



**V REUNION
SOCIEDAD CHILENA DE NEUROCIENCIA
COQUIMBO, CHILE.**

Lugar: Centro Convenciones HOTEL ENJOY de Coquimbo
Fecha: 23, 24 y 25 septiembre del 2009

PROGRAMA

I CONFERENCIAS PLENARIAS

Conferencia 1:

Conferencia Inaugural: Miércoles 23, 18:30-19:30

“Del frío al dolor: Mecanismos periféricos”. Dr. Carlos Belmonte
(Instituto de Neurociencias, Universidad Miguel Hernández España).

Conferencia 2:

Conferencia de perspectivas Básico-Clínicas: Jueves 24, 9:00-10:00

“Bases neurocognitivas de la Esquizofrenia”. Dr. Sophia Vinogradov
(Laboratory of Cognitive Neuroscience, Professor of Psychiatry, Department of Psychiatry, University of California, San Francisco, EEUU).

Conferencia 3:

Conferencia Premio Nacional de Ciencias 2008: Jueves 24, 18:30-19:30

“La Vía Wnt de Señalización Modula la Maduración de las Regiones Pre- y Postsinápticas del Sistema Nervioso de Vertebrados” Dr. Nibaldo C Inestrosa Centros de Regulación Celular y Patología “Joaquín V. Luco” (CRCP), de Envejecimiento y Regeneración (CARE) e Instituto Milenio MIFAB, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile.

Conferencia 4:

Conferencia nombramiento Miembro Emérito de la SCN: Viernes 25, 14:30-15:30

“El Sistema Nervioso como una arquitectura dinámica en tiempo cero”. Dr. Humberto Maturana (Instituto Matríztico)

Presenta: Dr: Pedro Maldonado

Conferencia 5:

Conferencia Viernes 25, 15:30-16:30

“Development of intracerebral gene & cell therapy strategies to treat neurological disease”. Dr. Alberto Martinez Serrano Centro de Biología Molecular Severo Ochoa. Universidad Autónoma de Madrid

II SIMPOSIOS

Simposio 1. Miércoles / Wednesday 23, 11:30-13:30

Coordina / Chair: Dr. Patricio Huerta

“PLASTICIDAD SINÁPTICA. SOBRE COMO LAS SINAPSIS SON CONSTRUIDAS Y FORTALECIDAS, PERO TAMBIEN DESTRUIDAS”

11:30-12:10 “Ensamble y plasticidad de las sinapsis” Dr. Jimena Sierralta (ICBM, Facultad de Medicina, Universidad de Chile)

12:10-12:50 “Ontogenia de la plasticidad sináptica en la corteza cerebral” Dr. Alfredo Kirkwood (John Hopkins University)

12:50-13:30 “Enfermedades de la sinapsis corticales” Dr. Patricio Huerta (Cornell University)

Simposio 2. Jueves / Thursday 24, 11:30-13:30

Coordina / Chair: Dr. Juan Carlos Sáez

“CONNEXIN MUTATIONS AND DEAFNESS”

11:30-12:00 “The complex selectivity of information transfer through gap junctions and hemichannels: a new era in cellular integration” Dr. Bruce Nicholson (Department of Biochemistry, University of San Antonio, USA).

12:00-12:30 “Connexins and the cochlea” Dr. Juan C. Sáez, (Departamento de Ciencias Fisiológicas, Pontificia Universidad Católica de Chile).

12:30-13:00 “Deafness related mutations in the first transmembrane domain of Connexin26 unveil a fundamental role of this domain for the assembly and function of connexin channels” Dr. Agustín Martínez (Centro de Neurociencias, Universidad de Valparaíso, Chile)

13:00-13:30 “Biophysical properties of membrane channels constituted by connexin mutated at sites identified in human patients” Dr. Vytautas Verselis (Department of Neuroscience, Albert Einstein College of Medicine, NY, USA).

Simposio 3: Jueves / Thursday 24, 16:30-18:30

Coordina / Chair: **Dra. Rommy von Bernhardt**

"GLIAL CELL-NEURON INTERACTION IN THE NORMAL AND ABNORMAL FUNCTION OF THE NERVOUS SYSTEM".

16:30-17:00 "Modification of microglial function in response to glutamate and its participation in the neurodegenerative diseases" **Dr. Mami Noda** (Laboratory of Pathophysiology, Kyushu University, Fukuoka Japan).

17:00-17:30 "Key role of glial cells in the coupling of energy supply and demand in the brain". **Dr. Felipe Barros** (CECS, Valdivia)

17:30-18:00 "Glia-mediated neuroinflammation as disease mechanisms in neurodegenerative disorders". **Dr. Rommy von Bernhardt** (Facultad de Medicina, P. Universidad Católica de Chile).

18:00-18:30 "Schwann Cell to axon transfer of ribosomes: Toward a novel understanding of the role of glia in the nervous system" **Dr. Felipe Court** (Facultad de Ciencias Biológicas, P. Universidad Católica de Chile).

Simposio 4: Viernes / Friday 25, 9:00-11:00

Coordina / Chair: **Dr. Gricel Orellana**

"APORTES DE LAS NEUROCIENCIAS COGNITIVAS A LA INVESTIGACION EN ESQUIZOFRENIA"

9:00-9:40: "Auto-evaluación cognitiva en Esquizofrenia: Evidencia conductual y psicofisiológica". **Dr. Jaime Silva** (Departamento de Salud Mental y Psiquiatría, Facultad de Medicina, U. de la Frontera.)

9:40-10:20: "Déficit específico en la atención ejecutiva en primer episodio de Esquizofrenia" **Dr. Gricel Orellana** (Unidad de Neurociencias Cognitivas y Esquizofrenia. Depto. Psiquiatría Oriente, Facultad de Medicina, Universidad de Chile).

10:20-11:00 "Neuroplasticity-Based Cognitive Training in Schizophrenia". **Dr. Sophia Vinogradov** (Laboratory of Cognitive Neuroscience, Professor of Psychiatry, Department of Psychiatry, University of California, San Francisco, EEUU).

Simposio 5: Viernes / Friday 25, 11:30-13:30

Coordinan / Chair: **Drs. Julio Alcayaga y Rodrigo Iturriaga**

“RECEPTORES SENSORIALES EN CONDICIONES FISIOLÓGICAS Y PATOLÓGICAS”

11:30-11:54 “Contribución del Dr. Carlos Eyzaguirre a la Fisiología Sensorial” **Dr. Patricio Zapata** (Facultad de Medicina, Universidad del Desarrollo).

11:54-12:18 “Comunicación en un receptor sensorial secundario: El caso del cuerpo carotídeo”. **Dr. Julio Alcayaga** (Facultad de Ciencias, Universidad de Chile).

12:18-12:42 “Compromiso sensorial en la disfunción del ritmo respiratorio inducida por nicotina prenatal” **Dr. Jaime Eugenin** (Facultad de Química y Biología, Universidad de Santiago).

12:42-13:06 “Contribución del cuerpo carotideo a la hipertensión producida por la apnea obstructiva del sueño”. **Dr. Rodrigo Iturriaga** (Facultad de Ciencias Biológicas, P. Universidad Católica de Chile).

13:06-13:30 “Receptores sensoriales y sensaciones en la cornea” **Dr. Carlos Belmonte** (Instituto de Neurociencias, Universidad Miguel Hernández. Alicante, España).

III SESIONES COMUNICACIONES LIBRES

- | | | | |
|-----------|------------------|-----------|--------------------|
| 1. | Miércoles | 23 | 16:30-18:30 |
| 2. | Jueves | 24 | 10:00-11:00 |

IV SESIONES PANELES

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|-----------|------------------|-----------|--------------------|
| 1. | Miércoles | 23 | 14:30-16:30 |
| 2. | Jueves | 24 | 14:30-16:30 |
-

Comité Organizador V Reunión Anual SCN

Jaime Eugenin (Universidad de Santiago)

Fernando Torrealba (Pontificia Universidad Católica de Chile)

Pedro Maldonado (Universidad de Chile)

Comité Científico V Reunión Anual SCN

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Pedro Maldonado (Universidad de Chile).

RESUMEN PROGRAMA
V CONGRESO ANUAL DE LA SOCIEDAD CHILENA DE NEUROCIENCIA
HOTEL ENJOY COQUIMBO, CHILE, 23 al 25 SEPTIEMBRE, 2009

HORARIO	Miércoles 23	Jueves 24	Viernes 25
9:00 - 11:00	Inscripciones	Inscripciones	Inscripciones
9:00 - 10:00	Charlas Colegios	Conferencia 2	Simposio 4
10:00 - 11:00		Comunicaciones Libres 2	
11:00 - 11:30	Bienvenida	Coffee Break	Coffee Break
11:30 - 13:30	Simposio 1	Simposio 2	Simposio 5
13:30 - 14:30	Almuerzo	Almuerzo	Almuerzo
14:30 : 15:30	Paneles 1 Café	Paneles 2 Café	Conferencia 4
15:30 : 16:30			Conferencia 5
16:30 : 17:30	Comunicaciones Libres 1	Simposio 3	Reunión Ampliada SCN - Clausura Congreso
17:30- 18:30			
18:30 - 19:30	Conferencia 1	Conferencia 3	
20:15 - 22:00	Cocktail Recepción- CENA	Cocktail - CENA CLAUSURA	

RESUMENES /ABSTRACTS

I CONFERENCIAS PLENARIAS

Conferencia 1:

Conferencia Inaugural: Miércoles / Wednesday 23, 18:30-19:30

“Del frío al dolor: Mecanismos periféricos”. Dr. Carlos Belmonte (Instituto de Neurociencias, Universidad Miguel Hernández España).

CP1. Cold temperature can evoke a wide spectrum of perceptual sensations that range from freshness to unpleasant cold or overt pain. In mammals, the detection of cold temperature is accomplished by the activation of different subsets of sensory terminals innervating the skin and mucosae. Direct recordings of cold nerve endings, combined with studies of thermoreceptive neurons in culture, have allowed the characterization of ionic mechanisms involved in cold temperature sensing. In recent years, major progress has also taken place in the identification and operation of thermally gated ion channels, especially of the transient receptor potential (TRP) family. However, it is still uncertain how individual sensory endings can be activated with different thermal thresholds. We analyzed the gating by cold of specific ion channels and its relationship with the activation of particular sets of afferent fibers and concluded that cold thermotransduction is complex and involves the concerted operation of several ion channels. Excitatory effects of cationic channels (e.g., TRPs) balance their activity with several excitability brakes (e.g., potassium channels), leading to tunable levels of sensory thresholds and activity. Alteration in this fine balance may result in altered cold sensitivity, a frequent symptom in patients with peripheral nerve injury.

Conferencia 2:

Conferencia de perspectivas Básico-Clínicas: Jueves / Thursday 24, 9:00-10:00

“Bases neurocognitivas de la Esquizofrenia”. Dr. Sophia Vinogradov (Laboratory of Cognitive Neuroscience, Professor of Psychiatry, Department of Psychiatry, University of California, San Francisco, EEUU).

CP2. Schizophrenia is a severe and disabling psychiatric illness characterized by symptoms such as delusions and hallucinations, as well as a wide range of cognitive deficits. These deficits are significantly associated with poor functional outcome and contribute to billions of dollars in costs associated with unemployment and lost productivity. The U.S. National Institute of Mental Health has identified cognition as a critical treatment target in schizophrenia, but there is no coherent understanding of the origin of the cognitive impairments; they do not respond to currently available medications; and they show only modest improvements after conventional behavioral remediation. In recent years, a range of cognitive neuroscience approaches have begun to be applied to the study of schizophrenia, in the hope of elucidating the pathophysiologic underpinnings of the illness. We will selectively review research findings that indicate problems in early sensory processing, in attention and working memory, in semantic memory, in source memory, and in social cognition. We will then consider hypotheses that allow for a parsimonious understanding of this wide range of impairments, based on recent evidence from systems-based research in brain neuroplasticity mechanisms. This approach allows us to understand the developmental course of the illness, as well as its many complex clinical, neurocognitive, and neurobiological manifestations. It also points the way to translate principles of basic neuroscience into novel treatment interventions for schizophrenia that aim to reverse these impairments.

Conferencia 3:

Conferencia Premio Nacional de Ciencias: Jueves / Thursday 24, 18:30-19:30

“La Vía Wnt de Señalización Modula la Maduración de las Regiones Pre- y Postsinápticas del Sistema Nervioso de Vertebrados. Dr. Nibaldo C Inestrosa Centros de Regulación Celular y Patología “Joaquín V. Luco” (CRCP), de Envejecimiento y Regeneración (CARE) e Instituto Milenio MIFAB, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile.

