchell Center for Alzheimer's Disease and Related Brain Disorders, University of Texas Medical School at Houston, Houston, Texas. (3) Protein Misfolding Disorders Laboratory, Department of Neurology, University of Texas Medical Branch, Galveston, Texas.

More than 20 human diseases are included in the group of Protein Misfolding Disorders (PMDs), including Alzheimer's disease (AD), transmissible spongiform encephalopathies (prions), Parkinson's disease, Huntington disease, diabetes type-II, Amyotrophic Lateral Sclerosis, dialysis-related amyloidosis and others. The common feature of PMDs is the accumulation of misfolded proteins in diverse tissues, forming protein deposits called amyloid. Despite the diversity of clinical symptoms associated with different PMDs, many similarities in their mechanism suggest that distinct pathologies may cross talk at the molecular level. The main goal of this study was to analyze the interaction of the protein misfolding processes implicated in Alzheimer's and prion diseases. For this purpose, we inoculated prions in an Alzheimer's transgenic mouse model that develop typical AD features (Tg2576 mice model). We found that AD and prion pathologies synergistically interact to accelerate the onset of both diseases. Prion clinical signs appeared much faster after infection of animals that were simultaneously developing AD. Moreover, AD transgenic mice infected with prions had a significant higher load of amyloid plaques than non-infected mice. Histological and biochemical studies showed the association of the two misfolded proteins in the brain and in vitro experiments showed that protein misfolding can be enhanced by a cross-seeding mechanism.

These results suggest an interaction between AD and prion pathologies, indicating that one protein misfolding process may be an important risk factor for the development of a second one. Our findings may have important implications to understand the origin and progression

of PMDs. National Institutes of Health Grants AG028821 and NS050349. Mitchell Center for Alzheimer's Disease

CL1.6 Hiperglicemia aguda induce cambios estructurales en las células gliales y vascularización de la eminencia media. Acute hyperglycemia induces structural changes in median eminence glial cells and vasculature "Martínez F (1), Cortes-Campos C (1), Llanos P (1), Cifuentes M (2), García MA (1), Nualart F (1). (1) Laboratorio de Neurobiología y Células Madres, Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción. (2) Departamento de Biología Animal. Facultad de Ciencias. Universidad de Málaga.

The median eminence (ME) is located in the basal region of the third ventricle of the brain. Within the ME exist blood vessels lacking the blood-brain barrier thus allowing diffusion of glucose from the blood into brain tissue. The transfer of glucose is also limited by a second barrier (EM-CSF) formed by specialized glial cells, the tanycytes beta-2. Structural modifications of these cells could regulate the passage of glucose to the CSF, in order to stimulate glucosensing. We evaluated the effect of hyperglycemia on cell architecture and vascularization of the ME. we generate a hyperglycemia condition, via intraperitoneal injections of glucose, in Wistar rats which were sacrificed 30min after injection. The brain tissue was dissected for optical microscopy and electron microscopy analysis. Using 1?m and 40?m sections, we evaluated the presence of vimentin and GFAP in glial cells as well as changes in vasculature (GLUT1+ cells). Furthermore, ultrastructural studies were performed using TEM. We confirmed the presence of blood vessels unrestricted by a barrier in the ME. Hyperglycemia produced a significant increase in the arborization of glial cells in this region. Electron microscopy showed beta-2 tanycytes hyperplasia, increased peri-capillary spaces and increased in vesicles present in endothelial cells. Acute changes in glycemia induced structural changes in the ME which would maximize the transfer of glucose to regions of the hypothalamus involved in glucosensing.

Supported by Grants Ring ACT-02 (to FN and MAG), FONDECYT 1100705 (to MAG) and CONICYT PhD fellowship AT24080111 (to FM).

CL1.7 MODIFICACIONES POSTRANSDUCCIONALES EN LA REGULACION DE LA INACTIVACION DE CANALES DE CALCIO CAV2.3 POST-TRANSLATIONAL MODIFICATIONS IN THE REGULATION OF INACTIVATION OF CAV2.3 CALCIUM CHANNELS Vivian Gonzalez(1,2) , Erick Miranda-Laferte(3), Gustavo Contreras(1,2), Patricia Hidalgo(3) and Alan Neely(2) "(1)Programa de Doctorado en Ciencias Biológicas, Mención Neurociencias (2)Centro Interdisciplinario de Neurociencias de Valparaiso. Universidad de Valparaiso. (3) Institut für Neurophysiologie, Medizinische Hochschule Hannover

The Voltage-dependent calcium channels undergo two type of inactivation that are strongly influenced by accessory protein such as the calcium channel ?-subunit (CaV?) and calmodulin. While calmodulin is a determinant of calcium dependent-inactivation, CaVB regulates voltage-dependent inactivation. In the neuronal type of calcium channel CaV2.3, inactivation is accelerated by all CaV? except the palmitoylated splice variant CaV?2a. The prevalent view is that this membrane-anchoring post-translational modification keeps the inactivation domain from occluding the pore. However, as we recently reported ( ), inactivation of heterologously expressed CaV2.3 channels is slowed significantly by non-palmitoylated CaV??when injected as purified recombinant proteins on Xenopus laevis oocytes already expressing the pore forming subunit. This phenotype was reverted if recombinant proteins were injected together with CaV2.3 cRNA as if CaV? can switch the inactivation phenotype by some posttranslational modifications during channel biogenesis that is not accessible to the membrane anchored CaV?2a. Here we investigate the time course of the phenotype switch and its sensitivity to some kinases and palmitoylation inhibitors and report that, independent on the biogenesis of the pore-forming subunit, the inactivation-accelerating phenotype develops in about 24. Membrane anchored CaV?2a, that slows inactivation independent of the time of exposure, can be reverted to inactivation-accelerating phenotype by a short treatment of bromo-palmitate, a known inhibitor of

palmitoylation. This result suggests that membrane anchoring does not prevent the post-translational modification that confers the inactivation-accelerating phenotype to CaV? but occludes its effect. RED-24 and FONDECYT 1980635 to AN and a CONICYT Fellowship to GC.

COMUNICACIONES LIBRES
2. Jueves 30 10:00-11:00

CL2.1 Efectos de la ingesta de DHA sobre el deterioro que induce el estrés en el hipocampo y memoria de ratas. DHA intake effects on the stress-induced impairment in the rat hippocampus and memory. Pérez MA, Terreros G, Gonzalez N, Dagnino-Subiabre, A
Laboratory of Neurobiology and Behavior, Department of Biomedical Sciences, Faculty of Medicine, Universidad Católica del Norte, Coquimbo, Chile

Environment and diet may have a positive or negative effect on the brain. For instance, stress produces hippocampal dendritic atrophy and memory impairment. Decreased Intake of the docosahexaenoic acid (DHA), an essential fatty acid, produces the same stress effects, while DHA supplementation improves memory. The aim of this study was to evaluate the effects of DHA intake after weaning on the stress-induced both, memory impairment and CA3c hippocampal dendritic atrophy. We used Y-maze to measured memory and Golgi stain to measured the morphology of hippocampal CA3 piramidal neurons of the Sprague-Dawley rats. DHA intake significantly impaired memory of control rats (Different Score = control+DHA -18.11 +/- 5.52, control + Veh: 2.26 +/- 4.8, $p < 0.001$). Conversely, DHA intake increased the memory in stressed animals (Different Score = stress + DHA: 10.40 +/- 5.03, stress + Veh: -0.58 +/- 5.93, $p > 0.05$). At cellular level, DHA intake prevented the stress-induced dendritic atrophy in CA3c pyramidal neurons (stress + DHA: 1974 +/- 284.2 ?m; control + DHA: 1725 +/- 172.5 ?m). Our research shows that DHA intake supplementation in normal subjects may have negative effects on the brain, and positive effects in psychosocial stress and patients with major depression. Also, uncontrolled DHA administration in children's may produce severe cognitive deficit." This work was supported by "Anillo de Ciencia y Tecnología" (Nº ADI-09) and FONDECYT (Nº 1100413) grants (D-S, A).

CL2.2 Canales GABAA en el segmento inicial del axon controlan la excitabilidad de las neuronas granulares del Giro Dentado. GABAA receptors located at the axon initial segment control excitability of dentate granule neurons. Rojas P (1), Eisenman L (2), Mennerick (3). (1) CEAZA and Departamento de Química y Biología, Universidad de Santiago de Chile. (2) Department of Neurology, Washington University in St Louis Medical School. (3) Department of Psychiatry, Washington University in St Louis Medical School.

The site of action potential (AP) initiation is located at the axon initial segment (AIS) where a high density of voltage dependent channels is present, determining both threshold and the shape and frequency of APs. In many principal cells GABAergic inputs to the AIS and soma come from separate types of interneurons. Here we studied the effect of axonal versus somatic GABA receptor activation by local application of an exogenous GABAA receptor agonist to granule cells of the dentate gyrus, the gatekeepers of the hippocampal tri-synaptic circuit. Local application of 5 μM muscimol to the AIS robustly depolarized the voltage threshold for AP initiation (1.7 ± 0.2 mV, N=13) with relatively little change in input resistance (15%). Because presynaptic inputs to dentate granule neurons produce small EPSPs (0.1-0.5 mV), this threshold change is significant to granule cell function. Somatic application did not alter voltage threshold but strongly decreased input resistance (58 %), resulting in a large increase in rheobase. Interestingly, synaptic-like stimulus that were suprathreshold under basal conditions, failed to reach threshold with axonal muscimol application. With sustained current injections, axonal muscimol application decreased the number of APs generated at a fixed current amplitude These results shows that activation of GABAA channels are not restricted to single AP and can

have effects on temporal summation and repetitive firing. In summary, our results show that GABAA channels at the AIS have a role not only on the AP initiation, but can also modulate the firing pattern in granule cells. This work was supported by NIH grant NS54174 (SM), BioTecZA INNOVA-CORFO 06FC01IFC-71 (PR).

CL2.3 La activación de receptores adrenérgicos alpha2 disminuye los niveles extracelulares de dopamina en el septum lateral de la rata: esrtudios de mcirodiálisis con animales despiertos. The activation of alpha2-adrenergic receptors decreases extracellular levels of dopamine in the rat lateral septum: microdialysis studies in freely moving rats. Sotomayor-Zárate, R., Ibañez, M.R., Forray, M.I., Andrés, M.E. y Gysling, K. Millenium Science Initiative Nucleus "Stress and Addiction", Department of Cellular and Molecular Biology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile.

The lateral septum (LS) is a nucleus of the limbic system involved in the control of physiological processes related to cognitive, autonomic and neuroendocrine functions. The LS receives dopaminergic afferences from the ventral tegmental area (VTA).

Previously, we have shown that the increase in LS dopamine induced by systemic morphine depends on the decrease of VTA GABA (Sotomayor et al. J Neurosci Res. 2005). Additionally, we have also shown that systemic amphetamine does not increase LS dopamine due to the activation of LS GABA-B receptors (Sotomayor-Zárate, Araya et al, J Neurochem. 2010).

The purpose of the present study was to determine whether the activation of alpha2-adrenergic heterorepectors could regulate LS dopamine. To this end, a guide cannula was implanted in LS (angle of 14°, 0,2 mm anterior to bregma and 1,8 mm lateral and 3,8 mm ventral). The day of the experiment a microdialysis probe was inserted into the guide cannula. The results show that the infusion of 50 microM clonidine through the microdialysis probe decreases LS dopamine release. Thus, our results indicate that LS dopamine is regulated by the action of GABA-B and alpha2-adrenergic hetero-receptors. We like to suggest that noradrenaline controls LS dopamine, indirectly by increasing LS GABA extracellular levels and directly by activating alpha2-adrenergic hetero-receptors present in LS dopamine nerve terminals. Supported by: MSI N° P06/008-F and FONDECYT 1070340 to KG and FONDECYT 3095007 to R.S-Z.

CL2.4 Efectos de metilfenidato en el laberinto de Morris no son congruentes con plasticidad sináptica en el hipocampo de ratas jovenes Effects of Methylphenidate in the Morris water maze are not congruent with synaptic plasticity in the hippocampus of young rats Zeise ML (1), Arias-Cavieres A (2), Rozas C (3), Loyola S (3), Morales B (3)

(1) Escuela de Psicología, Fac. de Humanidades, Universidad de Santiago de Chile. (2) Fac. de Biología, U. de Valparaiso. (3) Dep. de Biología, Fac. de Química Biología, Universidad de Santiago de Chile Consumption of the psychostimulant Methylphenidate (MPH) in therapy and otherwise has risen in a dramatic way during the last 20 years. However, its direct effects on synaptic plasticity and learning are almost unknown.

We have found earlier that 3,4-Methylenedioxymethamphetamine (MDMA; extasy) in a dose-dependent manner reduces performance in the Morris water maze as well as hippocampal long-term potentiation (LTP), measured in slices from the animals tested before. A similar approach using methylphenidate did not yield parallel results, even though either psychostimulant augments LTP when applied acutely.

24 days old male rats received subcutaneous injections of MPH (2 and 10 mg/Kg) half an hour before being launched into a Morris water maze and latencies to reach a visible or invisible platform were measured. Controls and animals injected the lower dose performed virtually identically, whereas the higher dose led to locomotor unrest producing impairment even in reaching the visible platform. As sessions at the end revealed, in these animals no preference was seen for the quadrant where the platform had been situated, different from controls and rats who had received the lower dose demonstrating retrieval of the learned task. However, in slices from the same animals that had been tested before, LTP was

reduced in the hippocampi (CA1 region) from treated rats with little difference between the 2 mg/Kg and the 10 mg/Kg group.
Our results indicate that visuo-spatial learning is not modified by MPH, whereas synaptic plasticity is affected albeit in a different way when applied acutely or after prolonged administrations. DICYT Nº 020993Z, USACH (MLZ) FONDECYT 1080652 (BM, MLZ)"

PANELES
1. Miércoles 29 14:30-16:30

P1.1 Sobre el mecanismo de la depresión sináptica inducida por CaMKIIN, inhibidor endógeno de CaMKII On the mechanism of synaptic depression by CaMKIIN, an endogenous inhibitor of CaMKII. (1) C. Gouet, (2) B. Aburto, (1) C. Vergara, (1,2) M. Sanhueza (1) Dep. de Biología, Facultad de Ciencias, Universidad de Chile. (2) Instituto de Dinámica Celular y Biotecnología, Universidad de Chile.

Ca2+/Calmodulin-dependent protein kinase II (CaMKII) is intimately involved in learning and memory processes. Its participation in long-term potentiation (LTP), a cellular memory model, has been extensively studied. The relevance for LTP of the complex formed by CaMKII and NMDA receptor (NMDAR) in post-synaptic densities (PSD) has currently came up.
CaMKIIN, an endogenous protein of unknown function, specifically inhibits CaMKII by binding to the kinase´s site of interaction with the NR2B subunit of NMDAR. In different brain structures, including the hippocampus, CaMKII and CaMKIIN co-localize to the same cells. After a fear conditioning protocol, CaMKIIN mRNA is up-regulated in the hippocampus and amygdala. Furthermore, we recently reported that transient application of a peptide derived from CaMKIIN (CKIIN), made cell permeable by fusion to the antennapedia sequence, produces a persistent synaptic depression and facilitates further induction of LTP. Here, through a series of electrophysiological experiments in CA1 area, we explored the mechanism of synaptic depression induced by CKIIN. Our results indicate that CKIIN-induced depression is different from NMDAR-dependent long-term depression (LTD) and is not activity-dependent. In addition, the effect does not depend on intracellular or extracellular Ca2+ and does not require protein synthesis or degradation. By studying CKIIN-depression in animals of different ages, we showed that the amount of depression positively correlates with average CaMKII enrichment in PSD.
Overall, our results are consistent with a direct disruption of CaMKII-NMDAR complex stability by CKIIN and suggest a role for CaMKIIN protein as a synaptic transmission and plasticity regulator in the hippocampus. Fondecyt 1080630. Mideplan ICM P05-001-F

P1.2 Diferenciación hemisférica en el reconocimiento de expresiones faciales: el rol de los componentes espectrales. Hemispheric Differences in Discrimination of Facial Expressions: The Role of Spectral Components "A. HELO (1,2), J. P. OSSANDON (3), K. SAMESHIMA (4), E. MORYA (5), P. E. MALDONADO (1); (1) Dep. Fisiología y Biofisica Universidad de Chile. (2) Escuela de fonoaudiología Universidad de Chile. (3) Inst. dofs Cognitive Sci., Univ. of Osnabrück, Osnabrück, Germany. (4) Dep. Radiología, Facultad Med., Univ.de Sao Paulo. (4) Lab. Neurociencias, AASDAP, Enseñanza e Investigación. Inst., Hosp Sirio- Libanes.

Higher cognitive processing such as emotion recognition through facial expressions begins with the perception of a stimulus. This processing is lateralized to the right hemisphere. Nor is known how much of the visual processing is related to physical properties of stimuli. Early studies have shown that facial expressions processing is facilitated when presented at low frequencies. We conjecture that his may be due to the fact that the right hemisphere process more quickly, low spatial frequencies features. We conducted a discrimination study of facial expressions, presented with different spatial frequency components (unfiltered, low and high frequency band pass). These stimuli were presented either in the left or right visual field. We measured reaction times (RT),

discrimination performance and conducted electroencephalographic recordings. We found that neither facial expression nor the visual field location of images influenced the RT or performance of the subjects. Unfiltered images were discriminated better than filtered images (Anova,$P<0.01$). We also found that RT to high frequency images were larger than the RT for unfiltered images (Anova, $P<0.05$). The performance of subjects to stimuli at different spatial frequencies was not influenced by the visual field location. Event related potentials show that either P1 or N170 components were affected by the spatial frequency of images, P2 was significantly higher in the left hemisphere for high frequency mages (Anova, $P<0.05$). We conclude that both hemispheres contribute to facial expression discrimination by early processing of low frequency components, to then transfer to the left hemisphere processing high frequency features." Funded in part by Fondecyt ACT-66

P1.3 Corrientes de Calcio alteradas en línea celular derivada de corteza cerebral de ratón trisómico Ts16, un modelo del síndrome de Down. ALTERED CALCIUM CURRENTS IN A CELL LINE DERIVED FROM CEREBRAL CORTEX OF TRISOMY 16 MOUSE, A MODEL OF DOWN SYNDROME. Acuña M (1,3), Cárdenas AM (2), Bacigalupo J (3), Delgado R (3), Segura-Aguilar J (1), Caviedes R (1), Caviedes P (1) "(1) Program of Molecular and Clinical Pharmacology, ICBM, Faculty of Medicine, University of Chile. (2) CINV, University of Valparaíso. (3) Department of Biology & Milenio Institute ICDB, Faculty of Sciences, U. of Chile.

Human Down Syndrome (DS) is determined by the presence of an extra copy of autosome 21. It is expressed by multiple abnormalities, being mental retardation the most striking feature, plus an early onset of Alzheimer's disease-like pathology. The condition results in altered electrical membrane properties of fetal neurons, which are qualitatively identical to those of trisomy 16 fetal mice (Ts16), an animal model of the DS condition. Indeed, Ts16 cultured dorsal root ganglia (DRG) neurons exhibited a reduction in Voltage-gated Ca2+ current (ICa) amplitude and slower activation kinetics. Conversely, Ts16 hippocampal cultured neurons showed increased ICa amplitude. Since Ts16 animals are unviable, we have established immortalized cell lines from the central nervous system (CNS) of Ts16 and normal littermates. Using the whole-cell patch-clamp technique, we studied ICa in a cell line derived from the cerebral cortex of a Ts16 fetus (named CTb), in comparison to a cerebral cortex cell line from a euploid littermate (named CNh). Current activation occurs at -40mV in both cell lines, when stepped from a holding level of -80 mV. Trisomic cells exhibited a 2.4-fold increase in the maximal ICa density (CNh= -1,54 ± 0,53 pA/pF; CTb= -3,72 ± 0,63 pA/pF; $p=0.01$, $n=3$). These results suggest that the CTb cell line presents altered ICa currents, which may in turn contribute to impaired cell function. These cell lines represent a model for the study of DS-related impairments of electrical membrane properties in CNS neurons. Funded by: Fondecyt 1090160 VID, U. of Chile (Contingency grants) MIDEPLAN ICM P05-001-F

P1.4 Efecto de la estimulación acústica contralateral en los potenciales cocleares de chinchilla despierta y anestesiada Effect of contralateral acoustic stimulation on cochlear potentials in awake and anesthetized chinchilla. Aedo C (1), Tapia E (1), Pavez E (2), Robles L (3) "(1) Student 5th year Tecnología Médica, ORL, Facultad de Medicina, U. de Chile. (2) Escuela Tecnología Médica, ORL, Facultad de Medicina, U. de Chile. (3) Programa de Fisiología y Biofísica, ICBM, Facultad de Medicina, U. de Chile.