CP3. La vía de señalización Wnt regula el desarrollo y función del sistema nervioso de vertebrados, lo que se ha establecido fundamentalmente por estudios realizados durante el desarrollo embrionario. Tanto la expresión de ligandos Wnt como de los componentes de la misma en el sistema nervioso del adulto, sugiere un papel para esta vía en la mantención de la estructura y función sináptica. Nosotros hemos estudiado el como estos ligandos operan en la región pre- y postsináptica, para modular el ensamblaje y la estructuración de la sinapsis. Hasta el momento hemos a podido establecer que Wnt-7a, un ligando de la vía Wnt canónica, induce la agregación de “APC” (un componente del complejo de destrucción de β -catenina) con receptores nicotínicos de acetilcolina presinápticos. Además, Wnt-7a estimula la agregación de proteínas de vesículas sinápticas y zonas activas, e induce el reciclaje y la exocytosis de vesículas sinápticas, sugiriendo una función de este ligando en el ensamblaje y función del terminal presináptico. Por su parte, el ligando no-canónico Wnt-5a, induce la acumulación de receptores de glutamato tipo NMDA, junto a proteínas de la densidad postsináptica, en particular la PSD-95. Este efecto es mediado por NO, Ca²⁺ y JNK. También se ha establecido que el ligando Wnt-5a modula el tráfico del receptor GABA(A) a la membrana postsináptica. Es también interesante mencionar que oligómeros del péptido- β -amiloide (A β) de la enfermedad de Alzheimer, se unen específicamente a la región postsináptica, generando cambios en varios de sus componentes. Todos ellos pueden ser prevenidos por la presencia del ligando Wnt-5a. Recientemente hemos establecido que Neuroligina-1, sería un receptor sináptico para el péptido A β en las sinapsis excitatorias. Nuestros estudios establecen que la vía Wnt de señalización regula las regiones pre- y postsinápticas de las sinapsis del sistema nerviosos central de los vertebrados.

Conferencia 4:

Conferencia nombramiento Miembro Emérito de la SCN: Viernes / Friday 25, 14:30-15:30

“El Sistema Nervioso como una arquitectura dinámica en tiempo cero”. Dr. Humberto Maturana (Instituto Matríztico)

CP4. Presenta: Dr: Pedro Maldonado

Conferencia 5:

Conferencia; Viernes / Friday 25, 15:30-16:30

“Development of intracerebral gene & cell therapy strategies to treat neurological disease”. Dr. Alberto Martinez Serrano. Centro de Biología Molecular Severo Ochoa. Universidad Autónoma de Madrid

CP5. Preclinical research using fresh grafted tissue dates back to four decades ago. Precursor/Progenitor/Stem neural cells were incorporated to the neuro-regeneration research, in the context of ex-vivo gene therapy, by the early 90's. Later on, the concepts of gene therapy and cell therapy got merged, since the stem cells, by themselves, did show their therapeutic potential in the in vivo setting, regardless of any genetic modification. By the late 90's, the successful expansion of ES was described; a finding that triggered the interest of many research groups in stem cell research, for the development of putative therapies for degenerative disorders but little if any progress has been made in the clinical setting. In the context of developing new strategies for Central Nervous System (CNS) diseases, the main road-blocks in this research are a) how to regulate the proliferative capacity of stem cells; b) how to get stem cell derivatives, of whatever

accessible source, differentiating to reach the desired mature functional neural phenotype once they are grafted, in vivo, and c) how to instruct stem cells to differentiate into the desired neural lineage and not in other cell types. In recent years, investigating how to generate functional dopaminergic neurons for cell replacement in Parkinson's disease (PD) models, we found that Bcl-XL protein was able to enhance the capacity of forebrain and midbrain neural stem cells (NSCs) to generate dopaminergic neurons. We then investigated the actions of Bcl-XL in these NSCs preparations, finding that: a) Bcl-XL reduces cell death during differentiation by both the usual anti-apoptotic mechanisms, but also through other yet undefined actions, b) that Bcl-XL induces a bias in the generation of neurons with respect to glial cells, through the activation of neurogenic and dopaminergic developmental genes and c) that, at short time points, ventral mesencephalic neural stem cells induce partial behavioural recovery in parkinsonian rats after transplantation. These results will be discussed in the context of pre-clinical research in PD and possible future applications in the clinics.

II SIMPOSIOS

Simposio 1. Miércoles / Wednesday 23, 11:30-13:30

Coordina / Chair: **Dr. Patricio Huerta**

“PLASTICIDAD SINÁPTICA. SOBRE COMO LAS SINAPSIS SON CONSTRUIDAS Y FORTALECIDAS, PERO TAMBIEN DESTRUIDAS”

The process of synaptic plasticity can be described simply as a change in the way synapses work. Synapses are the elementary structural units by which neurons communicate with each other. A century ago, Santiago Ramón y Cajal postulated their existence, but it took the work of pioneers, such as Bernard Katz, Julius Axelrod and John Eccles, to demonstrate that synapses are central ingredients in brain function. Recent decades have witnessed tremendous progress in our understanding of synaptic plasticity, mainly because of the advent of novel techniques in molecular genetics, structural biology, electrophysiology and cellular imaging. In this symposium, we will present current investigations from our laboratories that illuminate how invertebrate and mammalian synapses control their plasticity in healthy and pathological conditions.

11:30-12:10 Proteínas MAGUK en la formación y función sináptica: desde los genes al comportamiento. **Dr. Jimena Sierralta** (Universidad de Chile)

S1.1 [MAGUK proteins in synaptic formation and function: from genes to behavior] Synaptic function is crucially dependent on the spatial organization of the pre- and postsynaptic apparatuses and the juxtaposition of both membrane compartments. This is achieved by a precise arrangement of a scaffolding network at the sub-membrane region of each cell, which binds to specific synaptic partners, thus restricting their localization within synaptic compartments. Our model organism is *Drosophila* and we use the larval neuromuscular synapse, a glutamatergic synapse as a model of mammalian central synapses. Among the proteins enriched in the neuromuscular synapse is DLG a scaffold protein of the SAP-MAGUKs family, which includes PSD-95, SAP97/hDLG, SAP102, and Chapsyn110 in mammals. *dlg* in *Drosophila* has two main protein products as the result of two different transcription start sites. DLGA, which resembles PSD95 structure with a short N terminal domain and DLGS97, which resembles SAP97 with a long N terminal domain bearing an L27 domain. We have generated specific mutants for the two main products of *dlg*, DLGA and DLGS97. Taking advantage of these genetic tools we study the function of *dlg* products on the normal activity of the synapse and on its plasticity. Our results demonstrate essential roles of *dlg* products on synaptic structure and function. In this work we used multiple approaches: electrophysiological recording of the NMJ to study synaptic physiology and short term synaptic plasticity, behavioral studies to study neuronal circuits and long term synaptic plasticity and immunolocalization associated to mutants and transgenic animals to study the interaction of proteins forming synaptic complexes *in vivo*.

12:10-12:50 “Control adaptativo de la plasticidad sináptica” **Dr. Alfredo Kirkwood** (John Hopkins University)

S1.2 Long-term potentiation (LTP) and long-term depression (LTD) are currently the most comprehensive models of synaptic plasticity to account for the storage of new memories in the brain. It is also widely accepted that patterns of activation of the NMDA-receptors specifies the polarity of plasticity (whether potentiation or depression), and that altered NMDAR-dependent plasticity is the basis for cognitive deficits in several neurological conditions. In this talk I will discuss evidence challenging the notion that NMDAR functionality alone is sufficient to fully account for synaptic plasticity and memory acquisition.

First I will show that neuromodulators (i.e the cholinergic and noradrenergic systems that convey information on the individual's behavioral state) specify the polarity of NMDAR-dependent plasticity. Basically, the same pattern of NMDAR-activation results in LTP or LTD depending on the balance of cholinergic/noradrenergic activation. One implication of these findings is that memories might be codified differently depending on the state of the individual.

In the second part I will discuss the synaptic basis for impaired and preserved learning in a rat model of aging. We found that NMDAR-dependent forms of LTP and LTD are uniformly reduced in the aged population and do not account for the differences in learning performance. What makes the difference are the NMDAR-independent forms of LTP and LTD, which are selectively enhanced in aged individuals with preserved learning. Thus, preserved learning in aging likely involves an adaptive switch in mechanism of plasticity rather than preserving “youthful” mechanisms. Altogether, the two examples presented underscore the necessity of expanding the current view of plasticity and learning.

12:50-13:30 “Enfermedades de la sinapsis corticales” **Dr. Patricio Huerta** (Cornell University)

S1.3 The diseases of the mind can be conceptualized as disorders of the synapses within the brain. Synapses are the elementary processing units of neurons; thus it is hardly surprising that their malfunction leads to major neuropsychiatric conditions. In this talk, I will present exemplars from our studies of Alzheimer's disease, stroke and lupus (an autoimmune disorder) describing the mechanisms by which cortical synapses become dysregulated during these pathological states. Excitatory synapses comprise ~70% of brain synapses, use glutamate as the transmitter and have three effectors: α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors, N-methyl-D-aspartate receptors, and metabotropic receptors. It has become clear that these postsynaptic receptors are the early targets for malfunction. I will discuss the receptor-mediated cascades that are affected in each disease; including drastic alterations in synaptic plasticity, abnormal homeostatic synaptic processes, excitotoxicity and mitochondrial stress leading to apoptosis. I will also present our studies showing how inhibitory synapses become dysregulated during brain diseases. Inhibitory synapses represent ~15% of brain synapses, use γ -aminobutyric acid (GABA) as the transmitter and have two effectors: GABA-A receptors and GABA-B receptors. Although epilepsy is a major syndrome involving inhibitory synapses, we will instead discuss recent results showing how selective populations of GABAergic cells are affected in autism and post-traumatic stress disorder. The increasing understanding of the synaptic processes at the root of brain illnesses opens novel avenues for therapy, based on highly specific drugs targeted to synapses.

Simposio 2. Jueves / Thursday 24, 11:30-13:30

Coordina / Chair: **Dr. Juan Carlos Sáez**

“CONNEXIN MUTATIONS AND DEAFNESS”

11:30-12:00 “Deafness mutation Cx26A88S induces cell death due to impairment of the slow gating of hCx26 hemichannel / La mutacion Cx26A88S que causan la sordera, induce muerte celular por deterioracion de regulacion lenta de las hemicanales de hCx26.” **Dr. Bruce Nicholson** (Xu, J.¹, Brenner, R.¹, Nicholson, B.J.² ¹Department of Physiology, University of Texas Health Science Center at San Antonio; ² Department of Biochemistry, University of Texas Health Science Center at San Antonio, USA).

S2.1 Gap junctions can be gated by transjunctional voltage (V_j), pH, intracellular [Ca²⁺] and phosphorylation. V_j gating has fast and slow components, which transit the channels from the open to a subconductance (residual state) or fully closed state, respectively. These same fast and slow gating components are also seen in hemichannels in response to changes in membrane potential. We show that the Cx26A88S mutation, which causes sensorineural deafness, specifically impairs the slow gate response of hemichannels that allows them to fully close. This leads to increased cell death, even in normal Ca⁺⁺ which closes wt hemichannels. The mutation does not affect hemichannel fast voltage gating, or V_j gating of gap junction channels, which is dominated by the fast gating phenotype. This is the first implication of a role for the slow voltage gating of hemichannels in the etiology of deafness, possibly through the induction of persistent leakiness and cell death. The recently published structural model of Cx26 channels suggests a mechanism for this effect, where S88 in M2 could form an inter-helical H-bond with R143 in M3, preventing the movement about P87 that has been implicated in V_j gating. This deafness mutation in Cx26 is actually the wild-type residue in Cx50, which form hemichannels with a persistent sub-conductance state. Furthermore, Cx50S89A that mimics wtCx26 shows depolarization activation and establishes a slow gating response that completely closes the channel. Thus, a disease associated mutant phenotype in the ear (Cx26) has actually been “captured” for the wild-type function of Cx50 in the lens. Work funded by NIH-NIGMS and NIH-NCI

12:00-12:30 “Connexin channels in the transduction mechanism for hearing and their regulation in ototoxicity and inflammation / Canales formados por conexinas en el mecanismo de transducción auditiva y sus regulaciones en ototoxicidad e inflamación” **Dr. Juan C. Sáez**, (Figueroa V¹, Sánchez, HA², Martínez, AD¹ and Sáez JC², ¹Centro Interdisciplinario de Neurociencias de Valparaíso, Universidad de Valparaíso y ²Departamento de Ciencias Fisiológicas, Universidad Católica de Chile.).

S2.2 Mutations in connexins (Cxs 26, 30 and 43) produce congenital prelingual syndromic and non-syndromic hear loss. In the adult human cochlea, Cxs are expressed in the stria vascularis, basement membrane, limbus and spiral prominence. Cx43 is found mainly in the stria vascularis and limbus and Cxs 26 and 30 are present in all structures. Factors that induce postlingual hearing loss are diverse and the most common and/or characterized are exposure to aminoglycosides, infections and trauma. Although these factors act on different molecular targets their effect on the transduction machinery of the hearing system include intercellular communication pathways based on channels constituted of Cxs. In HeLa Cx26 or Cx43 transfectants the effects of the aminoglycose gentamycin, LPS (bacterial endotoxin) and hypoxia-reoxygenation on the functional state of gap junction channel (GJCH; dye coupling) and hemichannels (uptake of ethidium) have been studied. Concentrations of gentamycin expected to occur during its therapeutic use reduced the functional activity of GJCH and hemichannels. Whereas, hypoxia-reoxygenation and LPS treatment reduced GJCH function but increased the activity of hemichannels. Lost of GJCH function between supporting cells and fibrocytes should reduce the recycling of K⁺ from hair cells back to the endolymph and prevent intercellular transfer of second messengers such as IP₃. The increase hemichannel

activity observed under proinflammatory conditions could lead to excessive ATP release; toxic for adult hair cells. Therefore, dysfunction of Cx based channels induced by toxic antibiotics and pro-inflammatory conditions might at least partially explain the hear loss induced by these agents. FONDECYT 1090573 and 1070591

12:30-13:00 " Deafness-related mutations in the TM1 of Cx26 unveil structural and functional differences between gap junction channels and hemichannels. / Mutaciones de sordera en el TM1 de la Cx26 revelan diferencias estructurales y funcionales entre uniones en hendidura y hemicanales." **Dr. Agustín Martínez** (Jara O¹, Acuña R¹, Maripillan J¹, Sáez JC² and Martínez AD¹, ¹Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso. ²Departamento de Ciencias Fisiológicas, Universidad Católica de Chile)

S2.3 More than one hundred different missense mutations in the connexin26 (Cx26) gene have been associated to congenital deafness; some of them are point mutations in the first transmembrane domain (TM1). Recent functional and structural X-Ray crystallography studies of Cx26 gap junction channels (GJCh), suggest that TM1 is a pore lining alpha helices. The pore narrowest diameter is located in the transition between TM1 and the extracellular loop-1; therefore this Cx region could be part of the selectivity filter. In addition, this Cx region has been propose to be important for loop gating, a type of voltage dependent gating mechanism. Loop gating may keep unopposed hemichannels closed under normal extracellular calcium conditions. We have study this region and found that amino acid residues V37-A40 are involved in homo-dimerization of the Cx26-TM1. Cx26 with replacement of domain V37-A40 by a sequence of the TM1 of Cx43 (Cx26-GTAV) failed to form GJCh. Wild type Cx26-GFP, but not Cx43-GFP, rescued Cx26-GTAV phenotype, allowing the formation of normal gap junction plaques and suggesting that both proteins are compatible to oligomerize. The recessive deafness-related mutations, Cx26V37I and Cx26A40G produce a type of non-syndromic deafness and non-functional GJCh when express in *Xenopus* oocytes. One possibility is that these mutations affect interactions between subunits within a hemichannel because at this position the pore lining residues of two Cx sub-units are in close proximity. Differences in GJCh and hemichannels permeability as a result of Cx26A40G and Cx26V37I mutations suggest that the structure of this connexin domain differs between GJCh and hemichannels. FONDECYT 1090573 and 1070591, Anillo Redes-24

13:00-13:30 "A Cx26 mutation that causes severe KID is pore-lining and alters Ca²⁺ permeability and regulation of hemichannels / Una mutacion en Cx26 que causa síndrome KID se ubica en el poro de los hemicanales y altera su permeabilidad y regulacion por Ca²⁺" **Dr. Vytautas Verselis** (Sanchez HA¹, White TW², Verselis VK¹, ¹Dominick P. Purpura Dept. of Neuroscience. ²Dept. of Physiology and Biophysics, Stony Brook University Medical Center. Department of Neuroscience, Albert Einstein College of Medicine, NY, USA)

S2.4 Mutations in GJB2, which encodes Cx26, are one of the most common causes of syndromic and non-syndromic deafness in humans. Although more than 100 mutants have been identified, a number that cause syndromic deafness associated with severe skin disorders localize to the region that borders the first transmembrane (TM1) and the first extracellular loop (E1) domains. Previously, using the substituted cysteine accessibility method (SCAM) applied to single Cx46 hemichannels, we showed that the extracellular half of TM1 and the proximal portion of E1 are pore-lining and participate in regulating hemichannel opening by voltage and extracellular Ca²⁺. A recently published crystal structure of Cx26 at 3.5 Å resolution is in agreement with our model of the pore. Here we applied SCAM to human Cx26 hemichannels and demonstrate that a homologous set of residues in Cx26 contribute to the pore. At one position, G45, a G to E missense mutation (G45E) is associated with a fatal form of keratitis-ichthiosis-deafness (KID). We found that G45E hemichannels tend to be more open at resting membrane potentials. The Ca²⁺ concentration at which conductance was reduced to half-maximal shifted ~0.2 to 0.4 mM. More dramatically, we found G45E hemichannels displayed a substantial increase in Ca²⁺ permeability. Mutational analysis supports an

electrostatic effect of the side chains at this position. These results are consistent with G45E being a gain-of-function mutant that imparts both reduced Ca²⁺ regulation and a large increase in Ca²⁺ permeability, both of which can contribute to accelerated cell death. NIGMS 54179 to V.K.V. NIDCD 006652 to T.W.W.

Simposio 3. Jueves / Thursday 24, 16:30-18:30
Coordina / Chair: **Dr. Rommy von Bernhardt**

“GLIAL CELL-NEURON INTERACTION IN THE NORMAL AND ABNORMAL FUNCTION OF THE NERVOUS SYSTEM”.

Glial cell are critical participants in every major aspect of brain physiological function and disease. Far more important than once thought, glial cells not only powerfully modulate synaptic function, neuronal maintenance, brain homeostasis and response to injury, but they also respond to neural activity. In this symposium, we will discuss how microglial cells' response to neuronal activity can activate neurodegenerative mechanisms, and the importance of glial cells in the pathogenesis of neurodegenerative diseases, the key role played by astrocytes in neurometabolic coupling of the brain, and the regulatory cross talk among schwann cells and neurons. The prominent functions served by glial suggest that the brain can not be regarded as a circuitry of neuronal contacts, but as an integrated network of interactive neurons and glia.

16:30-17:00 “Modification of microglial function in response to glutamate and its possible participation in the neurodegenerative diseases / Modificación de la función microglial en respuesta a glutamato, y su posible participación en las enfermedades neurodegenerativas” **Dr Mami Noda**. (Laboratory of Pathophysiology, Kyushu University, Fukuoka Japan)

S3.1 Decreased expression of AMPA-type of glutamate (Glu) receptor subunit GluR2 and GluR2/3 was reported in brains of Alzheimer's disease (AD) and sporadic Creutzfeldt-Jakob disease (CJD). It was postulated that the presence of the GluR2 subunit may provide a neuroprotective role by limiting the flow of extracellular Ca²⁺, whereas the reduction of GluR2 may contribute to the vulnerability via a mechanism involving an increase in intracellular Ca²⁺ and destabilization of intracellular Ca²⁺ homeostasis. Cultured microglial cells also express AMPA receptors but its functional importance has not been cleared yet. Here we examined the functional change induced by Glu before and after activation of microglia by lipopolysaccharide (LPS) and how they were modified in GluR2-deficient mice. Electrophysiological measurements showed that Glu-induced currents in the presence of cyclothiazide (CTZ), an inhibitor of AMPA receptor desensitization, were significantly inhibited in activated microglia. Immunocytochemical staining showed that GluR2 were translocated from cytoplasm to cell membrane after activation of microglia. Using GluR2 knock-out (ko) mice, no inhibition of Glu-induced currents by activation of microglia was observed. The amounts of Glu-induced pro-inflammatory cytokines were significantly increased in microglia from GluR2-ko mice. These results suggest that GluR2 contribute to frigidness to Glu in activated microglia and dysfunction or decreased expression of GluR2 may cause excess release of inflammatory cytokines from microglia, causing additional Glu neurotoxicity under pathologic conditions.

17:00-17:30 “Key role of glial cells in the coupling of energy supply and demand in the brain”. **Dr Felipe Barros**. (¹Barros LF, ¹Bittner CX, ^{1,2}Loaiza A, ^{1,2}Ruminot I, ^{1,2}Larenas V, ^{1,2}Sotelo T, ^{1,2}Moldenhauer H; ¹Centro de Estudios Científicos (CECS) ²Universidad Austral de Chile)

S3.2 Brain activity is accompanied by fast fluctuations in local energy metabolism and blood flow. Long-standing questions in this field relate to the mechanisms that match energy demand and supply, in particular to the proposed role glutamate and astrocytes in the coupling. This presentation will describe a new FRET-based microscope technique that is capable of measuring the absolute rate of glycolysis in single cells and with resolution of seconds, as compared with that of existing isotopic methods (PET and autoradiography) that require cell populations and minutes. Applied to astrocytes in

culture and in brain slices, the method showed that the rate of glycolysis can be activated by several fold and within seconds by increases in extracellular K⁺ similar to those measured in the brain interstice during synaptic activity. No activation of glycolysis was observed in response to glutamate, which nevertheless caused Na⁺ overload and acidification consistent with effective engagement of the Na⁺/glutamate cotransporter. The effect of K⁺ on glucose metabolism was readily reversible, and was synergistically mediated by Na⁺/K⁺ ATPase pump activation and depolarization-induced alkalization (DIA), a phenomenon of hitherto unknown physiological significance. A rapid activation of astrocytic glycolysis was also observed in brain slices exposed to exogenous K⁺ and during electrical stimulation. These results reveal a novel phenomenon linking synaptic activity to timely local production of lactate by astrocytes and ascribe interstitial K⁺ and astrocytic pH central roles in neurometabolic coupling and neurovascular coupling.

17:30-18:00 "Glia-mediated neuroinflammation as disease mechanism in neurodegenerative disorders / Neuroinflamación mediada por la glía como mecanismo patológico en las enfermedades neurodegenerativas". **Dr Rommy von Bernhardt**. (Depto. Neurología, Fac. Medicina, Pontificia Universidad Católica de Chile)

S3.3 Alzheimer's disease neuroinflammation depends heavily on glial activation. Unbalance between inflammatory and neuroprotective-modulatory activity of glia can lead to neurotoxicity. Such unbalance could be promoted by conditions like hypoxia and inflammation, which could modify expression of Scavenger Receptors (SRs) involved in binding of A β . We assessed production of NO, expression of SRs and activation of inflammation pathways in glial cultures exposed to inflammatory molecules, hypoxia, SR-A ligands and A β . Hypoxia+inflammation increased 14-fold NO production by astrocytes and 140-fold by microglia. A 2.3-fold increase compared to normoxic proinflammation. Hypoxia induced SR-BI, whereas decreased proinflammation-induced SR-MARCO and SR-BI expression. SR-A ligands increased NO production by microglia but not astrocytes. SR-A ligands and IFN γ +LPS increased expression of IL-1 α by astrocytes, effect that was potentiated by A β and greatly enhanced in SR-A KO mice, whereas NO production was abolished in KO mice. IFN γ +LPS also increased expression of TGF- β , which induced a 2.5-fold increase of SR-A in a Smad3 dependent manner, whereas SR-MARCO remained unchanged and SR-BI expression decreased by 87% in microglia. Our results suggest that hypoxia increase glial reactivity potentiating induction of NO and decreasing SR-BI and SR-MARCO expression induced by IFN γ +LPS. Activation of SR-A mediates various glial responses that could be involved in modulation of neuroinflammation. Whereas the antiinflammatory cytokine TGF- β increases SR-A while decreases SR-BI expression. FONDECYT 1090353 y proyecto Puente 01/08 VRAID PUC.