Inner and outer hair cells are the receptor cells in which mechano-electrical transduction takes place in the mammalian cochlea. Outer hair cells receive important efferent innervation from the superior olivary nuclei through the medial olivocochlear (MOC) fibers, and can modulate the responses of inner hair cells, and hence of the whole afferent auditory via. Activation of the MOC efferent system, in anesthetized animals, by acoustic contralateral stimulation causes an amplitude decrease of the compound action potentials (CAP) elicited by tones in the ipsilateral cochlea. However, since most studies on the effects of acoustic contralateral stimulation in cochlear sensitivity have been made in anesthetized animals, we have carried out a comparative study of these effects in the awake and

anesthetized chinchilla. We used nine healthy adult chinchillas (Chinchilla laniger) with chronically implanted electrodes in the two round windows. We recorded CAP and cochlear microphonic (CM) potentials in response to ipsilateral tones (15 ms duration, 1-6 KHz frequencies and 30-60 dB SPL intensities), previous to and after contralateral tone stimulation (500 ms duration, 40-90 dB SPL intensities) in, both, awake and anesthetized animals. Results show that contralateral acoustic stimulation causes decreases in CAP amplitudes that vary between 1 and 10 dB, and that have mean values 1-3 dB larger in awake than in anesthetized animals. In both cases the greatest effects are tuned to the frequency of contralateral stimulation, for intensities at 40-90 dB SPL. CM potentials did not show a statistically significant effect neither for awake, nor for anesthetized animals.

Supported by Fondecyt Project Nº 1080227

P1.5 Herramientas moleculares para analizar in vivo la función de la señalización Wnt en la unión neuromuscular de vertebrados. Molecular tools to analyze the in vivo role of Wnt signaling at the vertebrate neuromuscular junction. Albistur M, Teneb J, Sandoval D, Marcellini S, Henríquez JP* Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción. Concepción, CHILE. (*jhenriquez@udec.cl)

Wnt3 is expressed in mouse motoneurons during neuromuscular junction (NMJ) formation. Exposure to Wnt3 induces early events of postsynaptic differentiation in mouse-derived cultured muscle cells. In turn, overexpression of Frizzled-9 (Fz9) receptor in muscle cells decreases postsynaptic differentiation. Nonetheless, the in vivo role of Wnt ligands and their receptors at the vertebrate NMJ is still unknown. To analyze the possible function of Wnt3 and Fz9 at the NMJ, we designed gain-of-function experiments through conventional in vivo electroporation. Even though this powerful technique has been widely employed to analyze gene function in vivo, it fails to retain transgene expression for long periods of time, as they are not integrated into the genome. To overcome this issue, we designed the pT2K-CAG-T/A vector that allows: a) the easy ligation of PCR products, and b) the stable integration of cDNAs into the chromosomes of host cells through the Tol2 transposon element. Expression of the transgene is under the control of the CAG enhancer and chick β-actin promoter to achieve efficient overexpression in vivo. On the other hand, the mCherry-2A-xHA cassette allows the co-expression, from a single open reading frame, of a membrane-bound red fluorescent protein and an HA-tagged form of any protein of interest, that are subsequently separated by the intervening 2A self-cleaving peptide. The combination of these molecular tools results in the stable expression of GFP in cell lines. Moreover, fluorescently trackeable cDNAs coding for Wnt3 and Fz9 were expressed by in vivo electroporation in chick developing motoneurons and post-natal skeletal muscle, respectively." This work has been supported by FONDECYT 1100326 grant to J.P.H. The authors are indebted to the support of friends and colleagues after the damage caused by the earthquake on February 27th, 2010.

P1.6 Modelo comparativo de fagocitosis y degradación de Aβ mediado por un mecanismo dependiente de MMP-9 en microglia y macrófagos periféricos Comparative model of Aβ phagocytosis and degradation by a MMP-9 mediated mechanism in microglia and peripheral macrophages. Arroyo P. , von Bernhardi R. Pontificia Universidad Católica de Chile School of Medicine, SANTIAGO, CHILE

β-Amyloid (Aβ) accumulation and senile plaque formation are one of the neuropathological hallmarks of Alzheimer's disease (AD). However, there is no compelling evidence that Aβ production is increased in the most common late-onset form of AD, suggesting that insufficient or impaired Aβ clearance can also drive Aβ deposition and amyloid plaque formation. Microglia are found surrounding amyloid plaques, and Aβ phagocytosis is triggered when activated, but it is unclear if microglia are effective degrading Aβ. In Contrast, peripheral macrophages can surround Aβ-containing plaques and have been reported to prevent the formation and even to eliminate amyloid deposits. An alternative mechanism for Aβ clearance is the extracellular Aβ degradation by proteases. Recent

reports indicating that MMP-9 can degrade fibrillar Aβ led us to evaluate if this protease play a role in Aβ clearance. Our aim was to compare the efficiency of peripheral macrophages and microglia to phagocyte and degrade Aβ trough MMP-9-mediated mechanism. We compared MMP-9 expression in vitro in both cell types under inflammatory conditions induced by Lipopolysaccharide (LPS). Stimulation of macrophages and microglia with LPS or Aβ led to an increase in MMP-9 expression that was similar for both cell types (54% v/s 62% increase respectively). Aβ phagocytosis assays showed a similar phagocytic capacity in both cell types in noninflammatory conditions. When we used ilomastat, a metalloprotease inhibitor, a decrease in phagocyted Aβ was observed. Our results show that MMP-9 expression increased under inflammatory conditions and Aβ stimulation, also inhibition of MMP-9 decrease phagocitosys suggesting a role if MMP-9 in Aβ phagocytosis. Must be considered that several factors associated with AD pathology are involved in the modulation of MMP-9 expression and activity, like cell aging, apoE isoform, and the inflammatory conditions.Supported by Fondecyt 1090353

P1.7 Participación de diferentes áreas cerebrales en dos modelos de estrés repetido en ratas adultas. Involvement of different brain areas in two repetitive stress models in adult rats. Ampuero E. (1), Santibañez, M. (1), Rubio, J. (1), Diaz-Veliz G. (2), Forray M. (3) and Wyneken U (1).
(1) Laboratorio de Neurociencia, Facultad de Medicina, Universidad de los Andes. (2) Laboratorio de Farmacología del Comportamiento, ICBM, Universidad de Chile. (3) Departamento de Farmacia, Pontificia Universidad Católica de Chile.

Repetitive stress is an animal model that reproduces clinical symptoms present in major depression. Stress can be induced by movement restraint either in small cages or by immobilization in plastic bags. We studied the effects of two types of antidepressant drugs, fluoxetine (flx), a selective serotonin reuptake inhibitor, and reboxetine (rbx), a selective noradrenaline reuptake inhibitor, on depressive-like behaviors in rats. Following 10 days of stress, flx(0.7 or 3.5mg/kg,i.p.) or rbx(0.128 or 0.64mg/Kg,i.p.) were administered from days 11 to 38, concomitant with stress application. The corresponding control groups were included. On days 11 and 39, depressive-like behaviors were measured. Anxiety was evaluated by novelty suppressed feeding and the elevated plus-maze whereas learned helplessness was evaluated by the tail suspension and forced swimming tests. To gain insight into CNS regions activated by stress, fos-like immunoreactivity was measured. Our results showed that 10 days of stress were sufficient to induce anxiety and learned helplessness in both models. However, only immobilization induced persistent behavioral changes. Moreover, flx reverted exclusively depressive-like behaviors induced by restriction whereas rbx reverted these behaviors when induced by immobilization. In addition, c-fos immunoreactivity was expressed differentially in several brain areas, including the dentate gyrus and the retrosplenial cortex. These results suggest that restriction and immobilization correspond to different models of depression that can be distinguished by c-fos immunorectivity and a selective sensitivity to serotonin or noradrenaline-based antidepressant drugs. A better comprehension of biological differences between subtypes of depression may guide clinical decisions on effective pharmacotherapy in the future. Funded by Anillo09-2006(UW)

P1.8 Aumento en la proliferación de precursores neuronales bajo condiciones de ejercicio en modelo transgénico APPswe/PS1ΔE9 de la enfermedad de Alzheimer

Increased proliferation of neuronal precursors by running in the double transgenic APPswe/PS1ΔE9 mice model of Alzheimer's disease. Aranguiz FC, Slater PG, Varela-Nallar L and Inestrosa NC. Centro de Envejecimiento y Regeneración (CARE), Facultad de Ciencias Biológicas. PUC.

Adult neurogenesis is the process by which new neurons are generated and incorporated into the adult brain, this process occurs in both, the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus. The present study is focused on the SGZ, where adult neurogenesis has been associated to memory and

learning. Neural precursor proliferation was evaluated in 8 monthold wild-type mice and in the double transgenic APPswe/PS1ΔE9 mouse model of Alzheimer disease (AD) by the incorporation of the thymidine analog BrdU, which incorporates into DNA of proliferating cells during the S-phase. A significant reduction was observed in the double transgenic mice compared to aged-matched wild-type animals. Previously, it has been described that running increases neurogenesis in the SGZ. Therefore, we evaluated if running could increase neural precursor proliferation in the AD model. Animals were subjected to voluntary wheel running for 13 days, which increased the proliferation of neural precursor in wild-type and AD mice. Our results suggest that exercise increases neurogenesis in double trangenic mice that already show cognitive impairment and the neuropathological markers of AD, and suggest that neurogenesis could play a role in memory improvement previously described in AD animals exposed to voluntary exercise. Currently, we are analyzing the potential role of the non-canonical Wnt signaling in neurogenesis in the presence or absence of exercise. "Basal (PFB12/2007); Proyecto Inserción CONICYT (79090027).

P1.9 Oligomeros del peptido Aβ altera la plasticidad sinaptica y la funcion cognitiva en Octodon degus. The β-amyloid impairs synaptic plasticity and cognitive function in the aged Octodon degus. Ardiles AO (1), Elgueta C (1), Tapia-Rojas C (2), Inestrosa NC (2), Kirkwood A (3), Palacios AG (1)"(1) Centro Interdisciplinario de Neurociencia de Valparaiso, Universidad de Valparaiso, Valparaiso, Chile (2) Centro de Envejecimiento y Regeneración (CARE), Centro de Regulación Celular y Patología Joaquín V. Luco (CRCP), MIFAB, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile (3) Mind/Brain Institute and Department of Neurosciences, Johns Hopkins University, Baltimore MD, USA

The search of suitable animal models for the study of Alzheimer's disease (AD) has lead to the engineering of transgenic mouse bearing mutations for genes that encode proteins involved in AD. These models develop Aβ deposits and reproduce, in some cases, neurological symptoms of AD, such as neuronal loss, synaptic density reduction and dystrophic neurites. However, most of these animals lack of all essential hallmarks of AD, specially, not shown neurofibrillary tangles. The recent reports of non-transgenic animals models, presenting the natural hallmark of the diseases, have represented significant progress. Here we show that the rodent Octodon degus develops with age the neuropathological signs of Alzheimer's disease, as intracellular and extracellular amyloid deposits and intracellular accumulations of the Tau protein. Aged degus from 12 to 60 months exhibit a decreased cognitive capacity; reduced synaptic transmission and plasticity in hippocampus CA1 area, compared to young 6 months old degus. However, no differences were observed in the paired-pulse facilitation and the loss of presynaptic elements in the hippocampus. Conversely, postsynaptic structural elements were reduced in aged but not in young degus suggesting that postsynaptic impairments could explain the synaptic deficits. Interestingly, these cognitive impairments correlate with the presence of amyloid oligomers. These results suggest that aged Octodon degus constitutes an excellent model for the study of Alzheimer's disease. This proyect was supported by FIRCA NIH R03 TW007171-01 (AK),
CONICYT AT 24091109 (AOA)

P1.10 Modificación por SUMO regula la actividad transcripcional de Nurr1 SUMO modification regulates the transcriptional activity of Nurr1. Arredondo C, Andrés M E. Nucleus Millennium in Stress and Addiction. Department of Cell and Molecular Biology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile

Nurr1 is a transcription factor, orphan member of the nuclear receptor superfamily, highly expressed in midbrain dopaminergic neurons. Nurr1 is crucial for the development and survival of dopaminergic neurons. Nurr1 regulates the expression of key dopaminergic genes like tyrosine hydroxylase and the dopamine transporter, among others. However, it is unknown how the transcriptional activity of Nurr1 is regulated. SUMOylation is a post-

translational modification of proteins regulating different biological processes, but it is particularly relevant in the regulation of transcription. The SUMOylation process is characterized by the reversible conjugation of a SUMO peptide (SUMO-1, SUMO-2 and SUMO-3) to lysine residues in SUMO consensus motif (V/L/I-KXE) of substrate proteins. Nurr1 have several lysines with high score for SUMOylation and our previous work showed that the replacement of these lysines changes the transcriptional activity of Nurr1. Here, we demonstrate that Nurr1 is SUMOylated by SUMO-2. Wild type PIASg enhances Nurr1 SUMOylation. However, PIASg mutated on the LXXLL motif, which abolish the interaction with Nurr1 is not able to enhance Nurr1 SUMOylation. By using mammalian gene reporter assays, we also demonstrate that SUMO peptides and SUMO-specific proteases regulates the transcriptional activity induced by Nurr1. In conclusion, our results demonstrate that SUMOylation regulates Nurr1-dependent transcriptional activity. Funded by FONDECYT Nº 1070349 and Nucleus-Millennium Project Nº P06/008-F.

P1.11 Disfunción en la endocitosis de receptores en líneas neuronales de ratón trisómico 16, modelo animal del síndrome de Down. Dysfunction of receptor endocytosis in neuronal cell lines derived from a trisomy 16 mouse, an animal model of Down Syndrome. Báez-Matus X (1), Guerra MJ (1), Segura J (2), Caviedes P (2), Cárdenas AM (1). (1) Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso. (2) Programa de Farmacología Molecular y Clínica, ICBM, Facultad de Medicina, Universidad de Chile.

La endocitosis es un proceso celular crítico para la función neuronal, que permite el reciclaje de vesículas sinápticas y regula el número de receptores y canales iónicos en la membrana celular. Por tanto, su disfunción provoca alteraciones en la función y plasticidad sináptica. A ese respecto, se reconocen alteraciones en la función endocítica tanto en el Síndrome de Down como en la enfermedad de Alzheimer. Sin embargo, el mecanismo molecular que subyace a la disfunción endocítica en estas patologías no ha sido esclarecido. En el presente trabajo evaluamos la endocitosis mediada por receptor en una línea celular de hipocampo de ratón con trisomía 16 (HTk), un modelo animal de la trisomía 21 humana (Síndrome de Down). La endocitosis de las células HTk fue comparada con aquella de una línea celular de hipocampo de ratón normal (H1b). La endocitosis de receptores de transferrina fue cuantifica mediante análisis de inmunobloting de extracto de proteínas de células tratadas con transferrina-biotina. Nuestros resultados muestran que la razón transferrina internalizada/transferrina unida a la membrana se encuentra significativamente aumentada en las células trisómicas HTk. Las razones transferrina internalizada/transferrina unida a la membrana para las células H1b y HTk fueron de 0.24±0.05 y 0.51±0.11 ($p<0.05$), respectivamente. Esta disfunción endocítica podría producir alteraciones en el reciclaje de vesículas y en el procesamiento de la proteína precursora de amiloide observado en células de individuos con condición trisómica. Proyecto Fondecyt 1090160, Programa Contingencia (VID, U. de Chile).

P1.12 Caracterización electroencefalográfica de kindling por pentilentetrazol. Electroencephalographic characterization of pentylenetetrazole kindling. Bartsch V.(1), Diaz J.(2), Ibañez F.(2), Ocampo-Garcés A.(2), Wyneken U.(1). (1) Laboratorio de Neurociencias, Universidad de Los Andes, Chile. (2) Laboratorio de Sueño y Cronobiología, Universidad de Chile, Chile.

Epilepsy is a highly prevalent syndrome characterized by neuronal hiperexcitability that causes spontaneous and recurrent neuronal firing. In acquired epilepsies, the medial temporal lobe most frequently develops as an epileptic focus. The mechanisms underlying temporal lobe (TL) epileptogenesis are not well understood and animals models could be useful to develop pharmacological interventions. The aim of our study was to characterize the temporal course of the development of TL epilepsy by electroencephalographic methods in a pharmacological kindling model. For this, male Sprague Dawley rats were implanted with electrodes for chronic polysomnographic (EEG and EMG) recordings. Rats were injected with 10 pentylenetetrazole (PTZ) doses (20 mg/Kg) at 48 hour intervals.

Polysomnographic records were obtained for two hours per day as well as 24 days after finishing the last PTZ injection. Epileptic discharges (ED) were detected from the first day of injection. Fourier analysis revealed that ED´s spectral power was primarily in the theta band (4-10Hz). ED detection was automated based on an algorithm considering ED amplitude. The mean ED duration was of 4,62±0,97s and the total ED time increased with successive injections by 48,6 s/day.24 days after finishing the kindling protocol, the theta band spectral power increased significantly, reflecting the positive feedback of the epileptogenesis process. We have therefore developed a powerful methodology of epileptic EEG analysis in the rat to be used in pharmacological studies. Acknowdedgements: Fondecyt 1100322(UW), Fondecyt 1100245 (AO). Fondecyt 1100322(UW) Fondecyt 1100245 (AO)

P1.13 Expresión del receptor 5-HT1A en el raphe bulbar de raton durante el periodo postnatal temprano es modificada por nicotina prenatal. Expression of 5-HT1A receptor in mouse medullary raphe during early postnatal period is modified by prenatal nicotine Beltrán-Castillo, S. Llona, I. Eugenín, J. "Laboratorio de Sistemas Neurales. Facultad de Química y Biología, Universidad de Santiago

Nicotine can affect the ontogeny of the serotonergic system, which can lead to failures in the chemosensory ventilatory responses. Hypoactivity of serotonergic raphe neurons is found in mice exposed prenatally to nicotine. Since 5-HT1A receptors contribute to the control of the firing rate of serotonergic neurons, we investigated whether prenatal to early postnatal nicotine exposure can alter the 5-HT1A receptor expression in the brainstem respiratory nuclei. Osmotic mini pumps releasing saline (controls) or nicotine tartrate at a rate of 60 mg Kg-1 day.1 during 28 days, were implanted subcutaneously in CF1 mice at their fifth day of pregnancy. The 5-HT1A expression was detected by western blot and immunocytochemistry in brainstem from 1, 3, 5, and 8 days old (P1, P3, P5, P8) control and nicotine exposed mouse neonates. Prenatal nicotine exposure increased the number of serotonergic neurons that express 5-HT1A receptors and the cellular content of these receptors at the rostral raphe obscurus nuclei in P1-P5 neonates. In P8 neonates, no difference was found between controls and nicotine exposed neonates. These results are coherent and can explain previous results showing hypoactivity of the neurons of the caudal raphe in neonatal mice exposed to prenatal nicotine. These results may be relevant for understanding the pathogeny of neonatal respiratory disorders associated with maternal smoking as the Sudden Infant Death Syndrome. FONDECYT #1090375

P1.14 Propiedades eléctricas de neuronas carotídeas y glosofaríngeas del ganglio petroso del conejo. Electrical properties of carotid and glossopharyngeal rabbit petrosal ganglion neurons Boncompte. G, Ortiz FC, Arroyo J, Alcayaga J. Laboratorio de Fisiología Celular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile

The petrosal ganglion (PG) contains the somatas of glossopharyngeal sensory neurons that innervate the carotid body and the pharynx and posterior third portion of the tongue. It has been suggested that afferent neuron´s electrical properties are correlated with their sensory modality. We tested this hypothesis using intracellular recordings of PG neurons and their responses to the electrical stimulation of the glossopaheryngeal (pharynx-toungue) and carotid (sinus-body) branches of the glossopharyngeal nerve. The rabbit PG, with its nerve branches, were placed in a recording chamber and superfused with Hanks´ solution at constant flow (~1.2 mL/min) and temperature (~ 37°C). The neurons were impaled with KCl (3M; R≈40 MΩ) filled microelectrodes and identified according to their peripheral projection as carotid (PGC) or glossopharyngeal (PGG). We found no significant differences ($p<0.05$) between the electrical properties measured of PGC and PGG neurons: resting membrane potential (-46.3 ± 2.0 vs -49.2 ± 2.2 mV), input resistance (26.3 ± 8.6 vs 27.0 ± 4.4 MΩ), membrane capacitance (93.8 ± 32.4 vs 65.8 ± 9.4 pF) and time constant (1.91 ± 0.50 vs 1.21 ± 0.15 ms), and action potential duration (3.12 ± 0.68 vs 2.19 ± 0.18 ms). According to their conduction velocities, PGC and PGG neurons could be grouped in two subpopulations (PGC: 7.48 ± 1.10 and 2.49 ± 0.31 m/s; PGG: 7.58 ±

0.20 and 4.69 ± 0.53 m/s). Our results indicate that PGC and PGG neurons present similar passive characteristics and suggest they differ in the conduction properties of the slowest conducting fibres." Founded by grant FONDECYT 1090157

P1.15 Serotonina modula la respiración ficticia via receptor 5-HT1A Serotonin modulates fictive respiration via 5-HT1A receptors. Bravo E., Beltrán S., Llona I., Eugenín J. Laboratorio de Sistemas Neurales, Facultad de Química y Biología, Universidad de Santiago de Chile.