18:00-18:30 "Schwann Cell to Axon Transfer of Ribosomes: Toward a Novel Understanding of the Role of Glia in the Nervous System / Transferencia de Ribosomas desde la Celula de Schwann al Axon: Nuevos Roles de la celula Glial en el Sistema Nervioso" **Dr Felipe Court** (Departamento de Fisiologia, Facultad de Ciencias Biológicas, Pontificia Universidad Católica, Santiago, Chile)

S3.4 In the nervous system, the studies of signal processing and plastic phenomena have focused on synaptic mechanisms. In contrast, axons are considered a rather static array of lines of communication. In contrast to this view, we have proposed a model called "the autonomous axon", the main feature of which is that the axonal phenotype is dynamic, i.e., subject to local modifications in response to local challenges, without the immediate participation of the neuronal cell body. In this model, the Schwann cell is a major regulator of the axon. When the set of local regulators changes, so does the axonal phenotype. The axon is currently considered unable to synthesize proteins and to be fully dependent on its perikaryon. Recently, growing axons have been shown to contain ribosomes and to synthesize proteins. In adult mice, we found that peripheral axons contain ribosomes, that they are transferred from the associated Schwann cell, and that this transcellular transfer is up-regulated by nerve section and during axonal

regeneration. Therefore, the axon, which is anatomically a part of the nerve cell, is supported in such a basic function as protein synthesis by an associated cell. Preliminary evidence supports that messenger RNAs are also transferred to the axon along with ribosomes. The phenotype of a cell is the organization of its constituent proteins, which determines its functional capabilities. Thus the axon appears as a sort of joint venture of the nerve cell and the glial cell encompassing the axon as its phenotype is specified by the genome of two independent cells. This role of the glia endows our understanding of the nervous system with an unsuspected dimension. FONDECYT 1070377

Simposio 4: Viernes / Friday 25, 9:00-11:00
Coordina / Chair : Dr. Gricel Orellana

"APORTES DE LAS NEUROCIENCIAS COGNITIVAS A LA INVESTIGACION EN ESQUIZOFRENIA"

9:30-10:00: "Auto-evaluación cognitiva en Esquizofrenia: Evidencia conductual y psicofisiológica". Dr. Jaime Silva (Departamento de Salud Mental y Psiquiatría, Facultad de Medicina, U. de la Frontera.)

S4.1 Changes in personal identity have been described as a major component in the expression of schizophrenia. Considering current evidence that relates the cortical structures of the medial line with the self, we hypothesize that there are differences in the frontomedial electrophysiological activation in schizophrenics when compared to normal subjects during tasks that require this function. In an experimental design, 9 schizophrenic and 7 normal subjects participated in a personal traits attribution task regarding the attribution of the self, another person and a simple reading. Electric brain activity was recorded with a dense array of 128 electrodes, with evoked brain potentials being obtained for the three conditions. In this presentation, the electrophysiological and behavioral gathered in that experiment will be discussed and reviewed

10:00-10:30: "First episode of Schizophrenia: specific impairment in executive attention". Dr. Gricel Orellana (Orellana G¹, Slachevsky A² and Peña M³; ¹Department of West Psychiatry, Faculty of Medicine, University of Chile. ²Department of Neurology, Faculty of Medicine, University of Chile. ³School of Psychology, Faculty of Psychology, University Catholic and International School for Advanced Studies, Trieste, Italy)

S4.2 Objective: to clarify if patients in a first episode of schizophrenia (FES) have a specific impairment in attention. Attention, as Posner, are neural networks that would perform specialized functions such as: 1) achieving and maintaining the alert status, 2) orientation to sensory events and 3) executive control of the thoughts and feelings (executive attention). Specifically, this research seeks to: indicate which of the three networks of attention can function abnormally in FES. Participants and Methods: we evaluated 22 patients with schizophrenia between 17 to 29 years of age with a recent history of a psychotic outbreak only been treated with atypical neuroleptics. We conducted the following test: (1) a computer Attention Network Test (ANT) to assess the three attentional networks, (2) Dementia Rating Scale (DRS) and Raven's Progressive Matrices to assess global cognition. The healthy control group was matched by sex, age, educational level and received the same evaluation. Results: we assessed the efficiency of the segregated alerting, orienting, and executive control networks by measuring how response latencies were influenced by alert cues, spatial cues, and flanker congruency. In overall ANOVAs we found main effects of cue condition and flanker congruency. The data also revealed a significant interaction of group and flanker type, with schizophrenia patients taking longer and less accuracy on average in incongruent flanker. ANOVA revealed a main effect of Network and a significant interaction of Network X Group. The increase in the mean RT in the Conflict Network is significantly higher in FES with respect to controls, without significant differences between groups in Alert or Orienting Networks. Performances in executive attention are not correlated with DRS and Raven. Conclusions: FES patients showed a significant deficit in the executive network and no deficit in orienting or alerting network.

10:30-11:00 “Neuroplasticity and Schizophrenia”. **Dr. Sophia Vinogradov** (Laboratory of Cognitive Neuroscience, Professor of Psychiatry, Department of Psychiatry, University of California, San Francisco, EEUU).

S4.3 Verbal memory impairment in schizophrenia is a key rate-limiting factor for functional outcome, does not respond to currently available medications, and shows only modest improvement after conventional behavioral remediation. We investigated an innovative approach to the remediation of verbal memory in schizophrenia, based on principles derived from the basic neuroscience of learning-induced neuroplasticity. Fifty-five clinically stable schizophrenia subjects were randomly assigned to either 50 hours of computerized auditory training or a computer games control condition. Auditory training subjects engaged in a daily schedule of computerized exercises that placed implicit, increasing demands on auditory perception through progressively more difficult auditory/verbal working memory and verbal learning tasks. Relative to the control group, active training subjects showed significant gains in global cognition, verbal working memory, and verbal learning and memory, with treatment effect sizes of 0.55-0.89. They also showed reliable and significant improvement in the auditory psychophysical training aspect of the program; this improvement was significantly correlated with gains in verbal working memory and global cognition. We conclude that intensive training of early auditory processes and auditory/verbal learning results in substantial gains in verbal cognitive processes relevant to psychosocial functioning in schizophrenia. These gains may be due to a training method that addresses the early perceptual impairments in the illness, that exploits intact mechanisms of repetitive practice in schizophrenia, and that uses an intensive, adaptive training approach.

Simposio 5: Viernes / Friday 25, 11:30-13:30

Coordinan / Chair: **Dr. Julio Alcayaga and Dr. Rodrigo Iturriaga**

“RECEPTORES SENSORIALES EN CONDICIONES FISIOLÓGICAS Y PATOLÓGICAS”

Simposio sobre fisiología sensorial en honor a don Carlos Eyzaguirre por su aporte a la investigación en receptores mecanosensoriales (mecanorreceptor de crustáceos y husos musculares en anfibios y mamíferos), al establecimiento de la preparación in vitro para el estudio de la fisiología de los quimiorreceptores arteriales y a sus estudios sobre los mecanismos celulares y moleculares involucrados en dicha quimiorrecepción en mamíferos.

11:30-11:54 “Physiology of sensory receptors: The contribution of Carlos Eyzaguirre / Contribución del Dr. Carlos Eyzaguirre a la Fisiología Sensorial” **Dr. Patricio Zapata** (Medicina, Clínica Alemana / Facultad de Medicina, Universidad del Desarrollo).

S5.1 Carlos Eyzaguirre, as associate of Stephen Kuffler at Johns Hopkins University, performed the first intracellular recordings on mechanoreceptors, made possible by the presence of perikarya and dendrites within the crustacean stretch receptor. They obtained direct evidence of changes in membrane potential expressed as receptor (generator) potentials, as well as of inhibitory potentials produced by efferent nerve endings impinging upon receptor cells. This work resulted by 1955 in three influential papers in the Journal of General Physiology, extending for nearly 100 pages. After that, Eyzaguirre working mostly alone, initially at the Catholic University of Chile and then at the University of Utah, published five papers (1956-1960) on the physiology of amphibian and mammalian muscle mechanoreceptors. In 1961, four papers in the Journal of Physiology (London), reported on experiments performed by Eyzaguirre and associates on a new preparation: the cat's carotid body superfused in vitro. This was received with some reserve: how was it possible to maintain such an oxygen demanding organ for many hours without blood circulation? The answer was brief: if the carotid body continues providing sensory discharges and remains responsive to natural chemical stimuli, it must be well alive! From that moment, many young researchers went to Eyzaguirre's laboratory in Utah to collaborate in this scientific endeavour, with successive associations

resulting in approximately 115 scientific papers on this subject. Thus, Eyzaguirre may be well considered as the father of arterial chemosensory physiology.

11:54-12:18 "Synaptic transmitters in a secondary sensory receptor: the case of the carotid body / Comunicación en un receptor sensorial secundario: El caso del cuerpo carotídeo". **Dr. Julio Alcayaga** (Laboratorio de Fisiología Celular, Facultad de Ciencias, Universidad de Chile)

S5.2 Secondary, or compound, sensory receptors comprise a sensory cell synaptically connected to a sensory neuron. The carotid body (CB) receptor (glomus, type-I) cells are innervated by pseudomonopolar neurons located in the petrosal ganglion, constituting a compound receptor which afferent activity is driven by blood hypoxia, hypercapnia and acidification. Although glomus cell transduction mechanisms are fairly understood, the exact nature and function of many putative transmitters is unclear in the CB.

Acetylcholine (ACh) cannot account per se for the generation of CB activity, because of the inability of cholinergic receptor antagonists to abolish CB responses to natural stimuli. Dopamine (DA), present and released from the CB in response to stimulation, inhibits the afferent activity in most species, precluding considering DA as the CB excitatory transmitter. ATP increases the CB afferent activity and activates chemosensory neurons, and the "knock-out" of ionotropic nucleotide receptors largely impairs CB response to natural stimuli, indicating its participation in the generation of CB afferent activity. Combined blockade of ACh nicotinic and ATP ionotropic receptors abolishes responses of the CB in vitro and in reconstituted chemosensory units in tissue culture, but has little or no effect on the whole animal. In recent years GABA, serotonin, histamine and several peptides have been suggested to participate in the generation and/or maintenance of CB activity, but species differences have been largely underestimated.

Thus, transmission of chemosensory activity from receptor cells to sensory neurons in the CB appear to be mediated by several transmitter acting combined, both at pre- and post-synaptic sites.

12:18-12:42 "Sensorial alteration in the respiratory rhythm dysfunction induced by prenatal nicotina exposure / Compromiso sensorial en la disfunción del ritmo respiratorio inducida por nicotina prenatal" **Dr. Jaime Eugenin** (Facultad de Química y Biología, Universidad de Santiago)

S5.3 Prenatal nicotine exposure is proposed to be a link between smoking habit during pregnancy and sudden infant death syndrome (SIDS). Osmotic minipumps were implanted subcutaneously into 5-7 days pregnant mice to deliver saline (controls) or nicotine ditartrate 60 mg Kg⁻¹ day⁻¹. Using pletysmography and recording of fictive respiration in brainstem slices and in "en bloc" brainstem / spinal cord preparations, we observed that prenatal nicotine leads to blunted ventilatory responses to hypercarbia, and reduced central chemoreception responses, associated likely to muscarinic receptor downregulation in mouse neonates. In addition, hypoactivity of caudal raphe neurons seems to contribute to the depressed central chemosensory responses. These results indicate that nicotine exposure affects the respiratory rhythm pattern generator and causes a decline in central chemoreception during the early postnatal life. Consequently, breathing would become highly vulnerable, failing in responding to chemosensory demands. Such impairment could be related to the ventilatory abnormalities observed in SIDS.

12:42-13:06 "Contribution of the carotid body chemoreceptors to the autonomic dysfunction and hypertension induced by intermittent hypoxia in experimental models of sleep apnea / Contribución del cuerpo carotideo a la hipertensión producida por la apnea obstructiva del sueño". **Dr. Rodrigo Iturriaga** (Laboratorio de Neurobiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile)

S5. 4 The most usual form of hypoxia in humans is the chronic intermittent hypoxia (CIH) produced by the obstructive sleep apnea (OSA). The OSA syndrome is a highly prevalent sleep-breathing disorder considered as an independent risk factor for hypertension. Endothelial dysfunction, oxidative stress, inflammation, and sympathetic activation have been proposed as potential mechanisms involved in the hypertension. However,

evidence for a single pathogenic mechanism has been difficult to establish in OSA patients because of concomitant comorbidities. Thus, animal models have been developed to study the pathological consequences of CIH. Although, bilateral denervation of the carotid body (CB) prevents the progression of the hypertension in rats exposed to CIH, the proposal that arterial chemoreceptors play a role in the pathological consequences of OSA did not receive much attention. However, in the last decade an increasing body of evidence suggests that an abnormal enhanced CB chemoreceptor sensitivity to hypoxia contributes to the potentiation of the sympathetic discharges induced by CIH. Several studies have shown that patients with recently diagnosed OSA and animals exposed to CIH present potentiated sympathetic, vascular and ventilatory responses to acute hypoxia, suggesting that peripheral chemoreflexes are enhanced. More recently, recordings of CB chemosensory discharges in rats and cats provided direct evidence that CIH selectively enhanced the CB chemosensory discharge and the responses to hypoxia, indicating that CIH produces a long-term sensory potentiation of the CB chemoreceptor activity. The mechanisms underlying the CIH-induced CB chemosensory potentiation are not completely known. Recent evidence suggests that increased levels of reactive oxygen species and endothelin-1 within the CB contribute to enhance the chemosensory responses, but it is possible that pro-inflammatory molecules are also involved. Understanding how such signals interact with the CB chemoreceptor system will provide new insights into the generation of the cardiovascular OSA complications. FONDECYT 1070584.

13:06-13:30 “Corneal sensory neurons: From nerve endings to sensations / Receptores sensoriales y sensaciones en la cornea” Dr. Carlos Belmonte (Instituto de Neurociencias de Alicante Universidad Miguel Hernández-Consejo Superior de Investigaciones Científicas, Alicante, España)

S5.5 The cornea of the eye is innervated by various functional classes of primary sensory neurons. Mechanoreceptors represent about 20 % of the total, have high mechanical threshold, large receptive fields and conduction velocity in the A-delta range (<10m/s). The somas of mechanoreceptive neurons have fast action potentials, phasic discharges and marked inward rectification. Polymodal neurons, the most abundant type (70%) are activated by mechanical stimuli, temperatures over 39°C, exogenous irritants (acids, capsaicin) and a variety of endogenous mediators (prostaglandins, BK). They conduct in the A-delta or the C range, produce wide action potentials with a hump and do not rectify. Polymodal nociceptor terminals release CGRP at their endings. Corneal cold-sensitive neurons discharge spontaneously at temperatures of 33°C, often in bursts and increase the firing frequency with moderate cooling. The somas of these neurons that belong to the A-delta and C groups, are highly excitable but do not fire spontaneously, produce fast action potentials and exhibit a prominent rectification. A fraction of them also respond to heat and capsaicin. Corneal nerve endings possess TTX-resistant sodium channels that may sustain propagated action potentials. After surgical injury of the cornea, regenerating and sprouting axons exhibit an abnormal responsiveness to natural stimuli. In particular, cold-sensitive terminals show significantly increased spontaneous activity, decreased cooling thresholds and heightened maximum responses to cooling compared to nerve terminals of intact corneas. This may explain the development of dysesthesias following corneal refractive surgery. Supported by the Spanish MICINN (projects BFU2007-61855 and CONSOLIDER-INGENIO 2010, CSD2007-0002) and by the Fundación Marcelino Botín

III SESIONES COMUNICACIONES LIBRES

Miércoles / Wednesday 23 16:30-18:30

PREMIO JOAQUIN LUCO VALENZUELA: "Mejor Presentación de un Doctorando Año 2008"

C1. Inactivation of the Interoceptive Insula facilitates extinction of an amphetamine contextual memory

Contreras M.(1), Billeke P.(1), Vicencio S. (1) and Torrealba F. (1).

(1)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 interoceptive information is essential in maintaining an 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. We inactivated the primary interoceptive cortex in amphetamine-experienced rats using bilateral injections of a Na⁺ channel blocker (0.5% bupivacaine, 1 µl per side) that reversibly block cortical structures for about 40 min, during one extinction session. Insular cortex inactivation in amphetamine-experienced rats, in contrast with the bilateral injections of saline, facilitates extinction assessed by reduction of the time spent in the amphetamine-paired compartment. We conclude that the insular cortex is a key structure in perception of body signals that are important in maintaining an amphetamine contextual memory. Our results further suggest that the modulation of insula activity using non-invasive approaches should be considered as a therapeutic target to accelerate the extinction of memory addiction.

Fondecyt 1060476 and Iniciativa Científica Milenio.

C2. Fluoxetine induces structural plasticity in limbic cortices.

Ampuero E, Rubio FJ, Earle N y Wyneken U.

Laboratorio de Neurociencias, Universidad de los Andes Chile.

Fluoxetina (flx) es un fármaco antidepresivo prescrito para el tratamiento de la depresión. En el manejo terapéutico de la depresión, se describe un retardo entre el inicio del tratamiento y la disminución de los síntomas depresivos, lo que se debería a cambios adaptativos inducidos por tratamientos prolongados, pero no agudos. Nosotros postulamos que flx induce cambios en la morfología de las neuronas piramidales y el contenido de receptores para glutamato tipo NMDA (NMDAR) y AMPA (AMPA) en áreas corticales límbicas (corteza Prelímbica (PL) y corteza Retrosplenial Granularb (RSGb)). Se administró flx (0,7mg/kg,i.p.) durante 4 semanas y se evaluó el largo dendrítico, la densidad y morfología de espinas dendríticas mediante tinción de Golgi. La morfología de espinas varía desde delgadas a botón y callampa/segmentada, reflejando estas últimas sinapsis más estables y potenciadas. El contenido de las subunidades para NMDAR (NR1, NR2A y NR2B) y AMPAR (GluR1 y GluR2) se determinó mediante inmunohistoquímica. Flx disminuyó el largo dendrítico basal en ambas áreas corticales (en µm, PL: C=986,9±204,1; n=8; flx=624,8±72; n=23; p<0,05. RSGb: C=821,3±133,9; n=9; flx=499,1±54,3; n=15; p<0,05). Sin embargo, sólo en la RSGb flx aumentó tanto la densidad de espinas (nº/µm) (C=0,62±0,03, n=14; Flx=0,75±0,03,n=19, p<0,01) como el porcentaje de espinas tipo callampa (C=18,5±1,9%, n=14; flx= 26,1±1,8%, n=19, p<0,01), lo que se asoció a un aumento de células que expresan subunidades NR2A, GluR1 y GluR2. Nuestros resultados indican que flx regula la morfología de las neuronas excitadoras y sus sinapsis en la RSGb, lo que podría estar asociado a sus efectos terapéuticos (AnilloACT09-06). Proyecto Anillo ACT09-06 (UW), Beca Conicyt (EA)

C3. Sumo-1 and sumo specific-peptidase regulate Nurr1-dependent transcription

Arredondo C, Pereira L, Andrés M E.

Facultad de Ciencias Biológicas. Departamento de Biología Celular y Molecular. Pontificia Universidad Católica de Chile.

Nurr1 is an orphan nuclear receptor, essential for the origin and survival of midbrain dopaminergic neurons. Nurr1 is crucial for the expression of tyrosine hydroxylase, the dopamine transporter and the vesicular monoamine transporter-2. Our interest is to determine the mechanism involved in the regulation of Nurr1-dependent transcription. Sumoylation is a post-translational modification, where a peptide called SUMO binds to the target protein in a covalent and reversible reaction. We demonstrated that: 1) Nurr1 interacts and it is repressed by the E3-SUMO ligase PIASgamma, 2) Nurr1 has consensus sequences for sumoylation and 3) Mutants in consensus sequences for sumoylation changes the transcriptional activity of Nurr1. In this work we show by means of mammalian gene reporter assays that the transcriptional activity induced by Nurr1 is changed by the over-expression of SUMO-1 peptide and for Sumo-specific proteases. Also, by means of protein immunoprecipitation assays, we have detected a band, suggesting that it represents a sumoylated isoform of Nurr1. Another possible regulator of Nurr1 is the DJ-1 protein. This protein interacts with members of PIASgamma family, regulates sumoylation of proteins and is directly related to Parkinson disease. DJ-1 decreases the transcriptional activity induced by Nurr1 and this repression is added to the repression induced by PIASgamma. Our results suggest that the transcriptional activity of Nurr1 is regulated by sumoylation and by the presence of the DJ-1 protein.

C4. Effect of the Environment on the Dendritic Morphology of the Rat Auditory Cortex

Dagnino-Subiabre A (1), Bose M (2), Muñoz-Llancao P (1), Roychowdhury S (2), Nichol J (2), Salgado H (2), Kilgard M.P (3), Aboitiz F (4), Atzori M (2).

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The present study aimed to identify morphological correlates of environment-induced changes at excitatory synapses of the primary auditory cortex (A1). We used the Golgi-Cox stain technique to compare pyramidal cells dendritic properties of Sprague-Dawley rats exposed to different environmental manipulations. Sholl analysis, dendritic length measures, and spine density counts were used to monitor the effects of sensory deafness and an auditory version of environmental enrichment (EE). We found that deafness decreased apical dendritic length leaving basal dendritic length unchanged, whereas EE selectively increased basal dendritic length without changing apical dendritic length. On the contrary, deafness decreased while EE increased spine density in both basal and apical dendrites of A1 Layer 2/3 (LII/III) neurons. To determine whether stress contributed to the observed morphological changes in A1, we studied neural morphology in a restraint-induced model that lacked behaviorally relevant acoustic cues. We found that stress selectively decreased apical dendritic length in the auditory but not in the visual primary cortex. Similar to the acoustic manipulation, stress-induced changes in dendritic length possessed a layer specific pattern displaying LII/III neurons from stressed animals with normal apical dendrites but shorter basal dendrites, while infragranular neurons (Layers V and VI) displayed shorter apical dendrites but normal basal dendrites. The same treatment did not induce similar changes in the visual cortex, demonstrating that the auditory cortex is an exquisitely sensitive target of neocortical plasticity, and that prolonged exposure to different acoustic as well as emotional environmental manipulation may produce specific changes in dendritic shape and spine density. This work was supported by Anillo de Ciencia y Tecnología N° ADI-09 (Programa Bicentenario) and INNOVA N° 06FC01BC-71 (CORFO) grants to D-S. A. and NIH N° 5R01DC005986 and NARSAD Young Investigator grants to A.M.

C5. The oxidized form of vitamin C accelerates cellular death in neuronal models under oxidative stress

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La vitamina C reducida cumple importantes funciones antioxidativas. Esta vitamina puede ser oxidada a ácido deshidroascórbico (DHA) mediante el fenómeno de estrés oxidativo, incorporándose a las neuronas mediante transportadores GLUTs. Nuestro objetivo es determinar el efecto de la incorporación de DHA en modelos neuronales sometidos a estrés oxidativo. Caracterizamos los transportadores de DHA en células de linaje neuronal N2a y HN33.11, mediante RT-PCR, Western blot e inmunofluorescencia. La incorporación de DHA y glucosa fue analizada por ensayos de transporte. Sometimos luego las células a estrés oxidativo con H₂O₂ analizando la muerte celular en presencia y ausencia de DHA. Tanto células N2a como HN33.11 poseen los transportadores de DHA, GLUT1 y GLUT3. La incubación de estas células con H₂O₂ por 30 min, induce un 20% de muerte celular después de 6h en células N2a y un 60% de muerte celular después de 3h en células HN33.11. El tratamiento con DHA (30 min) después del daño oxidativo, favorece la muerte celular, aumentando a 60% la muerte celular en células N2a y en un 80% la muerte celular en células HN33.11. Al competir la incorporación de DHA mediante la incubación con células HL60, el proceso de muerte celular es revertido de manera significativa. Nuestros resultados sugieren que después de la oxidación extracelular de AA a DHA, durante un proceso oxidativo cerebral, DHA cumpliría una función pro-apoptótica. De esta forma, esta molécula debería ser rápidamente captada por los astrocitos para bloquear su efecto tóxico. Financiado por Proyecto Anillo ACT-02

C6. Mathematical model of Ih-dependent oscillations in cold thermotransduction

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Mouse cold sensitive (CS) nerve endings show a rhythmic spiking activity at normal skin temperature that switches to bursting mode when the temperature is lowered. It was recently shown (Orio et al., JPhysiol 587:1961, 2009) that cultured CS neurons express a prominent hyperpolarization activated current (I_h), mainly carried by HCN1 channels, supporting subthreshold resonance in the soma but not participating in the response threshold to cold. However, HCN1(-/-) mice have a deficit in cold perception. To gain further insight into what the role of I_h in thermotransduction may be, a mathematical model of temperature-modulated bursting was developed and studied. A previous temperature-modulated slow wave bursting model (Braun et al., 1998) was modified to include I_h and its parameters tuned to reproduce the firing pattern observed in CS receptors from mouse cornea at different static temperatures. In this new model, I_h dampens the membrane potential oscillation that underlies rhythmic activity, avoiding pronounced hyperpolarization of the membrane potential. When I_h is blocked, the membrane takes longer to depolarize again and start a new oscillation cycle, thus reducing the mean firing rate. When I_h parameters are changed to resemble the behavior of HCN2 channels, the model shows a higher mean firing rate at normal temperatures but not when cooled, thus showing a reduced slope of the firing rate vs temperature relationship. This is a plausible explanation for the altered thermal sensitivity of HCN1(-/-) mice, in which functional expression of HCN2 was detected in CS trigeminal neurons. PBCT PSD 20 (Conicyt, to P.O.) DIPUV 51/2007 (U. de Valparaíso, to P.O.) 020943MMR (VRID-USACH, to R.M.) BFU2007-61855 (Spanish MICINN, to F.V.) BFU2008-04425 (Spanish MICINN, to C.B.)