The respiratory rhythm is generated by a brainstem neural network whose main component is the ventral respiratory group (VRG). Serotonin, provided by raphe nucleus projections, modulates fictive respiration. Over-expression of serotoninergic receptor 5-HT1A is associated to a decrease in mice survival at early postnatal life. We have shown that prenatal nicotine exposure increases 5-HT1A receptor in raphe nuclei, decreases the frequency of discharge in raphe neurons, and reduces the respiratory response to hypercarbia in neonatal mice. In this work we evaluate the serotonin effect on fictive respiration in brainstem slices obtained from neonatal mice. CF1 neonates were anesthetized by cooling and ether inhalation. Their brainstem were removed and immersed in artificial cerebrospinal fluid (aCSF) supplemented with D-glucose, 30mM and equilibrated with O2:CO2 = 95%: 5%, (pH 7.4). Fictive respiration was recorded from VRG with suction electrode in 700 μm slice obtained with vibratome. In a typical experiment, the brainstem was superfused with aCSF at 28ºC during 10 minutes and then the medium was switched to aCSF containing serotonin or serotonin antagonists during 5 minutes followed by a period of washout of 15 minutes. Preliminary results show that serotonin (10-30 μM) increases the frequency of VRG rhythmic bursts by 45±5% . The increase in frequency was concentration-dependent and it was partially abolished by 5-HT1A receptor antagonist (NAN-190 30μM), and was completely abolished with a 5-HT antagonist (methysergide 10μM) . We conclude that serotonin modulates fictive respiration in brainstem slices acting in part through 5-HT1A receptor. Supported by Fondecyt: 1090375

P1.16 MDMA (3,4-METILENDIOXIMETAMFETAMINA,""ÉXTASIS"") Y SERTRALINA MEJORAN DESEMPEÑO VISUOESPACIAL DE CORTO Y LARGO PLAZO EN COMPARACIÓN A FLUOXETINA EN RATAS. MDMA (3,4-METHYLENEDIOXYMETHAMPHETAMINE,´ECSTASY´) AND SERTRALINE ENHANCE SHORT AND LONG LASTING VISUAL SPATIAL PERFORMANCE COMPARED TO FLUOXETINE IN RATS

Burgos H (1,3), Cofré C (2), Pedreros E (2), Torres S (2), Miño I (2), Concha G (2), Castillo A (1), Hernández A (4), Rebolledo-Fuentes M (4,5), Cassels BK (4,5), Sáez-Briones P (6,7) (1) Escuela de Psicología, Universidad Central de Chile. (2) Escuela de Psicología, Universidad de las Américas. (3) Escuela de Psicología, Universidad Autónoma de Chile. (4) Laboratorio de Neurobiología, Facultad de Química y Biología, USACH. (5) Laboratorio de Química Biodinámica, Depto. de Química, Facultad de Ciencias, Universidad de Chile. (6) Millennium Institute for Cell Dynamics and Biotechnology, Universidad de Chile. (7)Escuela de Medicina, Facultad de Ciencias Médicas, USACH.

Current evidence indicate that the entactogenic amphetamine MDMA (3,4-methylenedioxymethamphetamine,´ecstasy´) and the antidepressants sertraline and fluoxetine might induce cognitive impairments in humans, but published data regarding active doses and/or chronic administration regimens associated with this effect are controversial. In this work, fluoxetine (10 mg/kg), sertraline (10 mg/kg) and MDMA (0.25 mg/kg) were administered intraperitoneally to 30 male Sprague Dawley rats for 15 sessions. Each animal was evaluated at the radial octogonal Olton laberinth (4x4) for short- and long-term memory performance without interruption between short- and long-term phases to avoid possible cortical involvement. In each phase, the number of errors and the time required to complete the task were recorded. The data obtained indicated that MDMA and sertraline enhanced short-term performance, whereas only sertraline treated animals favoured long-term performance. In addition, the time required to complete both short- and long-term memory tasks were enhanced by MDMA only. Fluoxetine increased the

number of errors in the short-term performance, whereas sertraline and fluoxetine have no effect in altering the time to complete both the short- and long-term phases. Taken together, our results indicate that low doses of MDMA do not induce cognitive impairment and that sertraline and fluoxetine might alter differentially the visual spatial memory neurotransmission pathways. Supported by FONDECYT Grant 1070028, FONDECYT Grant 1085051, DICYT-Grant 021001SB and ICM-Grant P05-001-F.

P1.17 Expresión de RNAi contra proteínas relacionadas con la enfermedad de Alzheimer afecta diferencialmente comportamiento olfativo y desarrollo en Drosophila melanogaster. The expression of RNAi against Alzheimer disease-related proteins differentially affects olfactory behavior and development in Drosophila melanogaster Burgos-Sanchez P, Campusano JM Nucleo Científico Milenio Estrés y Adición (NEDA), Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

Presenilin, tau and presenilin enhancer (pen2) are proteins involved in Alzheimer's disease genesis, whose functions are still not fully understood. In the present study we used the Gal4/UAS system to direct the expression of RNAi for presenilin (CG18803; RNAi-psn), tau (CG31057; RNAi-tau) and pen2 (CG33198; RNAi-pen2) in Drosophila melanogaster CNS neurons and evaluated the effect of this genetic manipulation on fly growth and olfaction. In order to evaluate the effects on growth we registered the developmental time from egg to imago in RNAi expressing flies. Growth curves show that the development in flies expressing the RNAi-tau and RNAi-pen2 is slower than their genetic controls. In the case of the RNAi-tau flies, their development is arrested at pupal stage. To evaluate the olfactory behavior we performed a food trap assay. Results show that flies expressing RNAi-psn and RNAi-pen2 displayed a low rate of entry to the traps in comparison to the genetic control lines. Results obtained suggest that the expression of RNAi for presenilin, pen2 and tau differentially affect development and olfactory behavior in Drosophila melanogaster and suggest this is a good model to study the normal functions of these proteins in neurons. Fondecyt 1100965, VRAID 21-2009, ISN-CAEN Research Program.

P1.18 La exposición prenatal a nicotina modifica la expresión de los subtipos nAChRs en el tronco encéfalo de los ratones neonatos. Prenatal nicotine exposure modifies the expression of nAChRs subtypes in the brainstem of neonatal mice. Campos M, Gonzaléz-Rivas C, Eugenín J. Laboratorio Sistemas Neurales, Departamento de Biología, Facultad de Química y Biología, USACH.

Maternal smoking during pregnancy is the major risk factor for SIDS. Nicotine, a main teratogen component of cigarette smoke, may alter neural development through activation of acetylcholine nicotinic receptors (nAChRs). In the brain, prenatal nicotine exposure down regulates muscarinic receptors and up regulates nAChRs increasing alpha 4, beta 2 and alpha 7 mRNA. Here, we studied the effects of prenatal nicotine exposure on the expression of nAChRs subtypes α4 and α7 in brainstem slices of male and female neonatal mice (P0-P1, P3 and P8). Mini-osmotic pumps were implanted subcutaneously into 5 days pregnant mice to deliver nicotine ditartrate 60 mg/kg/day for up to 28 days. Newborns non-exposed (controls) and exposed to prenatal nicotine were anaesthetized and their CNS were removed. Brainstem was mounted on agar and 700 µm slices were obtained using a vibratome. The slice preparations were homogenizated in lysis buffer. Samples were separated by SDS/Page electrophoresis, blotted onto nitrocellulose membranes and incubated with antibodies against alpha 4, alpha 7 and Beta tubuline I and II. Preliminary results show that prenatal nicotine exposure diminished the expression of nAChRs subtype alpha 7 compared to control mice, difference which was less pronounced in males than females. In conclusion, this result indicates that prenatal exposure to nicotine significantly affects nAChRs subunit expression in the developing brainstem. This change in nAChRs expression may play a role in the pathogenia of respiratory dysfunctions in neonates exposed prenatally to cigarette smoking.

P1.19 Propiedades únicas introducidas por el ruido de canales en un modelo general de disparo en ráfagas. UNIQUE FEATURES INTRODUCED BY CHANNEL NOISE IN A GENERALIZED MODEL OF NEURONAL BURST GENERATION. Canales A, Orio P. Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso.

Stochastic opening and closing of ion channels (channel noise) is a major source of variability in the behavior of neurons and their integration properties. The effect of channel noise on action potential timing and threshold variability has been extensively studied and its biological relevance is undisputed. In the case of more complex phenomena, such as sub-threshold oscillations and bursting, it has been shown that noise interacts with non-linear dynamics shaping oscillatory regimes. However, it is not clear whether additive white noise introduced into the current equation (current noise), a common simplification of noise sources in neuron models, can reproduce accurately the effects of channel noise. Using mathematical simulations, we are studying the effect of channel noise in a generalized model of bursting driven by a temperature-modulated subthreshold oscillation. In the vicinity of a bifurcation between different regimes, either current or channel noise can change a tonic firing pattern into a bursting firing pattern. However, we have found that channel noise is more efficient in inducing this change, originating more regular bursting patterns than current noise and over a broader range of parameters. We also present phase-plane analyses that illustrate the difference between channel noise and current noise. We conclude that channel noise can shape dramatically the subthreshold dynamics of neurons and their integration properties, and that the effect of channel noise cannot be explained or reproduced in mathematical simulations by current noise. Fondecyt 11090308 and DIPUV 51/2007.

P1.20 Presencia del Canal de Cloruro activado por Ca2+ de la familia ClCa en neuronas sensoriales olfatorias de Rata. Presence of Ca2+ activated Cl- channels of the ClCa family in olfactory sensory neurons of Rat. Carolina González, Jorge Vera, Alejandra Riveros, Christian González-Billaud, María Rosa Bono, Juan Bacigalupo. Department of Biology, Faculty of Science, University of Chile and Institute for Cell Dynamics and Biotechnology, University of Chile.

Odor transduction takes place in the cilia of olfactory neurons (ORNs). The Ca2+-activated Cl- channel is a key component in this process. Odor binding to a specific receptor in the cilia of OSNs triggers a cAMP cascade which mediates the opening of a cationic cyclic nucleotide-gated channel (CNG), allowing Ca2+ influx. Ca2+ ions activate Cl- channels, generating a prominent Cl- efflux, which substantially contributes to the depolarization of the OSNs. From both channels, CNG has been well characterized molecularly, whereas the Ca2+-activated Cl- channels still remains controvensial. In order to identified this elusive channel, we investigated the possibility that any of the members of the ClCa family channels expressed in the rat olfactory epithelium, where ClCa4l and ClCa2, may be good candidates. By means nested RT-PCR of whole epithelia and single OSNs, we determined that both channels express in these neurons. Full length sequencing of amplified ClCa expressed in rat olfactory epithelium indicated that ClCa4l is the most abundant of both channels. Immunoblotting with an antibody against a peptide common to both channels revealed expression in the ciliary membrane, fact that was confirmed later by inmmunofluorescence in cryosections of the olfactory epithelium. Besides, through RT-PCR we detected TMEM16A and B in OE, but not Best-2, which is interesting since this one has been identified in mouse ORNs. Finally, the evidence raises the possibility that ClCa4l and perhaps also ClCa2 were the responsable of the odor transduction signal. Support: Supported by MIDEPLAN ICM-P05-001-F, FONDECYT 1080653, TW007920 Fogarty Int Center NIH.

P1.21 Pérdida permanente de la memoria contextual asociada a drogas por la inhibición de la síntesis de proteínas en la corteza insular. Permanent loss of drug contextual memory by protein synthesis inhibition in the insular cortex. Contreras M.,

Madrid C. and Torrealba F Depto. Ciencias Fisiológicas, Facultad de Fisiología, Pontificia. Universidad Católica de Chile, Santiago, Chile. Addiction profoundly alters motivational circuits so that drugs become powerful reinforcers of behavior. The interoceptive system continuously updates homeostatic and emotional information that are important elements in motivational decisions. We tested the idea that the insular cortex, key element of the interoceptive system, is essential in reconsolidation of the amphetamine contextual memory, assessed by the conditioned place preference paradigm. Place preference tests used a two-compartment biased apparatus, with one white compartment paired with amphetamine (or saline) administration, connected by a brown alley to a black compartment paired with saline. Amphetamine-treatment considerably increased the time spent by treated rats in the amphetamine-paired compartment, in contrast with saline control rats. Bilateral injection of the protein synthesis inhibitor anisomycin, but not saline injection, into the agranular insular cortex in amphetamine-experienced rats immediately after memory reactivation produced permanent amnesia. The permanent amnesia of the amphetamine contextual memory was accompanied by a reduction in immunoreactivity of Zif268, a protein necessary for the reconsolidation of drug-associated memories. The same treatment with anisomycin but in the absence of memory reactivation left memory intact and did not affect the expression of Zif268. In contrast, bilateral injection of AP5 into agranular insular cortex or anisomycin into the primary interoceptive cortex after memory reactivation produced transient amnesia. These data suggest that the insular cortex involvement in the drug-memory reconsolidation processes of pavlovian associations between environmental conditioned stimuli and the drug craving. Fondecyt 1060476, Iniciativa Científica Milenio P06/008F & CONICYT Anillo ACT-66

P1.22 La hipoxia intermitente crónica aumenta los niveles de expresión de citoquinas pro-inflamatorias en el cuerpo carotideo: Papel en la potenciación quimiosensorial. Chronic intermittent hypoxia increases the relative expression levels of pro-inflammatory cytokines in the rat carotid body: Role in chemosensory potentiation.

Del Rio R, Arias P, Iturriaga R. Lab. eurobiología, Facultad de Ciencias Biológicas. P. Universidad Católica de Chile. Santiago, Chile.

Exposure to chronic sustained hypoxia induces carotid body (CB) chemosensory potentiation, which has been associated to an increased expression of pro-inflammatory cytokines within the CB. Chronic intermittent hypoxia (CIH), a main feature of obstructive sleep apnea, produces sympathovagal dysfunction and hypertension attributed to CB chemosensory potentiation. The mechanisms underlying the enhanced CB chemosensory responses to hypoxia induced by CIH are not fully understood. We hypothesized that CIH may increase the expression of pro-inflammatory cytokines in the rat CB. In male Sprague-Dawley rats exposed to CIH (5% O2, 12 times/hr for 8 hr for 21 days) that develop hypertension, we studied the expression of TNF-α, IL-1β and IL-6 in the CB by semiquantitative immunoreactivity analysis. The immunoreactive staining was measured using a colour deconvolution algorithm with the IMAGE J software. CIH produced a significant increase in the relative immunoreactive levels of TNF-α (CIH vs. Sham, 41.2±0.8 and 15.0±0.9 a.u., $p<0.05$) and IL-1β (CIH vs. Sham, 44.2±1.4 and 22.6±0.5 a.u., $p<0.05$) in the glomus cells of the CB of CIH-treated rats, which correlates with the enhanced CB chemosensory responses to hypoxia ($r=0.70$ and $r=0.66$ for TNF-α and IL-1β, respectively). In contrast, we did not find any changes in the expression of IL-6 between CIH and sham groups. Our results suggest that TNF-α and IL-1β may be involved in the CB chemosensory potentiation induced by CIH. FONDECYT 1100405 and CONICYT AT#24091043.

P1.23 Análisis estadístico durante la exploración visual libre en imágenes de distinta categoría. Statistical analysis during free visual explorations in images of different categories.

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Eye movements are typically classified as fixational movements (microsaccade, tremor and drift) and saccades. It has been described that fixational durations follow a gamma or lognormal distribution mainly due to its skewed shape. Could these distributions reflect differences in visual behavior that depends on the character (natural and artificial) of the visual stimuli? In this work we make a statistical analysis of the distribution of fixations durations of 7 different image categories: Construction, landscape, fractal, white noise, pink noise, and blank and gray images. A total of 8 male and female subjects participate in the task, they freely viewed the images for approximately 5 seconds each while their eye movements were recorded with a 500Hz eye-tracking system (EyeLink II, SR Research). To assess the similarity of its visual behavior, fixation distributions were studied. A Kruskal-Wallis test between distributions of each pair of image was performed; the threshold of significance was set to 1% ($p<0.01$). For the couples of images fractal-construction, construction-landscape, fractal-landscape, and white-pink noise, the p-value was above the threshold ($p>>0.01$), so it can be assume that the samples in each pair are drawn from the same distribution. Remarkably, the pair landscape-fractal images ($p\square0.9$) induce a quantitative similar fixation distribution while construction-landscape, both common life scenes for all subjects, have a much weaker relation under this test. On the other hand, the pair blank-gray images had significantly different distributions ($p<0.01$), as each of this images tested against all the others (fractal, construction, landscape, and noises). This findings show that fixational eye-movements induced by images with apparent cognitive content for the subjects have similar distributions, which is not observed on those with no complex content. These results suggest that fixational eye movements could be used as markers for bottom-up versus top down visual processes. " This study was supported in part by Conicyt (C.D.-M.). The authors would like to thank JP Ossandón, AV Helo, and R Montefusco-Siegmund for making available the data. Funded in part by Fondecyt ACT-66

P1.24 La modulación por cobre de las descargas espontáneas de CA3 en rebanadas entorrino-hipocampales es eliminada por picrotoxina, bloqueador GABA-A . Modulation by copper of the spontaneous CA3 spiking activity in entorhino-hippocampal slices is prevented by the GABA-A blocker Picrotoxin. Díaz-Franulic I (1,3), Díaz M J (2), Sanhueza M (1), Vergara C (1). "(1) Depto Biología, Facultad de Ciencias, U. de Chile (2) Escuela de Bioquímica, Facultad de Ciencias, UNAB
(3) Programa de Doctorado en Neurociencias, U. de Valparaíso

A neuromodulatory role for endogenously released copper in the CNS has been proposed based, among other evidences, on its differential distribution in hippocampus zones and the differential effects that exogenously added copper has on the spontaneous spikes discharges in several hippocampal areas. The spiking activity of CA3 cells results from the balance of their inhibitory and excitatory synaptic inputs. Inhibition comes mostly from the medial entorhinal cortex (EC) with a relay at the stratum lacunosum moleculare and excitation comes from mossy fibers, lateral EC and CA3 reciprocal interactions. We previously reported that: copper is present in the CA3 field at the pyramidal and lacunosum moleculare layers and that CA3 spontaneous spiking frequency decreases in the presence of the specific copper chelator bathocuproine (BCS); effect that is lost after EC removal. Attempting to characterize the modulatory role of copper, we recorded extracellular spontaneous CA3 pyramidal cell spikes in horizontal slices exposed to BCS in the presence of the synaptic blockers CNQX (AMPA/kainate) or picrotoxin (GABAA). The horizontal slices preserve many of the EC-hippocampus connections. Unexpectedly, we found that spontaneous spiking frequency increased or decreased after exposure to CNQX or picrotoxin respectively. Exposure to BCS together with CNQX caused a diminution in activity towards control values. BCS added after picrotoxin caused no further changes on spiking frequency. This suggests that under our recording conditions the inhibitory tone in CA3 could be strongly regulated by the EC and that endogenously released copper could act selectively on gabaergic-driven inputs in the CA3 area. Funding Fondecyt 1080670

P1.25 Transmisión selectiva de la actividad visual aferente a través de la vía tectofugal en paloma. Selective transmission of visual afferent activity along the tectofugal pathway in pigeons Ernesto Durán, Cristián Morales, Elisa Sentis, Juan Carlos Letelier, Jorge Mpodozis, Gonzalo MarínDepartamento de Biología, Facultad de Ciencias, Universidad de Chile.

Visual attention depends on mechanisms that promote the transmission of selected visual inputs in detriment to unselected ones. Increasing the synchrony between neurons representing an object at one stage in a visual pathway may be one strategy to facilitate the transmission of that neural representation to the following stage. Bursting firing could be another strategy, as temporal summation increases synaptic efficacy. We have shown in pigeons that visual inputs arriving to the superficial layers of the optic tectum (TeO) receive a fast, bursting feedback from the nucleus isthmi pars parvocellularis (Ipc), located underneath the tectum. This bursting feedback controls the ascending output from TeO to the nucleus rotundus (Rt) in the thalamus, producing a close synchrony between both the bursting responses recorded in Rt and those responses and the bursting signals in Ipc.
In the present study, we investigated whether the synchronization between the Ipc and Rt propagates to the nucleus entopallium, where rotundal axons terminate. To that end, we made simultaneous recordings from Ipc and the main subdivisions in the entopallium in response to single and paired moving visual stimuli in anaesthetized pigeons. We find that single and multi-unit responses evoked by moving stimuli in the entopallium present a bursting profile closely synchronized to bursting signals recorded in Ipc. These results, added to previous findings, indicate that the feedback signals from Ipc selects the afferent activity propagating to higher visual areas, synchronizing the neural responses representing the selected stimuli at all stages in the tectofugal pathway. Fondecyt (to GM): 1080220. Fondecyt (to JM): 1080094"

P1.26 Rol del complejo CaMKII-NMDA en la mantención de la transmisión sináptica. A role of CaMKII-NMDA receptor complex in synaptic strength maintenance. Fernández G.(1), Stein I.(2), Otmakhov N.(3), Hell J.(2), Lisman J.(3), Sanhueza M.(1)(4)

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The enzyme CaMKII is a major component of postsynaptic densities and is essential for induction of long-term potentiation (LTP), where its binding to the NMDA receptor (NMDAR) is critical. We recently showed that transient application of a CaMKII inhibitor, derived from the endogenous protein CaMKIIN, produced a persistent synaptic depression. Furthermore, this treatment allowed reinduction of previously saturated LTP, suggesting a disruption of LTP maintenance. In vitro studies indicate that this peptide binds to CaMKII at the site of enzyme interaction with NR2B subunit of NMDAR, interfering with complex formation. To further evaluate the role of CaMKII-NMDAR complex at the synapse, we used another peptide derived from CaMKIIN, fused to the incorporation sequence of HIV (Tat) and a control peptide not affecting the complex. The new agent also caused synaptic inhibition, while the control drug had no effect. However, concentrations required were four times higher than for the original peptide and depression persistence after drug removal was more difficult to assess, due to changes in presynaptic excitability. Incubation of slices for longer periods and construction of input-output curves after treatment demonstrated persistent depression selectively induced by test peptide. On the other hand, biochemical assays showed a reduction of basal NMDA-CaMKII complex in slices treated with this drug. After-treatment induction of LTP was similar for both groups. Since test peptide inhibits LTP, this indicates that persistent synaptic depression is not due to its residual presence after washing. Our experiments suggest a role for CaMKII-NMDA complex in basal synaptic strength maintenance. Fondecyt 1080630, ICM P05-001-F"

P1.27 Anatomía y conectividad del órgano vomeronasal y sus estructuras asociadas en la nariz de Octodon degus (Rodentia; Hystricognathi) Anatomy and

connectivity of the vomeronasal organ and its associated structures in the nose of Octodon degus (Rodentia; Hystricognathi). Fernández P, Mpodozis J, Suárez R. Lab. Neurobiología y Biología del Conocer. Dep. de Biología. Fac. de Ciencias, U. de Chile.