C7. Synaptic clustering of PSD-95 is regulated by c-Abl through tyrosine phosphorylation

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The c-Abl tyrosine-kinase has been described to localize on synaptic compartments in adult mouse hippocampus and participate in post-synaptic assembly in the neuromuscular junction, where regulate the agrin-induced acetylcholine receptor clustering. These studies suggest a c-Abl role in synaptic structure and function in the central nervous system. The PSD-95 is an important post-synaptic scaffold protein that promotes synapse maturation but little is known about its regulation. We analyzed whether c-Abl is involved in the synaptic contact formation and clustering of PSD-95. We find that c-Abl levels in the rat hippocampus increase postnatally, with expression peaking at the first postnatal week. In 14DIV hippocampal cultures, c-Abl localizes primarily to the postsynaptic compartment, where it colocalizes with the postsynaptic scaffold protein PSD-95 in apposition to presynaptic markers. c-Abl associates with PSD-95 and chemical or genetic inhibition of c-Abl activity reduces PSD-95 tyrosine phosphorylation, leading to reduced PSD-95 clustering and reduced synapses in treated neurons. c-Abl can phosphorylate PSD-95 on tyrosine 533, and mutation of this residue reduces the PSD-95 clustering. Our results indicate that c-Abl regulates synapse formation by mediating tyrosine phosphorylation and clustering of PSD-95. FONDECYT 1080221/ FONDAP 13980001/ MECESUP-PUC0780

C8. Role of Dorsolateral Prefrontal Cortex in task type Wisconsin Card Sorting and Stroop Test. A study of Transcranial Magnetic Stimulation.

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La activación de la Corteza Prefrontal Dorsolateral derecha (CPFdl) en los procesos relacionados con la inhibición de la conducta ha sido ampliamente descrita tanto en sujetos normales como en ciertas condiciones patológicas. Sin embargo, existe controversia en el rol que esta estructura cumple durante estos procesos bajo distintos requerimientos cognitivos. En nuestro trabajo nosotros evaluamos la relevancia de la CPFdl en procesos relacionados con el control inhibitorio, utilizando las pruebas de Madrid Card Sorting Test y Stroop, en sujetos a los que se les aplicaba Estimulación Magnética Transcraneana (EMT) guiada por imágenes. Doce sujetos normales (3 hombres y 9 mujeres) fueron sometidos a una alteración transitoria y reversible de la actividad cortical en momentos específicos durante la ejecución de estas tareas cuando el control inhibitorio es requerido. El pulso magnético afectó el rendimiento conductual de los sujetos, disminuyendo sus tiempos de respuesta de manera selectiva durante el desarrollo de las pruebas entre las tres condiciones de estimulación, sin alterar el rendimiento en las pruebas control (MCST: EMTreal/placebo/SitioControl $p < 0.001$; Stroop25: Congruentes ($p = 0.03$); Incongruentes ($p = 0.01$) neutrales ($p = 0.001$). Nuestros resultados concuerdan con la visión de que la CPFdl es una estructura relevante en el control inhibitorio, y que la disminución de los tiempos de respuesta posterior a la alteración de la actividad de la CPFdl se relaciona con la facilitación de conductas impulsivas que tiene como eje central la perturbación del control inhibitorio. Fondecyt 1050721

III SESIONES COMUNICACIONES LIBRES

Jueves / Thursday 24 10:00-11:00

C9. In human spina bifida, ependymal denudation of Sylvius aqueduct is an ongoing process from pre to post-natal life

Guerra M (1), Rodríguez EM (1), Sival D(2), Jiménez AJ(3), Domínguez Pinos MD(3), den Dunnen WFA(2), Bátiz LF (1), Pérez-Fígares JM (2).

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Introduction: Foetal spina bifida aperta (SBA) is associated with cerebral pathology, such as hydrocephalus, heterotopias and cortical abnormalities. In human fetuses with SBA, abnormalities of the ventricular walls have been reported, such as loss of ventricular lining (neuroepithelial/ependymal denudation) in the telencephalon and the aqueduct. In order to investigate the process of neuroepithelial/ependymal denudation in the human aqueduct we have investigated the SA of five preterm SBA patients, collected at the University Medical Centre of Groningen, Holland. **Methods:** Classical histological stains and immunohistochemical staining for junction proteins (N-cadherin and neural cell adhesion molecule, NCAM), functional proteins (caveolin 1, Glut-1 and connexin 43) structural proteins (GFAP and β IVtubulin, a marker of cilia) and secretory proteins (S100) were performed in serial sections. **Results:** The areas of disruption of the ependymal lining were associated with (i) abnormal location in the ependyma of S100, connexin 43, N-cadherin and NCAM; (ii) formation of small and large rosettes in the subependymal region, which were reactive with anti- β IV-tubulin and anti-caveolin 1. Out of the five cases, the process of ependymal denudation was distinctly in progress in the 37, 39 and 40-week fetuses. **Conclusions:** (i) The process of ependymal denudation found in these human fetuses largely resembles that found in mutant animals (mice *hyh*) where the ependymal loss appears to be due to a failure in the cell junctions. (ii) Most likely this process would have continued after birth triggering aqueductal stenosis and hydrocephalus; (iii) in SBA patients the ependymal pathology (cell-junction pathology?) precedes hydrocephalus and may be (junction proteins?) genetically driven. Supported by Fondecyt 1070241 (EMR).

C10. Abnormal forms of SCO secretory proteins in the CSF of hydrocephalic patients

Muñoz RI(1), Sival D(4), Vío K(1), Jaramillo C(1), Ortega E(2), Jaque J(2), Guerra F(3), Sarnat H(5), Catañeyra-Perdomo A(6), Rodríguez EM(1)

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Introducción: El órgano subcomisural, juega un rol clave en procesos del desarrollo del sistema nervioso central. Evidencias obtenidas en modelos animales demostraron que alteraciones en las células del OSC y su secreción participan en la patogénesis de la hidrocefalia congénita. En la rata normal el OSC secreta proteínas que permanecen solubles en el LCR; se han observado cambios en estas proteínas en el LCR de ratas hidrocefálicas. **Objetivo:** Investigar si en el LCR de fetos/niños hidrocefálicos hay alteraciones en la proteínas secretadas por el OSC. **Métodos:** Muestras de LCR de fetos/niños hidrocefálicos se estudiaron mediante electroforesis en una y dos dimensiones e inmunoblott utilizando dos anticuerpos generados contra un extracto de OSC humano (anti-OSCh) y contra una secuencia peptídica de la principal proteína secretoria del OSC, la OSC-espondina (anti-P15). Los mismos anticuerpos se utilizaron para estudios inmunocitoquímicos del OSC humano fetal. En uno de los casos estudiados se realizó un estudio imagenológico y clínico en la etapa pre- y post-natal. **Resultados:** El estudio reveló: (1) Dos

compuestos OSC-reactivos de 220 y 180 kDa están sólo en el LCR de pacientes con hidrocefalia, incluyendo el caso clínico estudiado. El estudio inmunocitoquímico reveló que ambos anticuerpos inmunoreaccionan con células ependimarias del OSC y con una población de pinealocitos. Conclusiones: (1) El OSC humano secreta al LCR proteínas tipo SCO-espondina (primera evidencia). (2) Estas proteínas están alteradas en el LCR de fetos/niños hidrocefálicos. Financiamiento: Proyecto FONDECYT 1070241

C11. Effect of microglial activation on the transport dynamics of vitamin C.

Rodríguez E., Nualart F.

Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción. Los gliomas pueden ser activamente infiltrados por células microgliales, llegando a constituir hasta el 45% del tumor. Se desconoce si la activación microglial y la génesis de superóxido pueden inducir la oxidación de la vitamina C a ácido deshidro-ascórbico (DHA), sustrato preferentemente transportado por las células tumorales, aumentando así su capacidad antioxidativa y su resistencia a terapias citostáticas. Determinamos el efecto de la activación microglial sobre la producción de superóxido, el transporte de vitamina C y su acumulación intracelular. Utilizamos las líneas celulares de microglía N9, BV-2 y microglía primaria de rata. Analizamos la expresión de GLUTs y SVCTs mediante RT-PCR, Western blot, inmunocitoquímica y ensayos funcionales. Determinamos la producción de superóxido al activarlas con PMA, midiendo la reducción intracelular de NBT y la reducción extracelular de citocromo c. Los resultados muestran que la microglía expresa funcionalmente SVCT2, acumulando altos niveles de ácido ascórbico. También expresa GLUT1 y GLUT6, que le permiten captar niveles altos de DHA. Al activar la microglia con PMA, aumentan los niveles de oxidantes intracelulares y disminuye la vitamina C acumulada. Finalmente, la utilización de diferentes inhibidores permiten postular que la microglía activada oxida el ácido ascórbico intracelular a DHA, realizando eflujo a través de GLUTs. La génesis microglial de DHA, su eflujo y posterior incorporación a células tumorales podría incrementar la resistencia a terapias antitumorales. Financiado por proyecto Conicyt-PBCT Anillo ACT-2.

C12. Activation of a Wnt/ β -catenin pathway via Frizzled-9 inhibits acetylcholine receptor clustering in muscle cells.

Evelyn Avilés¹, Patricia Hanna¹, Daniel Sandoval¹, Giancarlo De Ferrari², Juan Pablo Henríquez¹

Research Ring for the Study of the Nervous System¹, Departments of Cell Biology¹, and of Biochemistry and Molecular Biology², Faculty of Biological Sciences, University of Concepcion, Concepcion, CHILE.

Wnt proteins play prominent roles in different aspects of neuronal development leading to the formation of complex neuronal circuits. In both, invertebrate and vertebrate organisms, Wnt signalling promotes and also inhibits the assembly of the neuromuscular junction (NMJ). At the vertebrate NMJ, previous findings suggest that motoneuron-derived Wnt3 acts through a noncanonical cascade to positively influence postsynaptic differentiation at areas of synaptic contact. Concomitantly, Wnt3a secreted from muscle cells activates a β -catenin pathway to negatively regulate postsynaptic differentiation at extrasynaptic domains. We have previously shown that overexpression of the Wnt receptor Frizzled-9 (Fz9) inhibited the ability of agrin, an organizer of postsynaptic differentiation, to cluster acetylcholine receptors (AChRs) on the surface of cultured myotubes. Here, we have analyzed the expression pattern of Fz9 during NMJ assembly, the signalling pathway activated by this receptor and the effect of silencing Fz9 on AChR clustering. Following RT-PCR, Western blot and immunohistochemical analyses, we show that Fz9 is dynamically expressed by muscle fibres during neuromuscular synaptogenesis. Interestingly, Fz9 localizes at synaptic and extrasynaptic domains of the mature NMJ. Overexpression of Fz9 induces the accumulation of cytosolic β -catenin in myotubes and the transcriptional activation of canonical Wnt signaling in the absence of exogenous Wnt ligands. In agreement with our previous findings, shRNA-mediated silencing of Fz9 stimulates agrin-dependent AChR clustering in myotubes. Altogether, our results suggest that muscle Wnt ligands activate a Fz9-dependent Wnt/ β -catenin pathway to inhibit AChR clustering at the vertebrate NMJ. Funded by Research Ring Grant ACT-02

IV SESIONES PANELES / POSTERS

P1. Electrophysiological study of the role of the insular cortex in the interoception process.

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The insular cortex (IC) is a structure from the anterior brain that receives and processes interoceptive signals. This cortex is thought to be monitoring the physiological parameters of the "milieu interne", having a key role in the behavior response to maintain the body homeostasis. We hypothesized that the electric activity of IC is correlated with the behavioral response of the rat to several interoceptive stimuli. We studied the electric activity pattern of the IC while inducing a visceral malaise state by the intraperitoneal injection of LiCl or hyperthermia. We also looked at the effect of microstimulation of this cortex on the behavior of the animal. For both extracellular recording and electric microstimulation of the IC we used a tetrode array in three rats. Preliminary results suggest that there is a diversity of firing patterns in response to LiCl injection or hyperthermia from the IC neurons. In addition, when we used the recording electrodes to microstimulate this cortex in the same animal, we found that this electrical stimulation produced the "lying on belly" behavior similar to that induced by LiCl injection. We conclude that electric activity of the IC is correlated with the behavioral response of the rat to these interoceptive stimuli. Financial support Fondecyt 1090101 and Iniciativa Milenio P06/008-F.

P2. Tuberalin II (p21), a prolactin releasing factor secreted by the pars tuberalis of the hypophysis?

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Introduction: The pars tuberalis (PT) of the hypophysis is formed by three cell types: cisternal cells, pars distalis-secretory cells (LH, FSH, TSH) and PT-specific cells. The latter display melatonin receptors and show ultrastructural changes associated to the light/dark cycle. There is some evidence that they secrete a prolactin releasing factor (PRF). In the laboratory, two putative tuberalins of 72 (tuberalin I) and 21 kDa (tuberalin II) have been identified and their presence in the blood of the hypothalamus adenohypophyseal portal system has been detected. Melatonin regulates the secretion of both proteins. The aim of the present investigation was to test whether some of the tuberalins is a PRF. Methods: The conditioned

medium of bovine PT-explants (CMPT) was analyzed by bidimensional electrophoresis (2D) and immunoblot with antibodies against tuberalins I and II. Further, PD explants were exposed to CMPT in the presence or absence of antibodies against tuberalin I and II. Prolactin concentration in the culture medium was analysed by ELISA. Results: (i) The CMPT increased the secretion of prolactin in a dose-dependent manner; (ii) The immunoadsorption of CMPT with anti-tuberalin II blocked the stimulatory effect, but its immunoadsorption with anti-tuberalin I or anti-Ig rat (control) did not; (iii) Bidimensional and immunoblot analysis of the CMPT with anti-tuberalin II showed 10 immunoreactive spots. Conclusions: (i) Tuberalin II has a PRF activity; (ii) Tuberalin II may be the hormone involved in the transduction of the photoperiod (melatonin) signal into the hypophysis, so an endocrine axis pineal-pars tuberalis-pars distalis is proposed. The purification of tuberalin II is in progress. Supported by DID-UACH S-2009/21 (MG) and Fondecyt 1070241 (EMR)

P3. Molecular interaction between type-2 CRH and dopamine D1 receptors in the rat ventral tegmental area of rat

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Molecular interaction of Adenosine A1 and A2A receptors fine-tunes glutamatergic neurotransmission in striatum (Rodrigues et al, 2005). Recently, it has been demonstrated a Corticotrophin Releasing Hormone (CRH)-mediated sensitization of glutamate release in the ventral tegmental area (VTA) of cocaine-experienced rats, due to the activation of VTA CRH receptor type-2 (CRH-R2) (Wang et al, 2007). The activation of Dopamine receptors type-1 (D1) also induces VTA glutamate release in cocaine treated rats (Duffy and Kalivas, 1998). The present study was undertaken to determine whether glutamate release sensitization in VTA of cocaine-experienced rats is due to a molecular interaction between CRH-R2 and D1 receptors. First, we co-transfected constructs of CRH-R2 and D1 receptors in HEK293 cells and used specific antibodies against both receptors for co-immunoprecipitation experiments. The results show the existence of molecular interaction between CRH-R2 and D1 receptors in HEK293 cells. Second, we performed double and triple immunofluorescence in rat VTA synaptosomes of control and cocaine-

treated rats. Antibodies against vesicular glutamate transporters type 1 and 2 (vGluT1 and vGluT2) were used to identify VTA glutamatergic terminals and antibodies against CRH-R2 and D1 receptors allowed studying their presynaptic localization. The results show that both CRH-R2 and D1 receptors co-localize in same vGluT2 containing VTA synaptosomes. Thus, these results give anatomical support for molecular interactions between CRH-R2 and D1 receptors co-localized in VTA glutamatergic afferences of non-cortical origin. It is tempting to suggest that the molecular interaction between CRH-R2 and D1 receptors underlie the sensitization of VTA glutamate release after cocaine administration. Funded by MSI grant N° 006M/008-F, FONDECYT 7080124 and FONDECYT.

P4. MDMA impairs visuo-spatial learning and memory in the Morris Water Maze and abolishes hippocampal LTP in rats without neurotoxic action.

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It is widely accepted that chronic 3,4-methylenedioxymethamphetamine (MDMA) treatment affects cognitive processes like learning and memory due to a neurotoxic action. We have studied the non-neurotoxic effects of MDMA on visuo-spatial learning and long term potentiation (LTP). Sprague-Dawley rats were injected with 0.2 and 2 mg/Kg MDMA twice a day s.c. and trained in the Morris Water Maze (MWM) during 8 days. After training, LTP was studied in hippocampal slices and contralateral hippocampus was used to test serotonergic neurotoxicity by HPLC. In visible platform experiments, treated rats shown a similar decrease in time necessary to reach the platform, (1st v.s 6th session. Control: 76±8s to 16±2s; MDMA 0.2mg/Kg: 71±8s to 24±2s. MDMA 2mg/Kg: 78±8s to 24±3s; n=16;p<0.01). Under invisible platform conditions, differences in the average times to reach the platform with MDMA 2mg/Kg were significant from 4th session (treated: 53±4s and 46±6s vs. control: 26±2s and 19±1s; n=16;p<0.01), suggesting that MDMA retarded the acquisition of the visuo-spatial task. When the platform was removed, a diminution in the average time of permanence in the tested quadrant was observed (Controls: 55±2s, MDMA 0.2mg/kg: 40±2s, and MDMA 2mg/kg: 37±2s;

n=16;p<0.01), suggesting that MDMA impaired recalling of learned position in MWM. MDMA abolished the induction of LTP in hippocampal slices (Controls: 147±6%; 0.2mg/Kg: 116±8% and 2mg/Kg MDMA: 105±8%; n=7,7;p<0.05). The hippocampus from treated rats and controls no showed differences significant in the 5HT levels (Controls: 344±95ng/g; 2mg/Kg MDMA: 389±78ng/g; n=7;p=0.36), suggesting that the observed effects of MDMA are not due to neurotoxicity. Support by: FONDECYT grant 1080652 (B.M), DICYT-USACH grant 020993Z (M.Z) and DICYT-USACH grant 020943MMR (R.M).

P5. Expression of scavenger receptors and phagocytosis of β -Amyloid by glial cells is modified in cells exposed to acidic conditions.

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Alzheimer's disease (AD) is the most common cause of dementia. Hallmarks of the disease, β -Amyloid (A β) plaques, intracellular neuritic tangles and vascular amyloidosis have been associated to oxidative damage and neurodegeneration. Hypoxia leads to the acidification of brain tissue and increased A β accumulation. Normal activated microglial cells and astrocytes can phagocytose A β . However, changes in pH secondary to hypoxia could lead to the impairment of A β clearance by glial cells and thus, enhance neurodegeneration.. Scavenger receptors (SR) are among the receptors mediating the uptake of fibrillar A β by microglia and astrocytes in vitro. The effect of pH on the expression of SR on microglia and astrocytes was evaluated in primary glial cultures by immunoblot. Glial function was evaluated by phagocytosis of A β . Results: The incubation on acidic medium (pH 6.9 or 6.5) for 24 - 48h led to an increased expression by microglia of SR-BI and SR-MARCO by 123±18% and 151±11% respectively. In astrocytes expression of SR-BI increased by 138±16%, whereas SR-MARCO decreased by 63.8±13%. Phagocytosis assays showed that A β uptake by microglia was maintained at the different pHs (pH 6.9 and 6.5), 79% and 71% (p>0,05) respectively over time. In contrast, phagocytosis of A β by astrocytes decreased over the same pH range 51% and 45% (p<0,001) respectively. Co-localization of A β with early endosomes was observed by immunofluorescence. Conclusion: Low pH did not reduce A β uptake by microglia. Whereas it reduced A β uptake by astrocytes. The observed changes in the expression of SR and phagocytosis suggest that A β clearance and perhaps also the activation of glial cells may be affected in acidosis, potentially favoring A β

deposition. Supported By: grant FONDECYT 1090353 to RvB.

P6. Localized activation of granular cells in the olfactory bulb by electrical stimulation.

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La neurogénesis en roedores adultos en la zona subventricular produce precursores neuronales que migran hacia bulbo olfatorio (BO) integrándose principalmente como interneuronas inhibitorias. Aún no se conoce si esta integración es localizada en la zona activada o distribuida en todo el BO. Para estudiar la distribución espacial de la integración de las nuevas neuronas hemos desarrollado un método para activar regionalmente el BO, utilizando estimulación eléctrica. Se emplearon 12 ratas adultas, de las cuales 3 fueron privadas unilateralmente el día postnatal 1 por cauterización de la narina izquierda. A las 6 semanas de vida se les implantó un electrodo bipolar localizado a 300, 500 o 800 μm de profundidad. Luego de un período de 4 días de acondicionamiento en la caja de estimulación, se estimularon mediante pulsos de corriente (1.5 mA, 100 μs) a 10 Hz durante 30 minutos, simulando la estimulación por odorante. Como control se utilizaron 2 ratas normales y una privada no estimuladas. Una hora después las ratas fueron sacrificadas y se determinó la expresión de C-fos. La densidad de células marcadas en BO de ratas normales y privadas estimuladas fue similar y significativamente mayor que la de BO de animales sin estimulación indicando que la estimulación eléctrica simula la estimulación producida por odorantes. Además, la reconstrucción tridimensional de la región marcada en las ratas estimuladas es similar y mayor en los BO de ratas normales y privadas estimuladas en comparación con los BO de ratas no estimuladas tanto normales como privadas. Financiado por Iniciativa Científica Milenio P04-068-F.

P7. The association synaptophysin / dynamin-1 defines the mode of exocytosis in chromaffin cells.

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Synaptophysin is an integral protein of synaptic vesicle membranes that has been shown to associate with dynamin-1 in a calcium-dependent manner and proposed to be necessary for a dynamin-dependent, but clathrin-independent mechanism of synaptic vesicle endocytosis. Such

mechanism appears to underlie a rapid mode of endocytosis described in chromaffin cells. This type of endocytosis seems to be the terminal step of a transient fusion mode of exocytosis, by which chromaffin vesicles partially release their content. To get additional insight into this mechanism of exocytosis, we studied how the interaction of synaptophysin with dynamin-1 defines the mode of exocytosis in chromaffin cells. Immunoprecipitation and pull-down assays revealed that the C-terminal of synaptophysin and the proline-rich domain (PRD) of dynamin-1 are involved in the association of these proteins. Furthermore, sucrose gradients revealed co-sedimentation of both proteins with chromaffin vesicles markers. Intracellular injection of antibodies against the PRD of dynamin-1 or the C-terminal of synaptophysin increased the duration and quantal size of individual exocytotic events induced by depolarization. It appears then that disruption of the function of these proteins induces a shift in the mode of exocytosis from transient fusion to full-collapse. Similar effects were observed when the recombinant C-terminal of synaptophysin, or a synthetic peptide that interrupted the association between these proteins, was injected. Together our results support the hypothesis that the interaction of synaptophysin with dynamin-1 is necessary for the transient fusion mode of exocytosis.

This work was supported by Fondecyt 1085266 and Proyecto Redes PBCT 24

P8. Temporal course of pentylentetrazole kindling in rats. Electroencephalographic analysis.