The vomeronasal system participates in the perception of pheromones and in the establishment of socio-sexual behaviors. The vomeronasal organ (VNO) of mammals varies in their anatomy and connectivity with the nasal cavity (NC) and mouth. The VNOs of Octodon degus consist of paired blind-ended tubes that run along the rostrocaudal axis of the basal septum, over the vomer bone. It opens to the anterior NC through the vomeronasal duct and groove. Each VNO has a thick crescent-shaped sensory epithelium (SE) at its medial aspect, composed of vomeronasal chemosensory neurons and supporting and basal cells, and a thinner non-sensory epithelium (NSE) located laterally. The SE is pseudostratified columnar while the NSE is stratified squamous. Glandular ducts to the VNO lumen are observed at the margins between epithelia. Large blood vessels and glandular tissue are located within the vomeronasal capsule and their distribution varies with both epithelia along the rostrocaudal axis. Advancing caudally, the paraseptal cartilage is ventrolaterally covered and, by the end of the VNO, is fully replaced by compact bone. The nasopalatine duct connects oral openings of the palatal lobes with the floor of the NC towards the VNO end; it is surrounded by cartilage and formed by respiratory pseudostratified epithelium at its dorsal extent, and oral squamous epithelium towards the palate. Further posteriorly, a septal window connects both NC ventrally. We discuss these findings in terms of access of semiochemicals and pheromones to the VNO and additional chemosensory neurons. Fondecyt Nº 1080094.

P1.28 Déficits neuroplásticos neocorticales inducidos en la rata por desnutrición fetal: Efecto compensatorio del enriquecimiento postnatal temprano Neocortical neuroplastic deficits induced by fetal undernutrition in the rat: Compensatory effect of early postnatal environmental enrichment Flores O (1), Pérez H (1), Valladares L (1), Puentes G (1), Hernández A (2) (1) Unidad de Neurociencia Nutricional, Lab. Nutrición y Regulación Metabólica, INTA, U. de Chile. (2) Lab. Neurobiología, Dep. Biología, Facultad de Química y Biología, U. de Santiago de Chile.

Rats born from mothers submitted to hypoproteic/isocaloric diet during gestation show significant alterations in central noradrenergic systems, together with decreased ability for developing long-term potentiation (LTP) in the neocortex and deficits in learning tasks. In contrast, enriched environments do exert plastic effects upon developing brain that could impact positively on cognitive abilities, possibly by stimulating beta-adrenergic receptors. We studied if an enriched environment (sensory-motor stimulation) implemented during the early postnatal life of the rat (from day 3 to weanling) could revert deficits of LTP induced in the frontal and occipital cortex of rat by protein malnutrition during fetal life, as well as its relationship with beta adrenergic receptors expression. LTP was evaluated in vivo in the rat frontal and occipital cortex at 60-65 days of age, and beta adrenergic receptor density was determined in the same cortical regions by means of radioligand-binding techniques. Results showed (i) that malnourished rats raised on a standard environment presented significant deficits of LTP and beta adrenoceptors in both cortical regions; (ii) that the enriched environment significantly increased the ability to develop LTP as well as the beta adrenoceptor density in the studied cortical regions. These results suggest that poly-stimulation during the suckling period in the rat could compensate for the deficits of neocortical neuroplasticity induced by prenatal nutritional deprivation, possibly through incrementing expression of neocortical beta adrenoceptors.Fondecyt 1080684

P1.29 Estudio de los efectos conductuales inducidos en la mosca Drosophila melanogaster por el tratamiento crónico con nicotina A study on the behavioral effects induced by chronic nicotine treatment in the fly Drosophila melanogaster Fuenzalida-Uribe N, Campusano JM Nucleo Científico Milenio Estrés y Adición (NEDA), Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile

Vertebrates respond to nicotine exposure displaying a set of behaviors that depend on the concentration of the drug and routes of administration, among other factors. This is also the case for invertebrates. For example, flies exposed to low concentrations of nicotine show increased locomotion and stereotypy, while at higher concentrations show hypokinesia or even death. Moreover, flies repetitively exposed to a single nicotine dose show behavioral sensitization, as reported in vertebrates. However, no enough information exists on the effects induced by chronic exposure to nicotine in flies. In this work, we decided to begin evaluating this issue by exposing flies to different concentrations of nicotine delivered in the food and evaluating their locomotor activity and olfactory discrimination.
Low concentration of nicotine (0.025 mg/mL) increases locomotor activity while at higher concentrations of nicotine (0.05 and 0.1 mg/mL) an alteration in locomotor activity is observed. This is accompanied by an impairment in their "hanging" ability at higher doses of the drug. On the other hand, a trap assay was used to evaluate olfactory discrimination. Results obtained show that low concentration of nicotine (0.025 mg/mL) slow down the entry of flies to the traps compared to untreated controls. These results show that chronic nicotine treatment induces behavioral effects, as it has been previously reported in vertebrates. This poses the fly Drosophila melanogaster as a good model to study the cell and molecular determinants of the behavioral effects induced by chronic nicotine treatment.
Fondecyt 1100965, MSI P06/008-F, VRAID 21-2009

P1.30 Actividad y distribución subnuclear de Nurr1 cuando se co-expresa con la SUMO-E3 ligasa PIASγ. Activity and sub-nuclear distribution of Nurr1 when co-expressed with the SUMO-E3 ligase PIASγ. Galdames L, Orellana M, Andrés, ME. Núcleo Científico Milenio Estrés y Adicción, Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. Santiago, Chile.

Aim: Nurr1 is an orphan nuclear receptor essential for the development of midbrain dopaminergic neurons. We reported previously that the SUMO-E3 ligase PIASγ is an interaction partner of Nurr1, with which co-exist in the nuclei of midbrain dopamine neurons. Here, we evaluated the effect of PIASγ on wild type Nurr1 transcriptional activity and its sumoylation mutants. We investigated the influence of histone deacetylases inhibitors on PIASγ repressive effect and the sub-nuclear distribution of Nurr1 and PIASγ wild-type and mutants. Methods: Co-transfecting Nurr1 (wild-type or sumo mutants) and PIASγ (wild type or mutant for the LXXLL motif) expression vectors and treatment with trichostatin-A in HEK-293 and PC12 cells. Luciferase activity, immunofluorescence and confocal microscopy were assessed. Results: Mutation of LXXLL motif of PIASγ abolished the repression induced over Nurr1-dependent transcriptional activity. Treatment with trichostatin-A did not modify the repressive effect of PIASγ over Nurr1, suggesting that PIASγ repressive effect is independent of histone deacetylases. Nurr1 wild type and sumoylation mutants are similarly repressed by PIASγ. PIASγ wild-type and LXXLL motif mutant co-localized with Nurr1 in the cell nuclei. In addition, Nurr1 mutants in SUMO motifs present a similar pattern of nuclear distribution co-localizing with PIASγ. Conclusions: These results indicate that PIASγ requires the LXXLL motif to repress Nurr1-dependent transcriptional activity. However, nuclear co-localization of Nurr1 with PIASγ is independent of the interaction between these proteins. Funded by FONDECYT N°1070349 and MSI N°P06/008-F

P1.31 Los receptores para aminas biogénicas afectan diferencialmente comportamientos agresivos en la mosca Drosophila melanogaster. Biogenic Amine Receptors differentially modify aggressive behaviors in the fly Drosophila melanogaster

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Biogenic amines modulate complex behaviors not only in vertebrates but also in invertebrates. In our lab we are interested in studying the contribution of biogenic amines

and particularly their receptors, in the generation of aggressive behaviors in the fly Drosophila melanogaster.
In this work we isolated male flies for 72 hours and then evaluated 5 well-described aggressive behaviors: slow and fast approach, wing threats, fencing and lunging. We also used the GAL4/UAS technique to knockdown the expression of the type 1 serotonin receptor (5HT1BR) and two octopamine receptors (OAMB and OctR2) in a brain region previously shown to be associated to aggressive behaviors in flies, the Mushroom Bodies (MB). RNAi-mediated knockdown of 5HT1BR reduces the incidence of two aggressive behaviors, fast approach and fencing, compared to wildtype flies. On the other hand, while reduction of OctR2 expression in MB reduces all aggressive behaviors studied, RNAi-mediated knockdown of OAMB expression increased the incidence of fast approach. These results seem not to be explained by an altered locomotor behaviour since no differences are detected between control and RNAi expressing flies in climbing assays. Our results suggest that different biogenic amine receptors differentially modulate aggressive behaviors in the fly Drosophila melanogaster. Fondecyt 1100965, MSI P06/008-F, VRAID 21-2009.

P1.32 Efectos de un ambiente enriquecido sobre la ansiedad inducida por el estrés. Enriched environment effects on the stress-induced anxiety. Gonzalez N, Pérez MA, Figueroa H, Dagnino-Subiabre A. Laboratory of Neurobiology and Behavior, Department of Biomedical Sciences, Faculty of Medicine, Universidad Católica del Norte, Coquimbo, Chile.

Chronic stress alters the amygdala structure and increases anxiety-like behavior, which is reverted following enriched environment (EE). In this study we incorporated these two paradigms to determine whether EE would prevent stress-induced anxiety. After weaning, male Sprague-Dawley rats were housed in a EE or standard condition (SC) for 17 days prior to and throughout of a restraint stress protocol. One day after restraint, anxiety was measured by time in the center of the open field (TCOF) and elevated plus maze (EPM). TCOF was decreased in stressed rats housed in SC than controls (stress: 6.2±1.3, control: 30±5.3, $p < 0.01$), showing more anxiety level. Conversely, TCOF was increased in stressed rats housed in EE than SC animals (stress: 23.9±8.7, control: 10.1±2.3, $p < 0.05$). The frequency of entries into the open-arm of the EPM was decreased in both stress+SC and stress+EE (stress+SC: 3.5±0.5, control+SC: 6±1.1, $p < 0.05$). Time into the open-arm for control rats housed in EE was significant less than control rats housed in SC (control+EE: 3.8±0.5, control+SC: 6±1.1, $p < 0.05$), showing that EE can have an anxiogenic effect as assayed in some behavioral paradigms. These results showed that EE in adulthood prevented anxiety-like behavior in a context less aversive (open field), but not in a more aversive context for the rats (EPM). Interestingly, EE had an anxiogenic effect on control animals measured by EPM. Our data suggest that enhanced opportunities for mental and/or physical activity in adulthood may help overcome potential cognitive deficits induced by stress. Acknowledgements: This work was supported by FONDECYT (Nº 1100413) and "Anillo de Ciencia y Tecnología" (Nº ADI-09) grants (D-S, A).

P1.33 El foco atencional mesencéfalico: ¿Glutamatergico o colinérgico? The midbrain attentional spotlight: Glutamatergic or cholinergic? Gonzalez-Cabrera C., Roth A., Mpodozis J., Marín G. Dep. Biologia. Facultad de Ciencias, U. de Chile.

The optic téctum (TeO) is the main visual center in the avian brain. Two nuclei of the isthmic complex, the isthmi pars parvocellularis (Ipc) and the isthmi pars semilunaris (SLu) receive visual input from TeO and send feedback projections to TeO in a precise topographic fashion. The Feedback signals from Ipc have a strong gating effect upon the ascending visual flow from the tectum to the thalamic nucleus rotundus, probably acting as attentional spotlights of selected visual stimuli. Ipc and SLu are both considered cholinergic because of their strong immuno-positivity for choline acetyl transferase (ChAT). However, we performed in situ hybridization assays to determine the presence of vesicular acetylcholine transporter (VAChT), which transports acetylcholine into synaptic vesicles, finding clear expression of VAChT mRNA in SLu neurons but not in Ipc. This result raises

the possibility that acetylcholine is released by an unknown non-vesicular mechanism, or that Ipc neurons have a different neurochemical identity. A recent study in pigeons found that Ipc neurons expresses mRNA for the vesicular glutamate transporter 2 (VGLUT2). As the mRNA probe used in that study considerably overlapped with others mRNA expressed in the chicken brain, we performed in situ hybridization assays in the chicken mesencephalon using specific RNA probes for ChAT and VGLUT2. We found an expression pattern compatible with glutamatergic and cholinergic identities for Ipc and SLu, respectively. These results may explain the strong gating affect mediated by this nucleus upon the ascending flow of retinotectal inputs. We gratefully thank to Dr. Alexander Vargas M. for his installations and technical support. We also thank to Solano Henriquez for his technical assistance.

P1.34 Neurogénesis en un ambiente tumoral. Neurogenesis in a tumor environment. Jara N, Rodríguez F, Cisternas P, Martínez F, Nualart F. Laboratorio de Neurobiología y Células Madres, Facultad de Ciencias Biológicas, Universidad de Concepción.

The adult mammalian brain maintains the generation of new neurons or neuroblasts by neural stem cells (NSC), which can be found mainly in the subventricular zone (SVZ) of the lateral ventricles (LV). It has been reported that the NSC can proliferate, self-renew and migrate, however these capabilities give them the potential to cause tumors in the SVZ. To analyze the effect of this type of tumors on adult neurogenesis, we induced a SVZ tumor in adult guinea pigs by stereotaxic injection of U87 cells (human glioblastoma multiforme cells) in one ventricle, leaving the contralateral ventricle as a control. Before injection we characterized U87 cells by immunocytochemistry and confocal microscopy, RT-PCR and Western blot. We confirmed that the U87 cell line expressed GFAP, βIII tubulin, and vimentin. In the tumor area, we observed a different extent of damage in the ependymal cells layer (ILB4 +) and the average number of neuroblasts (Tuj +) generated in the SVZ was four times greater than in the SVZ control. However, the average number of precursor cells (BrdU +) was similar in both conditions. Our data lead us to conclude that the tumor does not alter neurogenesis, however, the cells generated accumulate in the SVZ. We postulate that the tumor in the SVZ alter the SLIT1 gradient that induces neuronal migration. Supported by Grants FONDECYT 1100396 (to FN) and CONICYT PhD fellowships (to FR, PC, FM).

P1.35 LA INACTIVACION DE LA CORTEZA CON LIDOCAINA MODULA LAS RESPUESTAS COCLEARES EN CHINCHILLAS CORTICAL INACTIVATION WITH LIDOCAINE MODULATES COCHLEAR RESPONSES IN CHINCHILLAS León A (2), Elgueda D (2), Delano P H (1,2) "(1) Otorrinolaringología, Hospital Clínico de la Universidad de Chile. (2) Programa de Fisiología y Biofísica, ICBM. Facultad de Medicina, Universidad de Chile.

Auditory-cortex descending projections are constituted by pyramidal neurons which somas are located in cortical layer V. These neurons make synaptic contacts with medial olivocochlear neurons which innervate outer hair cells in the cochlear receptor. This means that there are only two synapses between a cortical pyramidal neuron and a cochlear hair cell. In spite of the clear anatomical relevance of the efferent system, its functional role remains unclear. We hypothesized that descending pathways from the auditory cortex regulate cochlear afferent responses through the olivocochlear system. We used fifteen anesthetized chinchillas (450-750), and simultaneously recorded cochlear (auditory-nerve compound action potential (CAP) and cochlear microphonics potential (CM)) and cortical potentials, before and after a microinjection of lidocaine or saline solution in the auditory cortex. We measured cochlear responses to auditory stimuli between 1000 to 8000 Hz and from 20 up to 90 dB SPL. We found that cortical inactivation produced significant changes (up to 4 dB) in the amplitude of CAP and CM in all chinchillas. The main observed effect was a CM decrease in 11 out of 15 chinchillas. Concomitantly to these CM decreases, we found CAP increases in five chinchillas and CAP decreases in six animals. Sixty minutes after the microinjection, amplitudes of cortical potentials were completely recovered, however only

partial recovery was observed in cochlear responses. These results show that there is a modulation of auditory periphery by the cerebral cortex. The diversity of the obtained effects suggests that there are several (at least two) functional pathways from cortex to cochlea. FONDECYT 11080033 to PHD. SOCHIORL 2007 to PHD."

P1.36 Modulación colinérgica de la actividad visual en el tectum óptico de las aves. Cholinergic modulation of visual activity in the avian optic tectum. Macarena Faunes, Juan Carlos Letelier, Jorge Mpodozis, Gonzalo Marín. Facultad de Ciencias, Universidad de Chile

The Optic Tectum of vertebrates (TeO) receives a prominent projection from the contralateral retina. In birds, TeO is reciprocally connected to the ipsilateral nucleus isthmi pars parvocellularis (Ipc) in a topographic and homotopic fashion. Ipc axons are choline acetyltransferase immunopositive and branch in various tectal layers, including superficial retino-recipient layers. It has been shown that Ipc produces a gating of the ascending visual activity from TeO to the thalamic nucleus rotundus.

To explore the neurochemical basis of such gating, we performed in vivo recordings of visual and spontaneous activity in TeO and Ipc of ketamine-anesthetized pigeons.

We show that inactivation of an Ipc locus by microinjecting CNQX (200µM), reduces the retino-tectal evoked potentials in the homotopic locus in TeO, while local over-activation of Ipc by microinjecting picrotoxine (200µM) increases visual responses in the homotopic locus in TeO. Moreover, visual responses in both TeO and Ipc are reduced by microinjection of atropine (100µM) in TeO and enhanced by microinjection of acetylcholine (5mM) or muscarine (500µM) in TeO. On the other hand, microinjection in TeO of acetylcholine (5mM) or nicotine (500µM) produces an increase in the spontaneous activity of Ipc, even if retino-tectal synapses are blocked by application of CNQX in TeO.

Our results suggest that the Ipc feedback upon TeO enhances visual responses via muscarinic receptors –presumably located in the retinal axon terminals or in tectal interneurons - and concomitantly prolongs its own activation via nicotinic receptors, presumably located in the tectal cells projecting to Ipc.FONDECYT 1080220 to GM. CONICYT to MF

P1.37 Las sustituciones específicas de antropoides en un nuevo enhancer del gen Dll1, inducen expresión heterocrónica y heterotópica en ratones transgénicos. Anthropoid specific substitutions of a novel DLL1 enhancer induce heterochronic and heterotopic neocortical expression in transgenic mice. Lopez-Leal R (1,2), Kamm GB (3), Rubinstein M(1,3) y Franchini LF(3). (1) Centro de Estudios Cientificos, Valdivia Chile. (2) Universidad Austral de Chile. (3) INGEBI-CONICET UBA.

The neocortex, the most prominent structural and functional innovation of the mammalian brain, has expanded independently in several mammalian lineages. In anthropoid primates this expansion reached a maximum in the human lineage composing around 80 % of the entire brain mass. We hypothesize that uncovering the genetic basis of primate brain enlargement and functionality will help to understand human brain evolution. We identified an accelerated region carrying 6 positively selected substitutions in the lineage leading to anthropoids upstream of Delta-like1 (DLL1), a gene involved in the proliferation/differentiation switch of neuronal precursors during brain development. The mouse and human orthologs of this region act as a developmental nervous system specific enhancer between E9.5 and E15.5 in transgenic mouse embryos. Swapping the six anthropoid-specific nucleotides into the mouse enhancer expanded the spatio-temporal expression domain of a reporter gene in the developing neocortex between E14.5 to E16.5 and at P1. Moreover, swapping the ancestral nucleotides back into the human ortholog impaired enhancer function within the entire nervous system beyond E14.5 indicating that these positively selected substitutions are critical for proper DLL1 expression at later stages of brain development. We propose that a spatio-temporal expansion of DLL1 expression driven by these six anthropoid-specific substitutions could have contributed to increase the

number of neural precursors in the telencephalon and, ultimately, to shape a bigger brain. CECS Transgenic Mouse Facility, CONICYT.

P1.38 El incremento de la potenciación de larga duración inducida por MDMA (éxtasis) involucra la fosforilación del residuo de serina 845 de la subunidad GLUR1 del receptor AMPA mediante PKA en el área CA1 del hipocampo de rata
THE INCREASE OF LONG TERM POTENTIATION INDUCED BY MDMA ("ECSTASY") INVOLVES PHOSPHORYLATION OF THE 845 SERINE RESIDUE IN GLUR1 SUBUNIT OF AMPA RECEPTOR VIA PKA IN THE CA1 AREA OF RAT HIPPOCAMPUS. Loyola S (1), Solano L (1), Rozas C (1), Arias-Cavieres A(3) , Zeise M (2) ,Morales B (1). (1) Department of Biology, Faculty of Chemistry and Biology, Universidad de Santiago de Chile, Santiago, Chile; (2) School Psychology, Universidad de Santiago de Chile, Santiago, Chile; (3) Faculty of Science, Universidad de Valparaíso, Valparaíso, Chile.

The MDMA or Ecstasy is an illegal drug consumed mainly by teenagers and young professionals for recreational purposes. Among its side effects detected in both humans and experimental animals are the impairment of memory and learning process. Therefore, it becomes necessary to study the mechanisms of action of this drug interfering with cognitive processes. Bearing this in mind, we focus our work on the effect of ecstasy on the long-term potentiation (LTP) in CA3-CA1 synapses of rat hippocampal slices. Previously, we have shown that MDMA superfusion of hippocampal slices increased the magnitude of LTP, involving serotonergic and dopaminergic systems through the activation of their receptors 5HT2A/2C and D1/D5, respectively. This was determined using specific antagonist of these receptors, which were able to selectively block the increase of LTP produced by the MDMA. Now, in this work we found that protein kinase A is also a key component in this mechanism since administration of a specific inhibitor of PKA (PKI) blocked the MDMA response. Furthermore, using the protocol of paired pulse facilitation it was determined that the PKA's action is postsynaptic. Finally, through western blot, we observed that MDMA increases the levels of phosphorylation of residue 845 in the GluR1 subunit of the AMPA receptor, which would produce an increase on the insertion of AMPA receptors in the postsynaptic membrane, thus allowing an increase of LTP in the CA3-CA1 synapses of the hippocampus.
FONDECYT 1080652, DYCIT-USACH

P1.39 La Inactivación de la Corteza Insular Posterior previene la expresión del miedo condicionado. The inactivation of the posterior insular cortex prevents the expression of conditioned fear Madrid C., Contreras M., Torrealba F. Departamento de Ciencias Fisiologicas, Facultad de Ciencias Biologicas, Pontifícia Universidad Católica de Chile.

The interoceptive region of the insular cortex is thought to be responsible for continuously monitoring internal physiological conditions, perhaps underlying the conscious perception of bodily responses, needs and emotions. We suggested that the insular cortex plays an important role in the retrieval of emotional memories, like those related to fear, based on insular connections with prefrontal cortices, visceral thalamus and limbic structures. Rats chronically cannulated in the interoceptive insula, were conditioned to fear. We used the Pavlovian fear conditioning, where an emotionally neutral conditioned stimulus (CS), 5 KHz tone for 20 sec. was paired to a mild (1mA, 1 sec) electric footshock, unconditioned stimulus (US). The CS acquires the capacity to elicit responses that typically occur in the presence of danger, such as freezing, described as a defensive behavior. In the first post-comditioning test, the animals showed a high freezing in the presence of the CS. Then the interoceptive insular cortex was inactivated for an extended period of time with Neosaxitoxin, a reversible voltage-dependent sodium channel blocker. Eight hours later conditioned rats had no freezing in response to CS, and they remained unresponsive for at least two weeks, which did not occur if Neosaxitoxin was infused in the primary somatosensory cortex. This study implicates for the first time the insular cortex in the expression of an emotional state, and also put the neosaxitoxin like promising tool to

reversibly inactivate in the mid-term brain structures in conjunction with behavioral studies of higher brain functions. Financed by Nucleo Milenio P06/008-F.

P1.40 Interacciones entre dos versiones del "Weather Prediction Task" Interactions in two versions of the "Weather Prediction Task". Manterola C. (1), Delgado C. (1, 2), Delano P.(1), Aylwin M.L.(1) (1) Fisiología y Biofísica. Facultad de Medicina. Universidad de Chile (2) Depto de Neurología y Neurocirugía. Hospital Clínico. Universidad de Chile

Implicit memory system refers to learning without awareness or intention, after repetitive training. Its neurological substrate is in the basal ganglia. One of its examples is probabilistic learning, which allows the prediction of the future by the observation of known elements. It has been evaluated in multiple occasions by the "Weather Prediction Task" (WPT), this task consist in the prediction of the weather between binary options. A set of cues, compose by tarot cards, are associated to a weather outcome. Participants receive probabilistic feedback after each response. The probabilistic nature of the task prevents attempts to explicitly memorize a solution (Knowlton 1994). It was been propose that learning strategies in normal subjects are based on strong cue-outcome association, favoring declarative memory (Lagnado 2006). In attempt to examine if the learning process used in the WPT involve implicit or explicit memory, we tested two different versions of WPT; one with strong cue-outcome association (Knowlton 1994), the other with weak cue-outcome association (Klimkowitz 2008). Twenty healthy participants between 20 – 35 years performed each version of the task. Accuracy, reaction time, strategies and awareness were analyzed using ANOVA. We found that accuracy improved significantly only in the strong cue-outcome association task. The patterns with stronger associations showed significant better performance. Both results would indicate the presence of explicit learning. In the version of weak associations, accuracy was negative correlated to response time, interaction that might be related with implicit memory.

PANELES
2. Jueves 30 14:30-16:30

P2.1 Contribución relativa de Ca2+ ATPasa e intercambiador Na+ /Ca2+ en la remoción de Ca2+ desde el cilio olfatorio. Relative contribution of Ca2+ ATPase and Na+ /Ca2+ exchanger in Ca2+ extrusion from olfactory cilia. María Graciela Delgado, Ricardo Delgado, and Juan Bacigalupo. Depto. de Biología, Facultad de Ciencias e Instituto Milenio ICDB, Universidad de Chile.

Odorants trigger a cyclic AMP signaling cascade which mediates the receptor potential in olfatory receptor neurons (ORNs). The excitatory receptor potential results from the dual contribution of a non-selective cationic current through cyclic nucleotide-gated (CNG) channels and a Cl- current through Ca2+–dependent Cl- channels (ClCa). Calcium increments within the cilia due to CNG channels opening causes the activation of ClCa. Clearance of Ca2+ relies on both transporters present in the ciliary membrane, a Na+/Ca2+ exchanger (NCX) and a plasma membrane Ca2+–ATPase (PMCA). To date, it is unclear the relative contribution of two Ca2+ transporters during the course of the receptor potential. Since NCX is electrogenic and PMCA is strongly Ca2+-dependent, their relative participation in Ca2+ clearance may vary along the receptor potential. We measured the transduction current from adult rat dissociated ORNs, under whole cell voltage clamp, in response to cAMP increments elicited by brief (100 ms) puffs of IBMX (a phosphodiesterase inhibitor) at different membrane potentials, ranged from -60 to 60 mV. Since the major component of this cAMP-induced current is carried by Cl- through ClCa, it can be used to monitor intraciliary Ca2+ changes. We measured the current relaxation time constant (τ) inhibiting either transporter separately or both of them together (NCX by replacing external Na+ by Li+, PMCA by omitting ATP). Inhibition of NCX or PMCA incremented τ, but inhibition of both transporters had a much stronger effect. Our observations indicate that both Ca2+ transporters similarly contribute to Ca2+ clearance from the chemosensory

cilia. MIDEPLAN: ICM P05-001-F, Fondecyt 1080653 and 1100682, FIRCA / NIH TW007920

P2.2 Src quinasas regulan la exocitosis en células cromafines. Src kinases regulate the exocytosis in chromaffin cells. María José Olivares, María José Guerra, Montserrat Hevia, Ana María Cárdenas. Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso.

Las Src quinasas comprende un grupo tirosina quinasas, de las cuales c-Src, Yes y Fyn se expresan ampliamente en el sistema nervioso, participan en procesos de plasticidad sináptica, y regulan la liberación de glutamato. Sin embargo, el mecanismo mediante el cual estas tirosinquinasas regulan la exocitosis se desconoce. Además de poseer un dominio catalítico, las Src quinasas poseen un dominio SH2 que une fosfotirosinas específicas de sus propios sustratos, y un dominio SH3 que se asocia a proteínas con regiones ricas en prolina (RRP). Entre los sustratos de las Src quinasas se encuentran sinaptofisina, una proteína de la membrana de vesículas sinápticas que posee un carboxilo citosólico rico en residuos tirosinas y la GTPasa dinamina, que posee una RRP. De acuerdo nuestros estudios previos, la asociación de sinaptofisina con dinamina regula la liberación quántica de adrenalina y noradrenalina desde células cromafines de la médula adrenal. Nuestros estudios preliminares en células cromafines muestran que cSrc interacciona, probablemente a través de sus dominios SH2 y SH3, con sinaptofisina y dinamina. Por otra parte, utilizando amperometría, observamos que la inyección intracelular de los dominios SH3 o SH2 de cSrc, o el tratamiento de las células con el inhibidor específico PP2, aumentan la duración de los eventos de exocitosis y disminuyen la cantidad de catecolaminas liberadas por evento. En conjunto nuestros resultados indican que las Src quinasas regulan la exocitosis, probablemente regulando la asociación de sinaptofisina con dinamina. Proyecto Fondecyt 1085266

P2.3 El Lecho de la Estría Terminal ejerce una influencia excitatoria mediada por CRF-R1 sobre neuronas dopaminérgicas del Área Tegmental Ventral. The bed nucleus of the stria terminalis exerts a CRF1 receptor-mediated excitatory influence on Ventral Tegmental Area dopaminergic neurons. Miranda A P (1,2), Forray M I (1,2), Gysling K(1,3). (1) Nucleus Millennium in Stress and Addiction. (2) Department of Pharmacy, Faculty of Chemistry. (3) Department of Cell and Molecular Biology, Faculty of Biological Sciences. Pontificia Universidad Católica de Chile.

The bed nucleus of the stria terminalis (BNST) has been involve in the excitatory regulation of the ventral tegmental area (VTA) dopaminergic neurons. It has been proposed that corticotropin-releasing factor (CRF) central circuits including the BNST play a mayor role in stress-induced drug seeking behavior. The present study was aimed at evaluating the role of glutamate (GLU) and CRF from the ventral-BNST in mediating activation of VTA dopaminergic neurons in naïve and repeatedly stressed rats. Specifically, we studied whether the activation of the ventral BNST exerts GLU- and/or CRF-mediated excitatory regulation of VTA dopaminergic activity. For these purpose, we stimulated the ventral-BNST by infusing 0.25 µL of a solution containing GLU (100 µM) and bicuculline (10 µM), and studied GLU and DA extracellular levels in the VTA by in vivo microdialysis. To study the participation of CRF1 receptors on this effect we perfused a CRF1 receptor antagonist (NBI27914) through the microdialysis probe located in the VTA. Our results show that local stimulation of the ventral-BNST increases VTA dopamine extracellular levels in both naïve and repeatedly stressed rats, without affecting glutamate extracellular levels. Interestingly, a significantly greater increase of dopamine extracellular levels was observed in repeatedly stressed rats. The effect of ventral-BNST activation on VTA dopamine extracellular levels was blocked by intra VTA perfusion of NBI27914. Thus, our results indicate that ventral-BNST exerts a CRF1 receptor-mediated excitatory influence on DA neurons. Moreover, repeated stress sensitizes the response of VTA DA neurons to ventral-BNST activation. Supported by grants FONDECYT Grant 1070369 & Millennium Science Initiative Grant Nº P06/008-F.

P2.4 Psicofísica y actividad de potenciales relacionados a evento del aprendizaje perceptual usando una aproximación ecológica. Psychophysics and event related potential activity of visual perceptual learning using an ecological approximation.

Montefusco-Siegmund R, Maldonado PE, Aylwin ML Programa de Fisiología y Biofísica, ICBM, Facultad de Medicina, Universidad de Chile.

Perceptual learning (PL) is the improvement of performance on a perceptual task after continuous practice, and is a manifestation of adult brain plasticity. PL has been studied in different sensory systems, where changes in behavior and neuronal activity in tasks based in different physical attributes of simple stimuli have been described. In natural situations, the PL involves more complex stimuli and ecological significance. Therefore, we developed a ""same/different"" discrimination task using 30 Kanji characters of 17 and 18 strokes that results in PL. Twelve subjects were trained for 9 sessions and the correct answers and reaction time were measured. At the 10th session, 15 trained characters and 15 untrained characters were presented. The subjects' performance on untrained stimuli was around 70% of the maximum achieved with trained characters, which reflects in part, PL with a significant component of explicit memory. As a control experiment, 8 subjects were trained using a different stimulus category (scrambled checkerboards) and at the 10th session, they were evaluated with Kanji characters. Using this approach, we can separate the two main components of learning: PL and procedural (eg. working memory, mental rotation and motor learning). Parallel evoked potential recordings exhibit changes in the amplitude of late components, which are shared by both trained and untrained stimuli, thus implying similar representations mechanisms for the learned category.Conicyt para estudios de doctorado. Iniciativa Científica Milenio P04-068F

P2.5 Curso temporal de la expresión de eNOS e iNOS y producción de óxido nítrico en el cuerpo carotídeo de ratas expuestas a hipoxia intermitente crónica. Temporal course of eNOS, iNOS expression and nitric oxide production in the carotid body of rats exposed to chronic intermittent hypoxia. Moya EA, Del Rio R, Iturriaga R. Laboratorio de Neurobiología, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Santiago, Chile.

Chronic intermittent hypoxia (CIH) a main feature of sleep apnea, is characterized by brief hypoxic episodes followed by normoxia. The carotid body (CB) is the main oxygen chemoreceptor, which senses arterial PO2 levels and produces reflex ventilatory responses to hypoxia. Rats exposed to CIH for 21 days show enhanced CB chemosensory responses to hypoxia and develop hypertension, but the mechanisms underlying the CB potentiation are not fully understood. Since a reduction of nitric oxide (NO) level has been involved in the hypertension and NO is an inhibitory modulator of CB chemoreception, we hypothesized that CIH may reduced the NO production in the CB of CIH-rats. Thus, we studied the CB chemosensory responses to hypoxia along with the immunoreactivity of endothelial (eNOS-ir) and inducible (iNOS-ir) nitric oxide synthases and NO production in the CB of male Sprague-Dawley rats (200g), exposed to 5%O2, 12 times/hour, 8 hours/day for 7-21 days. We found that 7 days of CIH potentiated CB chemosensory responses to hypoxia, which persisted until 21 days. The eNOS-ir decreased at the 7 day of CIH and tended to recover at 21 days. By contrast, the iNOS-ir only increased at the day 21 of CIH. In addition, we found a non-significant, but marked decrease of NO production in CBs from rats exposed to 7 days of CIH, which paralleled the reduced eNOS-ir. Thus, present results suggest that changes in NO levels and eNOS-ir after 7 days of CIH may partially contribute to the chemosensory potentiation. FONDECYT 1100405.

P2.6 Perfil lipídico de las membranas rabdoméricas de los fotorreceptores de Drosophila Lipid profile of Drosophila photoreceptor rhabdomeric membranes Muñoz Y (1,2), Sepúlveda M (1,2), Delgado R (1,2), Bacigalupo J (1,2), Roth A (1). (1) Depto. de Biología. Facultad de Ciencias, Universidad de Chile. (2) Instituto Milenio ICDB, Universidad de Chile.

The first member of TRP channel family was discovered in Drosophila, in a specialized structure of the photoreceptors termed rhabdomere, formed by ~30.000 tightly packed microvilli, where light transduction takes place. Phototransduction involves a PLC cascade which generates diacylglycerol (DAG) and IP3. DAG and its metabolic products, the polyunsaturated fatty acids (PUFAs), have been proposed to take part in TRP activation, but the underlying mechanism is unknown. Application of any these lipids to intact photoreceptors as well as to excised rhabdomeric membrane patches triggers TRP-dependent currents. A key question is whether light induces changes in the rhabdomere composition of these lipids and which of them may be implied in opening the TRP channels in their native membrane. To assess this problem we developed a membrane preparation enriched in rhabdomeres, obtained by differential centrifugation, in which the lipid profile could be determined. For this we performed a Folch´ lipid extraction, separation with aminopropyl columns and gas chromatography coupled to mass-spectrometry. Our results revealed the presence of different free fatty acids: myristic acid (c14:0), palmitic acid (c16:0), palmitoleic acid (c16:1Δ 9Z), estearic acid (c18:0), oleic acid (c18:1Δ 9Z), linoleic acid (c16:2Δ 9Z,12Z), arachidic acid (c20:0) and occasionally α-linolenic acid (c18:3Δ 9Z,12Z,15Z). We did not detect the presence of arachidonic acid (c20:4Δ 5Z,8Z,11Z,14Z) non docosahexaenoic acid (c22:6Δ 4Z,7Z,10Z,13Z,16Z,19Z). Our measurements have been done in light-exposed flies; experiments in light-insensitive flies for comparison of their lipid profiles are under way. Acknowledgements: FONDECYT Grant #1100730 and MIDEPLAN ICM-P05-001-F.

P2.7 El Receptor Scavenger de Clase A es necesario para una apropiada respuesta inflamatoria en astrocitos. Scavenger receptor class A is necessary for appropriate inflammatory response in astrocytes Murgas P, Godoy B, Soto D, Median E, von Bernhardi R Laboratorio de Neurociencias, Facultad de Medicina, Pontificia Universidad Católica de Chile

Scavenger receptor A (SR-A) was considered to be expressed almost exclusively in myeloid cells. It is involved in recognition of endogenous molecules and pathogens, and inflammatory activation. Here we describe for the first time, the presence of SR-A in astrocytes, modulators cells of the neuroinflammatory response. Astrocytes derived from SR-A-/- or SR-A+/+ mice were stimulated with LPS or Polyinositic acid (Poly I, a SR-family-A ligand). In contrast to microglial cells, which showed little dependence of SR-A, we demonstrated the participation of SR-A on the activation of NF-kB pathways, nitric oxide (NO) and the modulation of IL-1b production by astrocytes. MAPKs (JNK, ERK1/2) signaling pathways were evaluated. The absence of SR-A had no effect on the activation of ERK1/2 or JNK induced by inflammatory stimulation with LPS. However, the NF-kB transcripcional factor was unable to translocate into the nucleus in SR-A-/- astrocytes, whereas it normally translocated in SR-A+/+ astrocytes exposed to the same stimulus. At functional levels, astrocytes from SR-A-/- mice exposed to proinflammatory ligands produce 50% less NO than SR-A+/+ astrocytes, whereas NO production in microglia was equivalent for both genotypes. On the other hand, LPS or Poly I stimulated SR-A-/- astrocytes to produce 4-fold more IL-1b that their SR-A+/+ counterparts. In summary, our results show that stimulation of astrocytes from SR-A-/- animals with proinflammatory molecules activated MAPK but not NF-kB signaling, resulting in higher levels of IL-1b but lower NO production compared with SR-A+/+ astrocytes. All together, our results suggest that SR-A can modulate the inflammatory response of astrocytes. Support for grant FONDECYT 1090353 is acknowledged

P2.8 Potenciación de largo término induce cambios en la metilación del gen de reelin, en ratas Hippocampal long-term potentiation induces changes of DNA methylation in the gene for reelin in rats. Negrón I (1), Lizana P(1), Muñoz P (1,2). (1) Centro Interdisciplinario de Neurociencias de Valparaíso (CINV), Universidad de Valparaíso. (2) Centro Regional de Estudios en Alimentos Saludables (CREAS), Valparaíso.

Reelin gene is important for synaptic plasticity and long-term memory. However, it is unclear how these processes regulate the gene expression of reelin. Recent data have shown that epigenetic mechanisms, such as DNA-methylation, would be involved in the neuronal activity-dependent expression of this gene. In the present work, we assessed the relationship between synaptic plasticity and the epigenetic regulation of reelin expression. To that end, we applied tetanic stimulation in rat hippocampal brain slices to induce lasting long-term potentiation (L-LTP) a putative physiological substrate for memory. The degree of DNA methylation and reelin– containing mRNA transcripts, in microdissected CA1 tissue, was estimated with a methyl-sensitive PCR (MSP) using primer specific for the CpG islands located an internal region of the reelin gene and RT-PCR, respectively. We have found a significant decrease of the methylation and significant increase of the expression of the reelin gene, after two hours of LTP induction. On the other hand, we have found that slices superfused twenty minutes after LTP induction with 5AZA (30 μM), a demethylating agent, affected LTP maintenance. Interestingly, this effect was observed only when the drug was applied 20 min, but not 120 min after potentiation. Altogether, these data suggest that there is a critical period in which the activity-dependent expression of reelin could be sensitive to changes in DNA methylation. GORE - CONICYT R06I1004.

P2.9 Receptores CRH tipo 2, Orexina A y el transportador de glutamato vGluT2 son colocalizados en terminales nerviosos de VTA originados en el área del hipotálamo lateral. CRF type-2 receptors, orexin A and vGluT2 glutamate transporter are colocalized in VTA nerve terminals originated from the rat lateral hypothalamic area Noches V, Araya K, Gysling K. Millenium Science Initiative Nucleus "Stress and Addiction", Department of Cellular and Molecular Biology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile.

It has been shown that stressful stimuli induce the release of corticotrophin releasing factor (CRF) in ventral tegmental area (VTA) in control and cocaine-experienced rats. Interestingly, CRF induces the release of VTA glutamate only in cocaine-experienced rats through the activation of CRF type-2 receptors (CRF2). Furthermore, the activation of VTA CRF2 is essential for stress-induced relapse to drug seeking. However, there is not available anatomical evidence of the origin of VTA glutamatergic nerve terminals expressing CRF2. VTA receives glutamatergic afferences from cortical and subcortical brain regions. The most prominent subcortical VTA glutamatergic afference is originated in the lateral hypothalamic area (LHA) which is also the only brain area that express orexin neurons. It has been shown that LHA plays a role in addictive behavior. Thus, we decided to determine whether VTA glutamatergic nerve terminals bearing CRF2 are originated in LHA. To this end, we performed double and triple immunofluorescence in a VTA synaptosomal preparation devoid of postsynaptic elements using antibodies against orexin A, CRF2 and vGluT glutamate transporters. We have detected colocalization of CRF2 with orexin A and of CRF2 with vGluT2. Preliminary data with triple immunofluorescence show the existence of VTA synaptosomes co-expressing CRF2, orexin A and vGluT2. Thus, our results give anatomical support to suggest that LHA glutamatergic neurons are involved in stress-induced relapse to drug seeking. Supported by: MSI N°P06/008-F and FONDECYT 1070340

P2.10 Identificación del sueño MOR fásico y tónico en la rata Identifying tonic and phasic REM sleep in the rat Ocampo-Garcés A, Castro J, Espinoza D, Diaz J Programa de Fisiología y Biofísica,ICBM, Facultad de Medicina, Universidad de Chile

Tonic and phasic Rapid eye movement (REM) sleep has been described in the human polysomnogram as two alternating sub-components of REM sleep. Typical signs of tonic REM (tREM) sleep are desynchronized EEG and sustained muscle atonia. tREM sleep is interrupted by intervals known as phasic REM (pREM) sleep, characterized by intense REMs, associated to autonomic instability, and axial and distal muscle twitches. Although neurobiological and neurophysiological aspects of phasic phenomena of REM sleep has been studied in animal models (particularly cats and rats), there are no studies oriented to the chronobiological characterization of phasic and tonic components of REM sleep. Adult male

albino rats were chronically implanted for polysomnographic record, including EEG, electoculography (EOG) and muscular activity of the mystacial pad (whiskers EMG). Animals were maintained under a 12:12 LD cycle with food and water ad-libitum. Visual and automated sleep state scoring were performed. Our preliminary results suggest that mystacial pad activity is a confident marker of pREM sleep, whereas EOG is not. Phase-locking analysis and cluster analysis will be presented. Our purpose is to incorporate the dual (phasic and tonic) nature of REM sleep in explaining the chronobiology of mammalian sleep-wake cycle. FONDECYT 1100245

P2.11 Rol de conductancias termoreguladas en la respuesta dinámica de terminales nerviosos periféricos sensibles a frío. Una aproximación mediante modelos matemáticos. Role of thermoregulated conductances on the dynamic response of peripheral innocuous cold receptor terminals. A mathematical modeling approach. "Olivares E(1,2), Orio P (1). (1) Centro Interdisciplinario de Nurociencia, U/niversidad de Valparaíso. (2) Programa de Doctorado en ciencias, mención neurociencias, Universidad de Valparaíso

In mammals, cold transduction and coding occur in peripheral cold receptor terminals. Under stable thermal conditions, innocuous cold thermoreceptors present temperature-dependent firing patterns -the 'static response'- that range from regularly timed single spikes to periodic bursts. Upon a sudden decrease in temperature, the terminal shows a transient rise in mean frequency -the 'dynamic response'- characterized by an increase in bursting. The static response is thought to arise from a slow sub-threshold oscillation coupled to action potential generation mechanisms. Under this paradigm, current mathematical models are able to reproduce the static response of innocuous cold receptor terminals, however, they fail to reproduce the dynamic response. Interestingly, these models lack any conductance specifically modulated by temperature such as the TRPM8 or TREK1 ion channels, known to be expressed in innocuous cold thermoreceptors. We are using conductance-based modeling to test whether the dynamic response can be reproduced by the addition of TRPM8 and TREK1 conductances to a canonical model of temperature modulated bursting, the Huber&Braun model (Int.J.Bifurc.Chaos 8:881-889). We found that the solely inclusion of TRPM8 (cold-activated, depolarizing) conductance is not sufficient to reproduce the dynamical response, as the decrease in membrane resistance after TRPM8 activation reduces burst discharges in spite of depolarization. Compensation of membrane resistance changes by the inclusion of TREK1 (cold-inhibited, hyperpolarizing) conductance allows us to present a model that reproduces both the static and dynamic responses of innocuous cold terminals, with calcium-dependent adaptation of TRPM8 as the principal determinant of the transitory nature of the dynamic response.

CONICYT 21080289 Fellowship (EO), FONDECYT 11090308 and DIPUV(UV) 51-2007 grants (PO).

P2.12 Expresión funcional de un canal de potasio tipo TASK sensible a hipoxia en células quimiorreceptoras del cuerpo carotídeo de rata adulta. Functional expression of a hypoxia-sensitive TASK-like potassium channel in adult rat carotid body type-1 cells. ORTIZ FC, DEL RIO R, VARAS R, ITURRIAGA R. Lab Neurobiología. Facultad Ciencias Biológicas, P. Universidad Católica de Chile.

The carotid body (CB) is the main oxygen arterial chemoreceptor. The CB chemoreceptor (type-1) cells respond to hypoxia with a depolarization. In neonatal rats, a key step in this mechanism is the reversible inhibition of a TASK-like background potassium channel by hypoxia. However, in the adult rat CB there is no evidence of the functional presence of TASK-like K+ channels. Therefore we evaluated the presence of background potassium currents in type-1 cells dissociated from adult rat CBs. TASK-channel activity was recorded using the cell-attached voltage clamp technique. The channel was active at resting membrane potential and shows no/little voltage dependence in a wide range of membrane potentials (-120 mV to +30mV). The current shows burst discharges and flickery activity, a conductance of 15.4 ± 0.8 pS (symmetrical K+) and insensitivity to the

K-channels blockers tetraethylammonium and 4-aminopyridine. The current was not affected when Cl- was replaced by gluconate. Moreover, modifications of extracellular K+ concentration shift the I-V curve as expected from a highly selective potassium conductance. Acute hypoxic stimuli (PO2= 5 Torr), induced a strong inhibition on the K+ current (58.5 ± 7.6 %) in type-1 cells. We also performed co-immunolocalization of TASK-1, TASK-3 and the type-I cells marker tyrosine hydroxylase. Our results show that TASK-like channels are functionally expressed in the adult rat CB type-1 cells and suggest that they are involved in the response to acute hypoxic stimuli. FONDECYT 1100405

P2.13 Cambios en la via de señalizacion c-Abl en neuronas y astrocitos inducidos por hipoxia y citoquinas. Changes in c-Abl signaling pathway in neurons and astrocytes induced by hypoxia and cytokines. Osorio J (1,2), von Bernhardi R (2), Alvarez A (1) "(1) Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile
(2) Laboratorio de Neurociencia, Departamento de Neurología, Facultad de Medicina, Pontificia Universidad Católica de Chile

Alzheimer Disease (AD) is the most common neurodegenerative disease, affecting millions of people worldwide. Currently, there is no treatment to prevent or slow the disease progression. It is believed that cognitive impairment is triggered by the accumulation of amyloid-beta peptide (Abeta) forming toxic oligomers and amyloid fibrils, which are the main component of senile plaques. Reactive microglia and astrocytes surround Abeta deposits and appears to be responsible for AD-associated neuroinflammation. We have previously shown that the non-receptor tyrosine protein kinase c-Abl plays a central role in neurodegeneration induced by Abeta fibrils in both in vitro and in vivo models. Also, it has been shown that cytokines such as TNFalpha and TGFbeta, and oxidative stress, activate c-Abl signaling in non-neuronal cells. Because neuroinflammation and glial activation are central in AD pathology, we decided to evaluate in glial cells and neurons, if inflammatory mediators or oxidative stress, modulate the activation of c-Abl. We generated primary cultures of hippocampal neurons from rat embryos and treated them in vitro with different concentrations of TGFbeta. Astrocytes cultures were obtained from newborn rats and were subjected to cytokines treatment and hypoxia for different time lengths. After treatment, we evaluate c-Abl activation by assessing phosphorilated c-Abl by western blot. Our results show that TGFbeta activates c-Abl pathway in rat hipppocampal neurons. In contrast, we did not find c-Abl activation in astrocytes treated with TGFbeta nor subjected to hypoxia. Therefore astrocytes and neurons have a different response to hypoxia and cytokines.
Supported by grants FONDECYT 1090353 (RvB) and 1080221 (AA) and grant "Proyecto Interdisciplinario N° 08/2009" from the VRAID of the Pontificia Universidad Católica de Chile.

P2.14 EL PROCESAMIENTO PROTEOLÍTICO DE TAU POR CASPASA 3 ES INDUCIDO POR INFECCION NEURONAL CON HERPES SIMPLEX VIRUS TIPO 1

PROTEOLYTIC PROCESSING OF TAU BY CASPASE-3 IS INDUCED IN NEURONS INFECTED WITH HERPES SIMPLEX VIRUS TYPE 1 Otth C(1), Neira R (1), Lerchundi R (1), Valdivia S (1), Vio K (2), Concha M I (3), Zambrano A (4). "(1) Instituto de Microbiología Clínica, (2) Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile; (3) Instituto de Bioquímica e (4) Instituto de Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.

Objective: Herpes Simplex Virus Type 1 (HSV-1) is ubiquitous, neurotropic, and the most common pathogenic causes of sporadic acute encephalitis in humans. Herpes simplex encephalitis is associated with a high mortality rate and significant neurological, neuropsychological, and neurobehavioral sequelae, which afflict patients for life. HSV-1 infects limbic system structures in the central nervous system, and has been suggested as an environmental risk factor for Alzheimer's disease. In a previous study we demonstrated

that HSV-1 triggers hyperphosphorylation of tau epitopes serine 202/threonine 205 and serine 396/serine 404 in neuronal cultures, resembling what occurs in neurodegenerative diseases. Therefore, the aim of the present study was to further evaluate at a cellular level other possible mechanisms associated with neurodegeneration triggered by HSV-1 infection in primary neuronal cultures, such as caspase-3 induced cleavage of tau.
Methods: Western blot and immunofluorescence techniques were used to study caspase-3 activation and tau protein processing in mice neuronal primary cultures infected with HSV-1.
Results: It is shown that HSV-1 infection induced caspase-3 activation and cleavage of tau protein at aspartic acid 421, generating an epitope that is recognized by TauC3 antibody. In agreement with our previous study on tau hyperphosphorylation, tau cleavage was also observed during the first 4 hours of infection, before neuronal death takes place.
Conclusions: Neuronal HSV-1 infection induces caspase-3 activation triggering the cleavage of tau at its specific site. This tau processing has been previously demonstrated to increase the kinetics of tau aggregation and has been observed in neurodegenerative pathologies.
Funding FONDECYT 11080067, DID-UACH. Funding FONDECYT 11080067, DID-UACH.

P2.15 Efectos del estrés prenatal sobre aspectos morfológicos y neuroendócrinos en la progenie masculina de ratas Prenatal stress effects on morphological and neuroendocrine aspects of male rats progeny". "Pallarés ME (1). González-Calvar SI (2). Bourguignon NS (3). Adrover (E). Katunar MR (1). Baier CJ (1). Lux-Lantos V (3). Calandra RS (2). Antonelli MC(1). (1) IQUIFIB- Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires. Junín 956. Buenos Aires. Argentina.
(2)Laboratorio de Esteroides y (3) Laboratorio de Neuroendocrinología- IBYME. Vuelta de Obligado 2490. Buenos Aires. Argentina.

We have previously demonstrated that prenatal stress (PS) exerted an impairment of dopaminergic (DA) metabolism in limbic brain areas especially after puberty. In this study we evaluated hypothalamic- pituitary- testicular (HPT) axis status of males exposed to PS. Stress consisted on a 3 daily- 45min restraint session from day 14th of gestation to delivery. Anogenital distances and testis descent were measured at postnatal day (PND) 21. Luteinizing hormone (LH), Testosterone (T) and 5-□ Androstane- 3-□, 17□- Diol (DIOL) serum levels of 28, 35, 45, 60 and 75 days old male progeny were determined by radioimmunoassay. Testes of 35 and 60 days old animals were processed for histological morphometric measures. PS reduced anogenital distance of PND 21 male offspring and induced a 2-day delay in the completion of testis descent, in comparison to controls (C). Hormones serum levels analyses revealed that PS diminished LH at PND 28 and 75 (88 and 65,6%), decreased T serum levels at 75 days old rats (58,6%), and increased DIOL levels at PND 28 and 45 (61 and 63%). Additionally, the rate of spermatogenesis development was accelerated on PS rats and the mean tubular diameter was increased. However, the mean Leydig cell's number was reduced on PS rats. Our results demonstrate that stress during gestation induces long term effects on the male progeny HPT axis. Since it was reported that gonadal hormones produce plastic changes in the brain, a disbalanced hormone milieu might be responsible of the DA metabolism impairment observed in our previous studies.

P2.16 Déficit de aprendizaje visuo-espacial y de expresión neocortical de BDNF inducido por desnutrición prenatal: Efecto compensatorio del enriquecimiento postnatal temprano. Deficits of visuo-spatial learning and neocortical BDNF expression induced by prenatal undernutrition: Compensatory effect of early postnatal environmental enrichment Puentes G (1), Navarrete A (2), Martínez A (2), Castillo A (3), Flores O (1), Pérez H (1), Hernández A (4), Burgos H (3). "(1) Lab. Nutrición y Regulación Metabólica, INTA, Universidad de Chile; (2) Facultad de Educación, Universidad de los Lagos; (3) Escuela de Psicología, Universidad Autónoma de Chile; (4) Lab. Neurobiología, Universidad de Santiago de Chile.

Hypoproteic/isocaloric malnutrition during gestation lead to a significant decrease of callosal-cortical synapses in the rat, resulting in impaired neocortical LTP. In contrast, enriched environments exert favourable plastic effects on the brain and improve some cognitive processes, possibly by overexpressing neocortical brain-derived neurotrophic factor (BDNF) among other factors. We studied if an enriched environment (sensory-motor stimulation) implemented during the early postnatal life of rats (from day 3 to weanling) could reverse deficits of visuo-spatial learning induced by prenatal undernutrition, as well as the relationship with changes in BDNF in some brain regions related to cognition. Visuo-spatial learning was assessed by means of an eight-arm radial Olton maze, and BDNF expression was determined in the frontal and visual cortices and hippocampus by ELISA method. At 60-65 days of age, undernourished rats reared on a standard (non enriched) environment showed significant deficits in visuo-spatial performance and in neocortical, but not hippocampal, BDNF expression. In contrast, undernourished/enriched rats showed similar visuo-spatial performance to well-nourished counterparts, as well as similar (neocortex) or even higher (hippocampus) BDNF levels to normal/standard animals. These results suggest that enrichment during the lactating period in the rat could compensate for deficits of visuo-spatial learning induced by prenatal nutritional deprivation, possibly through incrementing the expression of BDNF neurotrophin in the neocortex and/or hippocampus. Fondecyt 1080684 and 1070028

P2.17 SPLICING ALTERNATIVO DEL mRNA DE NEUROPÉPTIDO B DE RATA

ALTERNATIVE SPLICING OF RAT NEUROPEPTIDE B Mrna. Quiroz G, Munita R, Galaz P, Blanco E, Ibáñez M A, Galdámes L, Andrés M A, Gysling K. Millenium Science Initiative Nucleus "Stress and Addiction", Department of Cellular and Molecular Biology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile.

Intron retention is a splicing variant that accounts for at least 14,8 % of the alternative splicing in the human transcriptome (Galante et al., 2004). These authors have shown that intron retention occurs preferentially in relatively short introns that also have a high content of GC. The alignment and analysis of the orthologous sequences of neuropeptide B (NPB) mRNA in rat, mouse and human (Fujii et al., 2002; Brezillon et al., 2003; Tanaka et al., 2003) indicate that the NPB mRNA is a good candidate to have intron retention. The purpose of the present study was to determine by RT-PCR and sequencing whether NPB present alternative splicing by intron retention. Results showed the existence of a NPB transcript with intron retention. Unexpectedly, results also showed the existence of an additional NPB transcript with an alternative 5' splice site. Thus, NPB presents 3 alternative transcripts suggesting that the expression of NPB could be modulated at this level. "Supported by: MSI N°P06/008-F and FONDECYT 1070340

P2.18 La exposición de largo plazo a dosis sub-agudas de diclorvos que inhiben específicamente a la acilpéptido hidrolasa y no a la acetilcolinesterasa produce efectos opuestos en procesos de plasticidad sináptica en ratas jóvenes y de mediana edad Long-term exposure to sub-acute doses of dichlorvos that inhibits specifically acylpeptide hydrolase and not acetylcholinesterase elicit opposite effects on synaptic plasticity processes in young and middle-aged rats. Rojas-Espina D., García-Rojo G., Sandoval R. y Pancetti F. Laboratorio de Neurotoxicología Ambiental, Departamento de Ciencias Biomédicas, Facultad de Medicina, Universidad Católica del Norte

Acylpeptide hydrolase (ACPH) belongs to the family of prolyl-oligopeptidases and catalyses the hydrolysis of N-acylated peptides to generate an acylated amino acid and a free N-terminal peptide. The physiological role of ACPH in neuronal tissue is unknown, however, it has been reported that ACPH can degrade the beta-amyloid peptide (Abeta) in vitro. Recently we have reported that in vitro short-term exposure of rat hippocampal slices to acute doses of the organophosphate molecule dichlorvos inhibits ACPH at a specific window of dosage and exposure time. A higher dose of dichlorvos or prolonged exposures causes the inhibition of both enzymes: ACPH and acetylcholinesterase (AChE). The specific inhibition of ACPH by dichlorvos is correlated with a significant increase of long-term

potentiation (LTP) through an indirect mechanism that involves the activation of postsynaptic □7 nicotinic receptors. In vivo long-term exposure (4 weeks) of young (3 weeks) male Sprague – Dawley rats to 0.1 mg/kg dichlorvos elicits an enhancement of LTP that is correlated with ACPH -but not AChE- inhibition, confirming in vivo the effect that we have described in vitro. However, when middle-aged rats (10-12 month-old) are exposed to the same dose and time-schedule used in young rats, the effect is inverted and the enhancement of LTP is abolished. We hypothesize that the opposite effect on LTP in young and middle-aged rats is being mediated by the accumulation of differential endogenous Abeta concentrations. Support by: VRIDT from Universidad Católica del Norte and INNOVA - Corfo

P2.19 El trauma in vitro aumenta la permeabilidad celular dependiente de hemicanales formados por conexinas. Trauma increases astroglial cell membrane permeability connexin based hemichannels in vitro. Rovegno M (1), Soto P (2), Sáez P (2), Sáez JC (2), von Bernhardi R (1). (1) Laboratorio de Neurociencias, Departamento de Neurología, Facultad de Medicina. (2) Departamento de Ciencias Fisiológicas, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

Introduction: Traumatic brain injury is a leading cause of morbidity and death. In trauma, connexins play an important role in the propagation of secondary damage. However, the exact participation of connexins, either as channels or hemichannels, remains unknown. Aim: To study the participation of connexins in cell communication among astrocytes post trauma in an in vitro model. Trauma was induced by scraping astrocyte-enriched monolayers derived from neonatal rats, in the presence of 60 mM extracellular potassium (K+e) to mimic conditions found in vivo after trauma. Results: Trauma plus high K+e increased astrocyte ethidium (Etd) uptake ($p<0.001$), revealing an increment in membrane permeability that was abolished by 200 μM La3+, a connexin hemichannel blocker ($p<0.001$; $n=10$). The use of a connexin gap-junction/hemichannel blocker (50 μM 18-β-glycyrrhetinic acid; β-GA) further decreased Etd uptake to values bellow basal condition ($p<0.001$; $n=3$). However, the magnitude of β-GA-induced blockade was more robust than that of La3+. Gap-junction-dependent cell coupling was estimated by dye (Lucifer yellow) transference, and no change was found in the dye coupling incidence or index ($n=10$). Conclusion: In this model of trauma, astroglial cell communication via gap junctions is not significantly affected but cells gain connexin hemichannel-mediated communication." Grant support from the Dirección de Investigación de la Facultad de Medicina-PUC and Fondecyt Grant 1090353 (to RvB) and 1070591 (to JCS) are acknowledged.

P2.20 El pH astrocítico como mediador del acoplamiento energético cerebral. Astrocytic pH as key mediator of energy coupling in the brain Ruminot I (1,3) and Barros L.F. (1,2). (1) Centro de Estudios Científicos (CECS) (2) Centro de Ingeniería de la Innovación del CECS (CIN) (3) Universidad Austral de Chile

fMRI and FDG-PET show local increases in blood flow and glucose uptake in response to neuronal activity, but the low resolution of these techniques does not allow to identify which cell type metabolizes the sugar, nor to elucidate the underlying biochemical mechanisms. Using FRET nanosensors and a novel method to measure the rate of glycolysis, we have previously observed that an increase in extracellular potassium provokes an acute and reversible stimulation of astrocytic glycolysis. In parallel, potassium induces a fast alkalinization mediated by the Na+/bicarbonate cotransporter (NBC). Considering the known pH sensitivity of phosphofructokinase, the enzyme that controls the glycolytic flux, we have proposed a causal relationship between potassium-induced alkalinization and glycolytic stimulation. In cultured astrocytes, both potassium-sensitive alkalinization and glycolysis activation were strongly inhibited in the absence of bicarbonate or in the presence of the NBC inhibitor SO859. These phenomena were not detected in HEK293 cells, but their glycolysis was found to be sensitive to pH. The transient expression of NBC in HEK293 cells was able to reproduce both alkalinization and glycolysis activation in

response to extracellular potassium. These data suggest a critical role for pH in metabolic coupling in the brain tissue, wherein NBC-dependent astrocytic alkalinization induced under neuronal activation, would be sufficient to activate astrocytic glycolysis acutely leading to the local release of lactate, molecule that has been proposed as neuronal fuel and modulator of local blood flow. Fondecyt 1100936 Conicyt AT 24091061

P2.21 Retirado

P2.22 EFECTO DE LA TRANSDUCCION LENTIVIRAL DE SVCT2 SOBRE LA ARBORIZACIÓN NEURONAL SVCT2 LENTIVIRAL TRANSDUCTION EFFECT ON NEURONAL BRANCHING. Salazar K (1), Ulloa V (1), Cortés-Campos C (1), Bongarzone E (2), García MA (1), Nualart F (1). (1) Department of Cellular Biology, University of Concepcion Chile. (2) University of Illinois Chicago.

Several studies have indicated that vitamin C promotes the functional maturity of postnatal neurons. Neuronal uptake of vitamin C is mediated by the sodium-vitamin C cotransporter 2 (SVCT2). In previous analyses, we demonstrated that overexpression of SVCT2 in N2a cells induced cell differentiation. However, there is not any data regarding SVCT2 function in primary cultures of cortical neurons. We generated fourth-generation lentivirus for SVCT2-EYFP and SVCT2sh-EYFP (negative dominant) transduction and analyzed the effect of SVCT2 overexpression in primary cultures of cortical neurons between a period of 24 to 96 h. Our analyses were performed using immunofluorescence, confocal microscopy, morphometric, RT-PCR and Western blot analysis. SVCT2-EYFP lentiviruses transduced MAP2- and TUJ1-positives neurons inducing primary and secondary neuronal branching, at 50% and 30%, respectively. Furthermore, SVCT2-EYFP positive branches co-localized with Piccolo and MAP1B positive pre-synaptic terminals. Neuronal branching was not observed after SVCT2sh-EYFP lentiviral transduction. SVCT2 over-expression promotes neuronal branching and stimulates the differentiation of primary cultures of cortical neurons. This study was sponsored by FONDECYT 1100396.

P2.23 Aproximación proteómica para estudiar los cambios plásticos inducidos por el tratamiento crónico con fluoxetina en el telencéfalo de ratas A proteomic approach to study plastic changes induced by chronic fluoxetine treatment in the rat telencephalon Sandoval M(1), Luarte A(1), Herrera-Molina R(2), Li KW (3), Smalla KH(2), Wyneken U(1) "(1) Laboratorio de Neurociencias, Universidad de los Andes, Santiago, Chile. (2) Leibniz institute for Neurobiology, Magdeburg, Germany. (3) Research Institute of Neurosciences, VU University, Amsterdam, The Netherlands

Pharmacotherapy with antidepressant drugs, such as fluoxetine (flx), is widely used for the treatment of major depression. As patients have to be treated for long times to observe a therapeutic effect, it is accepted that mood improvement is causally related with plastic changes in extensive brain circuits that occur with a slow time course. To get insight into such changes, we decided to analyze by mass spectrometry a microsomal subfraction obtained from rat telencephalon that contains trafficking organelles, synaptic vesicles and endocytic vesicles. Rats were treated for 2 weeks with saline or 0.7 mg/kg flx. Samples were submitted to 2D electrophoresis and the analysis of the relative abundance of proteins revealed changes in 14 proteins with the following primary functional roles: metabolic (n=5), signal transduction (n=3), protein folding (2) and other (n=4). The largest increase (7 times) occurred in Fructose-bisphosphate aldolase C (aldolase C) levels, a glycolytic enzyme present in astrocytes in the rat forebrain. By immunohistochemical analysis of the dorsal hippocampus, we could detect a decrease (43±2%) in the colocalization of this enzyme with GFAP (astrocyte marker). A diffuse, potentially extracellular staining appeared that invaded the CA1 pyramidal cell layer. Consistent with aldolase C secretion, it was increased in the cerebrospinal fluid by 2.61±0.27%fold over control ($p<0.01$). These results suggest that flx might regulate astrocyte metabolism and/or that aldolase C might have an extracellular signalling role, thereby modulating neuronal function. Support: Proyecto Anillo ACT09 (UW) and CONICYT Fellow (MS)

P2.24 Localización de la enzima acylpeptido hidrolasa en telencéfalo de ratas adultas: implicancias en procesos cognitivos y enfermedades neurodegenerativas

Localization of the enzyme acylpeptide hydrolase in adult rat forebrain: implicances in cognitive processes and neurodegenerative diseases Sandoval R (1), Navarro S (1), Wyneken U (2), Pancetti F (1) (1) Laboratorio de Neurotoxicología Ambiental, Dep. Ciencias Biomédicas, Facultad de Medicina, Universidad Católica del Norte. (2) Laboratorio de Neurociencias, Facultad de Medicina, Universidad de los Andes.

Acylpeptide hydrolase (ACPH) is an enzyme that belongs to the family of prolyl-oligopeptidases and catalyses the hydrolysis of several peptides that possess an acylated N-terminal amino acid, to generate an acylated amino acid and a free N-terminal peptide. ACPH also acts on bioactive peptides controlling their concentration and degrades oxidized proteins. At present, the physiological role of ACPH in neuronal tissue is unknown, however, it has been reported that ACPH isolated from conditioned culture medium of neuroblastoma cells can degrade the beta-amyloid peptide (Abeta) in vitro. Moreover, further information point out that ACPH hydrolyzes preferentially dimeric and trimeric forms of Abeta. On the other hand, data from our laboratory demonstrated that an ACPH specific inhibition increases long-term potentiation in young rats. These data demonstrate the relevance of ACPH in cognitive and neurodegenerative processes, and the importance of its localization on neuronal cells. Therefore, the aim of this work is to determine the subcellular localization of ACPH. Using synaptic fractionation we found that ACPH activity is enriched in synaptic membranes (SM). This activity is lost after hypo-osmotic lysis of SM at pH 8.1, a condition used to purify preferentially the postsynaptic machinery. When SM where lysed at pH 6.8, a condition used to enrich purified synapses with presynaptic components, ACPH activity was recovered, suggesting a presynaptic localization of this enzyme. These results would help to clarify the role of ACPH in the CNS and its possible implicances on neurodegenerative diseases. Proyecto Bicentenario PSD-11. Proyecto VRIDT-2010, Universidad Católica del Norte.

P2.25 Control transcripcional del promotor del gen del receptor tirosina quinasa RET de rata por el receptor nuclear huérfano Nurr1 Transcriptional control of the rat tyrosine kinase receptor RET gene promoter by the orphan nuclear receptor Nurr1 Saver M, Galleguillos D, Andrés ME Nucleus Millennium in Stress and Addiction & Department of Cell and Molecular Biology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile.

Nurr1 is an orphan nuclear receptor essential for the development of the midbrain dopaminergic neurons. Mice lacking Nurr1 die soon after birth and fail to express several characteristic genes of dopaminergic neurons. Nurr1 deficient mice also lack the expression of the transmembrane protein RET. The tyrosine kinase receptor RET is part of a protein complex acting as receptor for the Glial cell Derived Neurotrophic Factor. Both Nurr1 and RET are essential for the proper maintenance of the adult dopaminergic neurons in the midbrain. Reduction of Nurr1 expression in the adult brain diminished the abundance of RET mRNA and protein. Rat RET promoter harbours four NBRE-like elements between –2493 and -2163. Using chromatin immunoprecipitation assays we have found that Nurr1 binds in vivo to this segment of the rat RET promoter. In the present study we analyze the function of Nurr1 controlling the transcriptional activity of the rat RET gene promoter. Reporter vectors driven by 0.5 and 2.5 kb fragments of the rat RET promoter were transiently transfected into PC12 cells along with an expression vector for Nurr1. Results show that Nurr1 transactivate both reporters in a dose dependent manner. The 2.5 kb RET promoter show a higher response to Nurr1 than the 0.5 reporter, suggesting that the NBRE-like elements may play a role mediating Nurr1 effect. We also analyze the effect of another specific dopaminergic transcription factor, the homeodomain factor Pitx3 in the activity of Nurr1 over the rat RET gene promoter. FONDECYT 1070349 & Millennium Nucleus in Stress and Addiction MSI N° P06/008-F

P2.26 El receptor de la vía Wnt Frizzled-5 participa en la polarización de neuronas hipocampales de rata en cultivo. The Wnt receptor Frizzled-5 participates in the polarization of cultured hippocampal neurons. Slater PG (1), Varela-Nallar L (1), Aranguiz FC (1), González-Billault C (2), Inestrosa NC (1). (1) Centro envejecimiento y regeneración (CARE), Facultad de Ciencias Biológicas, PUC. (2) Laboratorio de Neurociencia, Facultad de Ciencias, Universidad de Chile.

The Wnt signaling pathway plays important roles during the development of the central nervous system, particularly in dendrite and axonal morphogenesis. In the present work we studied the expression and function of the Wnt receptor Frizzled-5 during the development of primary cultured rat hippocampal neurons. We found that Frizzled-5 is expressed in early stages of development, and it is localized intracellularly in the distal zone of all neuronal processes toghether with actin filaments. After the begining of dendritic development, Frizzled-5 expression remains mainly in the periferic zone of axonal growth cones, particulary in the tips of filipodiums where the receptor shows a vesicular patern. Tratment with Cytochalasin-D that disrupts actin filaments, result in the lost of Frizzled-5 distribution, suggesting an association of the receptor with the actin cytoskeleton. Moreover, Frizzled-5 over-expression affects neuronal morphology increasing dendritic branching and reducing axonal length. Besides, preliminary studies of Frizzled-5 knock-down with specific shRNAs show changes in the morphology of neurons which exhibit a reduced expression of the receptor. Our results suggest that Frizzled-5 plays a rol in the polarization and development of hippocampal neurons. Future experiments will help to elucidate whether Frizzled-5 function is mediated through its effect on actin citoskeleton. This work was supported by Basal proyect (CONICYT-PFB12/2007)

P2.27 Las células troncales mesenquimales inmunomodulan la activación glial en condiciones de neuroinflamación. Mesenquimal stem cells immunomodulate glial cell activation in neuroinflammatory conditions. Soler BM, von Bernhardi R. Lab. Neurociencia, Dpto. Neurología, Facultad de Medicina, Pontificia Universidad Católica de Chile. "Introduction: We hypothesize that glial dysfunction is responsible for the chronic inflammatory status in Alzheimer disease (AD), and could be relevant for Aβ accumulation, oxidative stress, synaptic dysfunction and neurodegeneration. It has been suggested that mesenchymal stem cells (MSC) could modulate inflammation in pathologies like multiple sclerosis, artritis and graft-vs-host disease. MSC therapy could exert a modulatory effect on CNS glial cells. Hypothesis. MSC modulate glial inflammatory activation, modifying their production of cytokines and phagocytosis. Methods. MSC were isolated from bone marrow of 4-8-week-old mice, and characterized at different passages (pass) by the expression of Qa-2, CD105 and CD34 using FACS, western blot, immunofluorescence microscopy and differentiation toward adipose and osteogenic cells. For the assessment of the capacity of MSC to modulate glia, MSC were stimulated with 1µg/ml LPS for 48h. Conditioned media was recollected and used to culture glia for 48h. We evaluated phagocytosis by an Aβ HilyteFluor uptake assay, and secretion of cytokines IL1β, TNFα, TGFβ by ELISA. Results: Expression of CD105 and Qa2 decreased, whereas CD34 increased from 4pass to 8pass, and we obtained differentiation to ostegenic and adipose cells evidencing the MSC nature of cells. Glial cultures exposed to MSC conditioned media increased TGFβ secretion by 71% and reduced TNFα and IL1β levels by 91% and 31% respectively, and increase 13-fold their phagocytic activity. Conclussion: MSC secreted soluble factors capable of regulating secretion of modulatory cytokines and fagocytosis of glia in an inflammatory in vitro model.

P2.28 Regulación de la producción de NO microglial por un factor soluble secretado constitutivamente por astrocitos Regulation of microglial cell NO production by a soluble factor constitutively produced by astrocytes Soto D, and von Bernhardi R. Dept. Neurology, Fac. Medicine, Pontificia Universidad Católica de Chile

Microglia are the macrophage cells resident of the CNS. Because its activation can lead to the release of citotoxic mediators, such as reactive nitrogen species, their activity is tightly regulated. The various factors responsible for such regulation are been only recently

investigated. Here, we report the presence of a soluble factor constitutively produced by astrocytes, which reduces the microglial cell activation response to 1µg/ml LPS. Using nitric oxide (NO) as read-out of inflammatory activation, we compared LPS induced production of nitrites (a stable NO metabolism product) by microglia, astrocytes, or mixed glial cultures. Astrocytes and mixed glial cultures had a similar kinetic pattern of NO production, which reached a peak of 25-30 µM at 24-48 h and then readily decreased to its basal level. In contrast, microglial cultures showed a persistent NO production lasting as long as 144 h, increasing up to 2.6 fold higher level of NO than the observed in mixed cultures. Furthermore, after washing and re-stimulating with LPS at 72 hr, astrocytes and mixed cultures became unresponsive to the inflammatory stimulation, which contrasted with the persistent of microglial cell cultures. Coherently, the addition of astrocytes, as well as conditioned media from astrocytes, to microglial cell cultures resulted in the inhibition of NO production by 66.8% ± 7.8 and 52.2% ± 6.9 respectively. Our data indicate that astrocytes modulate microglial cell production of NO through a constitutively secreted soluble factor; mechanism that could be relevant for the understanding the regulation of microglia and its role on neurodegenerative disease." Support from grants FONDECYT 1090353 (RvB) and INSERCIÓN CONICYT 79090028 (DS) are acknowledged.

P2.29 PHENOTYPIC RESCUE OF Mecp2-NULL MICE BY ISOFORM-SPECIFIC TRANSGENES PHENOTYPIC RESCUE OF Mecp2-NULL MICE BY ISOFORM-SPECIFIC TRANSGENES Soto-Covasich J (1), Kerr B (1), Saez MA (2), Young JI (1,3) (1) Centro de Estudios Científicos, Valdivia 5110246, Chile. (2) Universidad Austral de Chile, Valdivia, 5111100, Chile. (3) John P Hussman Institute for Human Genomics, University of Miami MillerSchool of Medicine, FL 33136, USA.

Rett syndrome (RTT) is a disorder that affects communication, movement and behavior. RTT patients are characterized by impaired language, stereotypic behaviors, frequent seizures, ataxia, sleep disturbances and onset of symptoms after a period of apparently normal development. RTT is caused by mutations in MECP2, an X chromosome gene encoding for MeCP2, a protein that regulates gene expression. MECP2 generates two alternative splice variants encoding two protein isoforms that differ only in the N-terminus. The RTT phenotype may occur in the presence of a functional MeCP2-e2 protein, since MeCP2-e1 specific mutations have been identified in patients diagnosed with RTT. This suggests that the two isoforms might be functionally distinct. Supporting this notion, the two variants show regional and age-related differences in transcript abundance. We generated transgenic mice expressing either MECP2-e1 or MECP2-e2 in brain cells, crossed them to mice lacking Mecp2 and compared the ability of each of the variants to compensate for the lack of the endogenous gene.

Our results showed that transgenic mice expressing either the MeCP2-e1 or MeCP2-e2 splice variant on an otherwise Mecp2 null background are phenotypically indistinguishable from wild-type mice. These results indicate that the two MeCP2 splice variants can substitute for each other and fulfill the basic functions of MeCP2 in the brain. Fondecyt 1100821

P2.30 Potenciales electroencefalográficos durante la atención a la preparación del movimiento como señales de control posible para interfases cerebro-máquina. Electroencephalographic potentials during attention to motor preparation as plausible signals for Brain Computer Interface (BCI) control. Tapia-Malebra C (1), Moenne C M (2), Avaria R (3), Ruiz-del-Solar J (4), Aboitiz F (5), Cosmelli D (6) (1) Programa de Doctorado en Ingeniería Electrica, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile (2) Programa de Doctorado en Ciencias de la Computación, Escuela de Ingeniería, Pontificia Universidad Católica de Chile (3) Centro de Investigación Avanzada en Educación (CIAE), Universidad de Chile (4) Dep. de Ingeniería Electrica, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile (5) Laboratorio de Neurociencia Cognitiva, Departamento de Psiquiatría, Escuela de Medicina, Pontificia Universidad Católica de Chile (6) Escuela de Psicología, Pontificia Universidad Católica de Chile

Traditional approaches to BCIs for limb control are based on the analysis and classification of EEG signals related either to the actual execution or the mental simulation of the intended movement. However, during complex voluntary action, rather than imagining the movement, and prior to its execution, one has to pay attention to its preparation. Indeed, it has been shown that preparing a motor action such as pressing a button, elicits brain activity that resembles that evoked during visuo-spatial attention shifts. This suggests that a potential candidate for the design and control of effective BCIs could be attention-related cortical activity during the preparatory phase of movement execution.
In this study we analyzed EEG and electromyographic (EMG) activity elicited when subjects were instructed to either "execute" or "prepare" left or right-hand finger movements. We were interested in determining whether cortical potentials could be used to discriminate, based both on traditional readiness-potentials and attention-related signals, which of the two possible fingers the subject had chosen to prepare or to move, and when. To accomplish this, we first preprocess the EEG and EMG signals using Independent Component Analysis (ICA to obtain a better differentiation of the cortical potentials associated to each action, and then we carry out the detection using two complementary methodologies, Linear Discriminant Analysis (LDA) and K-Nearest Neighbors (KNN). Our results suggest that early potentials that develop over central (readiness) and frontal (attentional) regions show hemispheric differences between target finger that could be exploited to drive and control brain-computer interfaces. This work is funded by FONDECYT project 1090612 and PBCT-CONICYT PSD72

P2.31 IDN5706 disminuye marcadores neuropatológicos y el déficit cognitivo e induce LTP en un transgénico modelo de la Enfermedad de Alzheimer. IDN5706 decreases neuropathological markers, cognitive deficit and induces LTP in brains of a double transgenic model of Alzheimer's disease. Tapia-Rojas C.(1), Serrano F. G. (1), Álvarez A. (2), Parodi J. (1), Varela-Nallar L. (1), Inestrosa N.C. (1) (1) Centro de Envejecimiento y Regeneración (CARE), Pontificia Universidad Católica de Chile. (2) Departamento de Biología Celular y Molecular, Pontificia Universidad Católica de Chile. Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive deterioration of cognitive abilities, the accumulation of senile plaques mainly composed of amyloid-β peptide (Aß) and neurofibrillary tangles formed by the hyperphosphorylated tau protein. In the present study, double transgenic APPswe/PSEN1ΔE9 mice model of AD were used to examine in vivo the ability of the compound IDN5706 to prevent the development of neuropathological markers of AD and the cognitive impairment. IDN5706 is a semi-synthetic derivative of hyperforin, one of the active components of St. John's wort (Hypericum perforatum). It was determined that treatment of 5 month-old mice with IDN5706 for 10 weeks significantly reduced the: (a) Aβ burden (number of amyloid plaques and Aβ oligomers), (b) tau hyperphosphorylation, (c) activation of astrocytes that occurs as a chronic inflammatory response, and (d) decrease of the post-synaptic proteins PSD95, NR2B, and GluR2 as well as the decrease of the pre-synaptic protein VGlut1. In addition, IDN5706 significantly reduced in a dose-dependent manner the spatial memory impairment as assessed by the Morris water maze and induced long-term potentiation (LTP). Our results suggest that IDN5706 might be considered as a potential therapeutic agent for the treatment of AD because it prevents the neuropathological, and cognitive alterations with a concomitant increases in synaptic plasticity. Supported by FONDEF (NºD07I1052) and Basal Grant (CONICYT-PFB12/2007).

P2.32 Cambios en la vía de TGFβ Smad3 dependientes del envejecimiento modifican el patrón de activación microglial. Aging dependent changes on TGFβ Smad3 pathway modify the pattern of microglial cell activation. Tichauer J, von Bernhardi R
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Aging is the main risk factor for Alzheimer Disease (AD). It is characterized by an increase in inflammatory signaling that could affect the modulation of glial cells function

and the cytotoxic activation. Previous studies show that astrocytes modulate microglia mediated cytotoxicity through the secretion of TGFβ. However, even though TGFβ levels are elevated, there is an over expression of inflammatory mediators and glial activation in aging. To understand this apparent contradiction, we evaluated changes of TGFβ-Smad3 signaling and its effect on microglial aging. Hypothesis: TGFβ Smad3 activation is decreased during aging changing microglial cell reactivity. Approach: We evaluated participation of TGFβ-Smad3 pathway on the modulation of nitrite secretion, super oxide production and phagocytosis in microglial cell cultures from mice at different ages previously treated with i.p. LPS for 48 h to induce a inflammatory status.Results: Basal nitrites were similar in microglial cultures of 2 and 12 months old mice. Only in microglia cultures from 2-month old mice 1µg/ml LPS induced nitrite secretion by 3.4 and 4.6 fold in PBS and LPS pretreated mice respectively. In contrast, ROS production was only increased in 12 months old mice by 4.5 and 3.3 fold in PBS and LPS pretreated mice. TGFβ decreased LPS induced effect on nitrite secretion in 2 months old mice microglia but ROS production at any age. Similarly, phagocytosis was increased by 25% by pretreatment with LPS and by 171% by exposure to TGFβ only in microglial cultures from 2 months old mice. We detected that pretreatment with LPS increased pSmad3 by 60% in the hippocampus of 2 months old, but not of 12 months old mice. Conclusion: TGFβ Smad3 pathway appears to be an important determinant for the pattern of microglial cell activation, which could be impaired in aging, favoring inflammation and neurodegenerative diseases. Supported By: grant FONDECYT 1090353 to RvB.

P2.33 Modelo computacional basado en conductancias de la resonancia eléctrica en rangos de frecuencia teta en neuronas de la amigdala olfatoria A computational conductance-based model of electrical resonance in the theta frequency range in olfactory amygdala neurons U. Pereira, M. Pezzoli, J. Bacigalupo and M. Sanhueza Departamento de Biología e Instituto Milenio ICDB, Fac. de Ciencias, Universidad de Chile.

We recently showed that a significant fraction of layer II pyramidal neurons of the cortical amygdala exhibit theta-range intrinsic sub-threshold oscillations in membrane potential. Furthermore, we now demonstrated that when neurons are stimulated with a sinusoidal current of linearly incrementing frequency (ZAP protocol), the voltage output had a maximal amplitude at the natural frequency (2-6 Hz), a phenomenon called electrical resonance. Resonant neurons showed an impedance spectrum resembling an electrical band-pass filter with a peak at the preferred frequencies. By using pharmacological tools, we identified a set of voltage-dependent conductances present in these cells and affecting impedance profiles: a fast persistent sodium conductance, an unspecific hyperpolarization-activated conductance and a muscarinic-regulated potassium conductance. To fully understand the biophysical mechanisms underlying intrinsic oscillations and resonance in these neurons, we developed a conductance-based model that included the mentioned conductances together with a leak conductance and Hodgkin & Huxley sodium and potassium channels. We implemented a single conic compartment model in NEURON platform emulating the soma of a pyramidal cell of the cortical amygdala. Using this model we performed in silico DC current injection and ZAP stimulation experiments to simulate our slice physiology experiments. Depolarization-induced sub-threshold theta oscillations were mimicked. The model also reproduced the theta-range resonant impedance profile and our pharmacological results, allowing quantitative determination of each conductance contribution to resonance. Remarkably, for supra-threshold ZAP stimulation, we reproduced selective spiking at resonance frequency. Thus, we developed a comprehensive conductance-based model that successfully accounts for the intrinsic dynamic properties of olfactory amygdala neurons." Funding: Proyecto Fondecyt 1080630, Mideplan ICM-P05-001-F.

P2.34 Dimetoato administrado prenatalmente persiste en crías macho de rata Sprague Dawley y afecta los procesos de memoria y aprendizaje. Prenatally

administered dimethoate persists in young Sprague Dawley male rats and affects memory and learning processes. Valdés M (1), Celis F (2), Martínez U (2), Villagrán J (2), Muñoz J (2), Contreras A (2), Díaz Fleming G (2), Feito R (3), Berrios C (1) y Valenzuela-Harrington M (1) "1 Laboratorio de Neurociencias, 2 Laboratorio de Espectroscopia Atómica y Molecular (CESPAM), y 3 Laboratorio de Reproducción e Histología, Departamento de Química y Biología, Facultad de Ciencias Naturales y Exactas. Universidad de Playa Ancha

Organophosphorus compounds are widely used to control agricultural pests. On the other hand, it has been clearly demonstrated that in mammal these compounds produce highly toxic effects because of their ability to inhibit the enzyme acetylcholinesterase irreversibly prolonging the effect of acetylcholine in the synaptic space. The aim of this study was to determine the effects of organophosphate pesticide dimethoate on the progeny of male rats exposed during neurulation on the capacities of memory and spatial learning. It was administered between 9 and 12 days of gestation with dose of 7, 28 and 40 mg / kg / day to rats of the Sprague Dawley strain. The male offsprings were tested for spatial learning and memory and Barnes maze, T maze and object recognition test. Upon completion of the experiments, samples were taken from different tissues (pituitary, cerebral cortex, liver, spleen and adipose tissue) and analyzed with Raman spectroscopy, showing the presence of Dimethoate in almost all tissues of the offsprings, which could explain cognitive alterations obtained during the performance of behavioral tests of Barnes and object recognition. Financiamiento: Proyecto CNEI 06-0910 Universidad de Playa Ancha

P2.35 Shunt ventrículo-peritoneal en ratas HTx hidrocefálicas produce la generación de anticuerpos contra proteínas presntes en el Líquido Cefalorraquídeo (LCR) Ventricular-peritoneal shunting in hydrocephalic HTx rats results in the generation of antibodies against proteins present in the cerebrospinal fluid (CFS) Vásquez, P(1), Rodríguez, S(1), Guerra, M(1), E. Ortega(2), Rodríguez, EM(1). (1)Instituto de Anatomía, Histología y Patología, (2)Instituo de Neurociencias Clínicas, Facultad de Medicina, Universidad Austral de Chile, Valdivia.

Background. Ventricular-peritoneal shunting and ventriculostomy are virtually the only treatment of hydrocephalus today. Considering that there are CSF-specific proteins, some of which could be regarded as foreign proteins for the immune system, we hypothesize that their permanent delivery to the peritoneum, a home-like place of the immune system, would result in the generation of auto-antibodies against such proteins. Methodology. The HTx rat is the only rat strain developing a hereditary congenital hydrocephalus. Ventricular-peritoneal shunting in HTx rats improves their survival time moderately. 90 HTx rats were subjected to ventricular-peritoneal shunting. Blood samples were collected before shunting and at various post-shunting periods. These samples were used a primary antiserum (i) to immunostain brain sections of normal and hydrocephalic HTX rats and, (ii) for immunoblotting of CSF and tissue extracts of various brain regions. Results. Post-shunting, but not pre-shunting, blood samples have antibodies reacting with proteins present in the CSF and brain tissue, ranging between 17 and 230 kDa. One of these proteins was identified as transthyretin (TTR). Post-shunting antibodies immunostained the choroid plexus and Purkinje's cells of the cerebellum. Conclusions. In HTx rats, ventricular-peritoneal shunting triggers the generation of antibodies against CSF-specific proteins, such as TTR, raising the question as to how much shunting is beneficial or deleterious for the clinical evolution of the disease. Fondecyt Nº 1070241(EMR).

P2.36 El hábito visual subterráneo conlleva a una disminución de la visión binocular: roedores sudamericanos Spalacopus cyanus y Ctenomys talarum como caso de estudio. Subterranean visual lifestyle leads to a decrease of binocular vision: South American rodents Spalacopus cyanus and Ctenomys talarum as a case in point. Vega-Zuniga T.(1), Medina F.(1), Palacios A.G.(2), Mpodozis J.(1) "(1)

Departamento de Biología, Facultad de Ciencias, Universidad de Chile (2) Centro de Neurociencias de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso

We studied the extent and position of the visual field, the distribution and densities of the retinal ganglion cells and the characteristics of the retinal central projections in two visually active subterranean species Spalacopus cyanus and Ctenomys talarum. The shape and extension of the visual fields were determined using ophtalmoscopic techniques. Counts and mapping of cells in the ganglion cell layer (GCL) were carried out in retinal whole mounts stained with nissl, and visual central projections were revealed by means of intraocular injections of CTB. The S. cyanus visual field has a 50° of frontal binocular overlap, slightly smaller than the 60° of overlap found in C. talarum. Both species have a well-developed visual streak with 5 isodensity curves, but an area centralis is only found in S.cyanus, which is located temporally. Retinal ganglion cells were ca. 72.000 in S. cyanus and 94.200 in C. talarum. The volumes of the contralateral LGN have no statistical differences between species. The volume of contralateral superior colliculus (SC) is larger in C. talarum compared to S. cyanus ($p \leq 0.05$, Mann Whitney). Interestingly, even when both animals show a considerable degree of binocular overlap, the ipsilateral projections to the LGN and SC are in both of them poorly developed (only traces in C.talarum). Our findings strongly suggest that subterranean visual behavior leads to a decrease of structures associated to binocular vision, at least in the case of these rodents. Fondecyt 1080094

P2.37 Alteraciones de la zona ventricular y subventricular de las eminencias ganglionicas en los ratones hidrocefálicos hyh están asociada a anormalidades de las neuronas GABAergicas corticales. The disruption of the ventricular (VZ) and subventricular (SVZ) zones of the ganglionic eminences in hydrocephalic hyh mice is associated to abnormalities in the cortical GABAergic neurons. Vio K, Ojeda A, Yulis C, Rodríguez E. Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral de Chile.

Background. The structure of the cerebral cortex results from the orderly migration of two major types of neurons, the glutamatergic projection neurons and the GABAergic interneurons. Most GABAergic neurons originate from the ganglionic eminences. They first migrate tangentially toward the pallium and then migrate radially through the developing brain cortex. The disruption of the VZ and SVZ of the palium and subpalium has been shown to occur at key developmental periods in the hydrocephalic mutant mouse hyh. The aim of the present investigation was to study whether such a disruption of the VZ and SVZ results in abnormalities of the GABAergic neurons populating the brain cortex. Material and Methods. The brain of non-hydrocephalic and hydrocephalic hyh mice at postnatal day 7 (n=20) were processed for immunocytochemistry and immunofluorescence using antibodies against GABA and the marker of neuronal nuclei NeuN. Sections processed for double immunofluorescence were inspected with an epifluorescence microscope provided with the multidimensional acquisition software AxioVision Rel. Single and overlay images were used for quantitative analyses of the whole populations of cortical neurons (NeuE-reactive) and that of GABAergic neurons. Absolute and relative cell density and intracortical distribution were recorded for the GABAergic neurons. Results. The mutant hyh mice were characterized by (i) a marked reduction in the width of the cerebral cortex; (ii) a reduction in the total number of GABAergic neurons; (iii) a reduction in the relative number of GABAergic neurons with respect to the total population of neurons (GABA/NeuE); (iv) an abnormal distribution of GABAergic neurons in the cortex layers, with a significant reduction in layers II and III and an increase in layer IV y V. Conclusion. The hyh mutation is associated with: (i) a decreased number of GABAergic neurons migrating from the ganglionic eminences; (ii) abnormal migration of GABAergic neurons through the developing brain cortex; (iii) in hyh mice disruption of the VZ and SVZ is associated to the onset of hydrocephalus and abnormal corticogenesis.Supported by Fondecyt 1070241

P2.38 ALTERACIONES EN EL TRÁFICO DE VITAMINA E EN MODELOS DE NIEMANN PICK TIPO C VITAMIN E TRAFFICKING ALTERATIONS IN NIEMANN PICK TYPE C MODELS

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Niemann-Pick type C (NPC) disease is an autosomal recessive and neurodegenerative lipidosis caused mainly by mutations in the npc1 gene. NPC disease is characterized by the accumulation of free cholesterol and glycosphingolipids in the endosomal-lysosomal system. NPC patients present a markedly progressive neuronal loss, mainly of cerebellar Purkinje neurons. We propose that NPC1, a cholesterol transport protein of the endocytic pathway, could be participating in the intracellular trafficking of alpha-tocopherol due to its great homology with the NPC1L1 protein which is known to transfer alpha-tocopherol in enterocytes. We also propose that there is an alpha-tocopherol trafficking alteration and entrapment into the endocytic pathway in NPC1-/- neurons leading to vitamin E deficiency because of the similarities found between NPC and the Ataxia with isolated Vitamin E Deficiency (AVED) diseases. Interestingly, in NPC1 KO mice we found increased levels of alpha-tocopherol in the cerebellum, despite an increase in oxidative damage. Moreover, we observed accumulation and entrapment of alpha-tocopherol in NPC neurons, mainly in the endocytic pathway. Furthermore, we have found decreased mRNA levels of the alpha-tocopherol transporter, alpha-Tocopherol Transfer Protein (aTTP), in the cerebellum of NPC mice. Together these results suggest that there is a vitamin E trafficking alteration in NPC cells that leads to vitamin E accumulation and entrapment in the endocytic pathway. This may result in a decreased bioavailability and antioxidant function of Vitamin E, contributing to the pathology of NPC disease. Ara Parseghian Medical Research Foundation, y Fondecyt 1080221 (AA) y 1070622 (SZ)

P2.39 EL RESIDUO CONSERVADO TRP-6.48 Y SU POSIBLE RELACIÓN CON LA SELECTIVIDAD FUNCIONAL INDUCIDA POR FENILALQUILAMINAS MONOAMINÉRGICAS EN RECEPTORES 5-HT2A HUMANOS THE CONSERVED TRP-6.48 AS A POSSIBLE ON/OFF SWITCH FOR FUNCTIONAL SELECTIVITY ELICITED BY MONOAMINERGIC PHENYLALKYLAMINES AT HUMAN 5-HT2A RECEPTORS.

Alveal-Fuentealba N (1), Zapata-Torres G (2,5), Miranda-Rojas S (2), Fierro-Huerta A (3,5), Sáez-Briones P (4,5) (1) Centro de Bioinformática y Simulación Molecular, Universidad de Talca. (2) Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. (3)Departamento de Ciencias del Ambiente, Facultad de Química y Biología, Universidad de Santiago de Chile. (4) Escuela de Medicina, Facultad de Ciencias Médicas, Universidad de Santiago de Chile. (5) Millennium Institute for Cell Dynamics and Biotechnology, Universidad de Chile. Functional selectivity is a ligand-dependent property by which different agonists act at the same G-protein-coupled receptor (GPCR) activating its signal transduction pathways in different unique combinations. Each combination leads to different therapeutic and/or to unexpected effects. Unfortunately, the molecular mechanisms associated with functional selectivity at GPCRs are not well understood and a clear consensus about their association with receptor activation is still lacking. In this regard, a number of conserved regions have been already identified in GPCRs and it has been proposed that their disruption induced by agonists might lead to a shift in the receptor active/inactive state ratio, enabling the occurrence of functional selectivity. A recent study has demonstrated that synthetic monoaminergic phenylalkylamines exhibit functional selectivity at central human metabotropic 5-HT2A receptors, inducing different magnitudes of activation of both the phospholipase C (PLC) and phospholipase A2 (PLA2) effector routes. In the present work, the latter hypothesis was tested using 10 ns molecular dynamics simulations at an homology-based model of the human 5-HT2A receptor and the monoaminergic phenylalkylamines 2C-N (2,5-dimethoxy-4-nitrophenylethylamine) and 2,5-DMA (2,5-dimethoxyphenylisopropylamine) as agonists. The results obtained indicate that

the conserved residue Trp-6.48 appears to adopt two different conformations depending of the attached ligand: whereas 2C-N does not promote any shift of the Trp side chain (holding it in a perpendicular orientation referred to the plasma membrane), 2,5-DMA shifts it toward a parallel orientation. As 2,5-DMA activates the PLC effector route only, the side chain shift reported of Trp-6.48 might be associated with this signal transduction pathway.

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P2.40 CONTEXTUAL FEAR EXTINCTION INCREASES RYANODINE RECEPTOR PROTEIN CONTENT IN THE RAT HIPPOCAMPUS Casas M, Haeger P, Morales P e Hidalgo C. CEMC e ICBM, Facultad de Medicina, Universidad de Chile.

Synaptic modifications implicated in the learning process require calcium-mediated intracellular signaling. Calcium signals generated by ryanodine receptor (RyR) activation play a key role in this process, but the detailed molecular events underlying RyR activation are not fully understood. In this work we tested the behavioral response (freezing) and measured RyR2 protein content in rat hippocampus obtained from rats subjected to a contextual fear conditioning (CFC) protocol. In the training session we used two 2 s stimuli (0.35 or 0.7 mA); 24 h later we re-exposed the rats to the training context for 3 minutes without electrical stimulation, and 5h later we measured RyR2 protein content. We found significant changes in RyR2 expression (2-fold) when using 0.7 mA, but not 0.35 mA as stimulus. We then re-exposed to the context for 3 minutes rats trained with 0.7 mA 24 h earlier. We found that at the end of this 3 minute period freezing was 64% and RyR2 content did not increase, while 5 h later in a behavioral test, freezing decreased to 47% and RyR2 protein content increased (2-fold). Extending the re-exposure time to 15 min caused a significant decrease in the behavioral response (about 35%) when measured at the end of this period, accompanied by a significant increase in RyR2 protein content (3-fold), measured 5 h later. Finally we measured freezing 29 h after training and found a high behavioral response (65%) in comparison with the other experiments that showed a decrease in freezing after the re-exposure session. These combined results suggest that re-exposure to the context after 24 h without electrical stimulation, depending on the duration of the re-exposure session, leads to fear memory extinction and increased RyR2 protein expression, which may result in enhanced calcium release mediated by RyR2 during the extinction process.

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P2.41 HIGH-FREQUENCY FIELD STIMULATION OF RAT PRIMARY HIPPOCAMPAL NEURONS MODULATES ROS-DEPENDENT RYR-MEDIATED CA^{2+} RELEASE AND RYR2 PROTEIN EXPRESSION [1]Espinoza I, [1]Adasme T, [1]Riquelme D, [1]Leal N, [1,2]Hidalgo J, [1,2]Hidalgo C, and [1,2]Carrasco MA. [1]CEMC & [2]ICBM, F. Medicina, U. Chile, Santiago, Chile

Neuronal electrical activity generates reactive oxygen species (ROS) and intracellular Ca^{2+} signals that induce local changes at the synaptic level and activate the expression of genes that are essential for synaptic plasticity. The activity-induced intracellular Ca^{2+} increase requires Ca^{2+} influx through different plasma membrane entry pathways, including voltage or neurotransmitter-activated Ca^{2+} channels. Ryanodine receptors (RyR) amplify and propagate Ca^{2+} entry signals via Ca^{2+}-induced Ca^{2+} release (CICR) to other cellular regions, including the nucleus. Here, we studied the participation of ROS and RyR activity in Ca^{2+} signal generation induced by a high-frequency field stimulation (HFS) protocol (50 Hz, 4000 pulses). The viability of the primary hippocampal culture was not affected by HFS, as assessed 24 h after stimulation with Propidium Iodide. Ca^{2+} signals were determined by time series confocal microscopy in primary hippocampal neurons preloaded with Fluo-4 AM. To prevent RyR activity or decrease intracellular ROS levels, neurons were preincubated for 1 h with 50 μM ryanodine or the antioxidant N-acetyl L-cysteine (NAC, 10 mM), respectively. Changes in RyR2 mRNA levels or protein content were evaluated by q-PCR and Western blot,

respectively. RyR2 distribution was also determined by immunocytochemistry. Our results show that HFS generated long-lasting Ca^{2+} signals that were inhibited by ryanodine or NAC, indicating RyR and ROS involvement in their generation. HFS also enhanced within 45 min the expression of the early gene *c-fos* and increased RyR2 protein content, measured 6 h after HFS; this RyR2 increase was suppressed by pre-incubation with NAC (1 h, 10 mM) before HFS. The present results show that HFS induced RyR-dependent Ca^{2+} signals, likely generated via CICR, which required cellular ROS production. Moreover, since the increase in RyR2 protein content induced by HFS also required ROS, we propose that HFS-induced RyR-mediated Ca^{2+} signals activate ROS-dependent pathways that enhance RyR2 protein expression.
Financial support**:** FONDECYT 1060177, FONDECYT-FONDAP 15010006

P2.42 Amyloid beta peptideoligomers stimulate RyR-mediated Ca+ release inducing mitochondrial fragmentation and prevent RyR- mediated dendritic spine remodeling produced by BDNF. SanMartín C[1], Paula-Lima A[1], Adasme T[1], Sebollela A[2], Hetz C[1,3], Carrasco M.A[1,4], Ferreira S.T[2] and Hidalgo C[1,4] [1]Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile; [2]Programa de Bioquímica e Biofísica Celular, Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, Brasil; Programas de [3]Biología Celular y Molecular and [4]Fisiología y Biofísica, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile.
Abnormal Ca^{2+} signaling is a central event in the pathology of Alzheimer´s disease (AD). Soluble oligomers of amyloid beta peptide (AβOs) - increasingly recognized as causative agents of AD - disrupt Ca^{2+} homeostasis and cause severe synapse deterioration. Here, we report that AβOs at sub-lethal concentrations bind to primary hippocampal neurons generating within seconds prolonged cytoplasmic Ca^{2+} signals. Incubation in Ca^{2+}-free solutions, ryanodine receptor (RyR) or N-methyl-D-aspartate receptor (NMDAR) inhibition or pre-incubation with the antioxidant N-acetylcysteine abolished AβOs-induced Ca^{2+} signals. In addition, after 6 h AβOs transiently decreased RyR2 and RyR3 mRNA expression and RyR2 protein content, and after 24 h promoted mitochondrial fragmentation without affecting mitochondrial ATP generation or inducing cytochrome C release. Inhibition of NMDAR abolished the RyR2 protein decrease while selective inhibition of RyR-mediated Ca^{2+} release with ryanodine partially protected against the RyR2 decrease and mitochondrial fragmentation induced by AβOs. Incubation with AβOs (6 h) eliminated the RyR2 protein increase induced by brain-derived nerve factor (BDNF) after 6 h, and prevented the dendritic spine remodeling induced within minutes by BDNF or the RyR agonist caffeine. Addition of BDNF to neurons incubated with AβOs for 24 h, which had similar RyR2 and slightly higher RyR3 protein content than controls, induced dendritic spine growth albeit at slower rates than in controls. These combined effects of AβOs added at sub-lethal concentrations – which include redox-sensitive stimulation of RyR-mediated Ca^{2+} release, decreased RyR2 protein expression, mitochondrial fragmentation and prevention of RyR-mediated spine remodeling - may contribute to impair synaptic plasticity in AD. Supported by FONDAP-CEMC 15010006.