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La epilepsia es un síndrome neurológico que se caracteriza por hiperexcitabilidad neuronal que lleva a descargas neuronales espontáneas y recurrentes. La epilepsia del lóbulo temporal (ELT) es la epilepsia adquirida más frecuente, en ella el foco epiléptico se ubica en estructuras telencefálicas temporales debido a una reorganización permanente de sus circuitos neuronales. Para estudiar la ELT, utilizamos el kindling farmacológico con el antagonista GABAérgico pentilentetrazol (PTZ), cuya administración repetitiva a dosis subconvulsivantes es epileptogénica. OBJETIVO: Caracterizar los cambios electroencefalográficos y curso temporal en este modelo de ELT. METODOLOGÍA: Ratas macho se trataron

intraperitonealmente con 10 dosis de PTZ (20mg/kg) cada 2 días. El día 8, se implantaron electrodos corticales y musculares para realizar registros electroencefalográficos y electromiográficos a partir del día 20. Los registros fueron analizados en LabWindows/CVI 5.0 y los espectros en IGORPro 6.0. RESULTADOS: Al analizar el número de episodios epileptoides presentes en los días 20, 30 y 44 no se obtienen diferencias estadísticamente significativas ($1,5 \pm 5,7$; $3,1 \pm 3,5$; $4,6 \pm 7,9$ episodios/10 minutos, respectivamente, $n=3$). La duración de estos episodios tampoco muestra diferencias. Espectralmente, en el día 20 los registros se caracterizan principalmente por un peak prominente y difuso en frecuencias delta (0.1-4Hz), acompañados ocasionalmente de un peak difuso en la banda theta (4-10Hz). En el día 44, la banda theta es prominente con peaks discretos centrados en 6 y 8 Hz. PROYECCIONES: Caracterizar electroencefalográficamente el kindling, permitirá comprender la evolución de la epileptogénesis en la ELT y evaluar alternativas farmacológicas tendientes a inhibir este proceso. FINANCIAMIENTO: Proyecto Anillo ACT09-2006 (UW) Proyecto Anillo ACT09-2006 (UW) Beca Conicyt (VB)

P9. p73 Protein isoforms regulate cytoskeleton dynamics in hippocampal neurons

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p73 is a transcription factor and a structural and functional homolog of the p53. There are different p73 protein isoforms: (1) the full-length, or TAp73 isoforms that have an N-terminal domain (TA), participate in DNA-damage-induced cell-cycle arrest or apoptosis, and (2) short-form isoforms or Δ Np73, which lack the TA domain. The Δ Np73 proteins cannot induce apoptosis, but instead appear to block the function of full-length forms showing antiapoptotic properties. Δ Np73 isoforms are the predominant isoforms expressed postnatally in nervous system, and their expression prevents neuronal apoptosis during development and is required for the long-term survival of neurons. Members of the p53 family members determine neuronal survival versus neuronal death in the developing and mature nervous system, whereas the role of p73 in neuronal differentiation and morphology has not been studied. We are interested in analyzing the role of p73 isoforms in neuronal differentiation and cytoskeletal dynamics. We used hippocampal neurons primary cultures of control and p73 knock out mice. First we analyzed

the distribution of p73 isoforms (both TAp73 and Δ Np73) in control hippocampal neurons and found TAp73 isoforms are present both in soma and neuronal processes, and they are especially concentrated in the growth cones. While, Δ Np73 isoforms did not show a special distribution pattern. Then we analyzed (by immunofluorescence) the neuronal morphology of p73 knock out and control neurons following the cytoskeletal proteins; tubulin, MAP-2, actin and tau, and found an increase in the actin cytoskeleton remodeling. Our results indicate that p73 could be involved in the development of neuronal morphology. FONDECYT 1080221

P10. The propeptide CART enters to the regulated secretory pathway through a specific sorting domain

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CART (Cocaine- and amphetamine- regulated transcript) neuropeptide is an anorexigenic and anxiogenic peptide. The mechanisms by which CART peptide is sorted into the regulated secretory pathway are presently unknown. Recently, it has been shown that a missense Leu34Phe mutation in human proCART determines obesity phenotype (Yanik et al, Endocrinology 147:39-43, 2006). It was suggested that Leu34Phe mutation in proCART induced misrouting of the peptide to constitutive secretory pathway. The purpose of the present study is to identify putative proCART sorting domains towards the regulated secretory pathway. We designed a strategy based on chimeric forms of human proCART, fused to enhanced green fluorescent protein (EGFP) and expression in PC12 cells. The results show that a chimera bearing a fragment of the N-terminal region of proCART colocalizes with secretogranin II, marker of the regulated secretory vesicles. This colocalization was confirmed by quantitative Van Steensel analysis. This sorting domain in proCART includes Leu34. However, the proCART_EGFP chimera with the unique Leu34Phe mutation maintains the sorting to the regulated secretory pathway, in PC12 cells. Thus, the results show the existence of a sorting domain to the regulated secretory pathway in the N-terminal region of proCART. Funded by MSI grant N° P06/008-F and FONDECYT grant N° 107340.

P11. Fictive respiration is modulated by purinergic receptors in brainstem slices from mouse neonates

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ATP and related nucleotides acting on purinergic receptors can modulate the respiratory rhythm. In brainstem slices, respiratory rhythm generation depends on the activity of the preBötzinger Complex (preBötC) in the ventral respiratory group (VRG). In the present work we characterized ATP actions upon fictive respiration generated by the isolated brainstem slices. Fictive respiration was recorded in brainstem slices of 700 μ m width from CF1 mouse neonates. Suction electrodes were placed on the VRG and connected to a differential amplifier. The signal was integrated, displayed, and analyzed with AD acquisition system. Stimulation was done by changing the basal superfusion medium (artificial cerebrospinal fluid equilibrated with O₂:CO₂ = 95%: 5%, (pH 7.4) at 20 C.) by adding 0.01-1 mM ATP and/or Cu²⁺ (blocker) or Zinc (selective facilitator) of P2X₄. Superfusion with 2 mM ATP increased the respiratory frequency from 7.0 ± 1.0 bursts/minute to 21.4 ± 3.0 and decreased the amplitude of the integrated inspiratory burst by 30%. The increase in respiratory frequency was prevented with 100 μ M oATP or 30 μ M BBG, (P2Xs receptors blockers) and also with 10 μ M Cu²⁺. In the presence of Zinc the respiratory frequency increased indicating that the respiratory activity is modulated by P2X₄ receptors. The presence of P2X₄ in the preBötC has been described (Funk et al., 2008). We now show by western blot the presence of P2X₇ and corroborated by RT-PCR the presence of both receptors in the brainstem of neonates mice. Our results suggest that ATP affects the frequency of fictive respiration mainly acting on P2X₄ and P2X₇ receptors, both of them present in the brainstem at the same rostrocaudal level of the preBötC. Previous reports indicate that in rats the purinergic modulation is driven mainly by P2Y₁ receptors (Funk et al. 2007), the present results indicates that different P₂ receptors are involved in the purinergic modulation of fictive respiration in mice. Support Contributed By: FONDECYT # 1090375 and DICYT-USACH postdoctoral grant.

P12. CYTOARCHITECTONIC AND IMMUNOHISTOCHEMICAL ANALYSIS OF SUBNUCLEAR ORGANIZATION IN THE HUMAN HABENULAE

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The habenulae forms part of an evolutionary conserved dorsal diencephalic conduction system of the vertebrate brain involved in varied biological functions, including generation of sleep patterns, stress response, modulation of olfactory response and reproductive behaviour. Notwithstanding the fact that the habenulae has also been implicated in neuropathological conditions such as psychosis, schizophrenia and addictive behaviours, little is known of its morphological and neurochemical organisation in humans. In this study we performed a cytoarchitectonic and immunocytochemical analysis focused on elucidating the spatial organization of cell bodies and nerve fiber bundles within the human habenular region. Twenty diencephalic post-mortem male samples were serially cut and histologically processed with Nissl, Weigert, Kluver-Barrera and immunohistochemical techniques. We found that the human habenulae consists of a medial and a lateral subdivision, each containing 5 discernible subnuclei. The medial subdivision lies adjacent to the wall of the third ventricle and contains densely packed small sized cells. The lateral subdivision lies between the medial region and the thalamus and shows a more heterogenous cell population, with larger sized cells loosely distributed within the neuropile. The gross outline of these subnuclear regions was confirmed by neurofilament, GABA-rb1, calbindine and ChAT immunoexpression patterns. The two main divisions observed in the human habenula share similar morphological and cytoarchitectonic characteristic with those previously described in rodents. The establishment of these subnuclear regions in the human specie constitute a first step in the understanding of their biological functions. Funding: HHMI, CONICYT (PBCT ACT47, PBCT Red6), ICM P04-048-F, EU FP6-2004-NEST-PATH EDCBNL, FONDECYT 1020902.

P13. Influence of chronic administration of modafinil on rat visuospatial memory.

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Modafinil is known as an activator of the central nervous system (CNS). It is clinically prescribed for sleep disorders as hypersomnia and even for cognitive and attentional deficits. Clinical evidence reported favorable results of modafinil on processes

depending on the frontal cortex and cognitive control, which are related with short-term rather than long-term learning performance. Seven adult Sprague-Dawley rats were given 5.7 mg/kg/day i.p. of modafinil during 15 days, while other seven rats receiving saline served as controls. They were tested on an eight-arm radial maze according to the protocol of Bustos et al. (2003) that discriminates short-term (STM) and long-term memory (LTM). Errors and time-to-goal were counted during two experimental situations: 4 arms closed for a 5 minutes maximal period, and the 8 arms open totalizing 10 minutes in the maze. Results showed that modafinil reduced both the errors and time-to-goal, especially during the last sessions, indicating that chronic modafinil enhanced memory processing. The involvement of catecholaminergic and serotonergic systems in modulating the effects of modafinil on visuospatial performance is discussed. Funded by grant Fondecyt 1070028 and a grant from the School of Psychology, University of the Americas.

P14. Changes in acylpeptide hydrolase activity during ontogeny: possible implications in cognition

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Acylpeptide hydrolase (ACPH) is an enzyme belonging 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. It is believed that ACPH acts on bioactive peptides possibly controlling their concentration. At present, the physiological role of ACPH in neuronal tissue is unknown, however, it has been reported that ACPH can degrade the beta-amyloid peptide in vitro, and that the inhibition of ACPH by the organophosphate dichlorvos induces a significant increase of LTP at a specific window of dosage and exposure time through an indirect mechanism that involves the activation of postsynaptic $\alpha 7$ nicotinic receptors. Moreover, preliminary studies about the profile of ACPH activity in synaptic fractions suggest a presynaptic localization of the enzyme. However, little it is known about the activity of this enzyme during ontogeny and specifically its implications during aging. Therefore, our aim is to determine whether the function of ACPH is modified with aging. To achieve this we perform activity profiles of ACPH in male rats at different age stages (1, 3, 6, 8 and 12 months). At three months ACPH activity decreases in an 81%

compared with 1 month ($p < 0.01$), and remains constant until 8 months. At 12 months its activity decreases again in a 35% compared with 3 months. These results suggest that ACPH activity changes during ontogeny and a relation between ACPH activity, aging and changes in cognitive function during ontogeny must be studied. Support by: Programa Bicentenario en Ciencia y Tecnología PSD11 and DGIP from Universidad Católica del Norte

P15. TGF β 1 Activation of ERK MAP Kinase Signaling Pathway Is Associated To Recovery From Hypoxia-Induced Depression Of Synaptic Efficacy In Young And Aging Rats.

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Transforming growth factor $\beta 1$ (TGF β 1) is involved in many physiological and pathophysiological processes, including cell growth, differentiation, inflammation, and tissue repair. TGF β 1 is minimally present in the intact brain, but its expression increases strongly after trauma, excitotoxicity, and ischemia/hypoxia. We demonstrated that exposure of hippocampal slices from young rats to TGF β 1 in vitro modifies synaptic response to hypoxia, making their response similar to that observed in untreated aging rats. In aging rats no differences in synaptic responses to hypoxia in control and TGF β 1-treated hippocampal slices were found. Here, we studied TGF β 1 activation of Erk1/2 and Smad2/3 signaling pathways in slices in control and TGF β 1-treated hippocampal slices from young and aging rats. Hippocampal slices of young and aging rats were incubated for 12 hours with 1 ng/ml TGF β 1 and then homogenized in lysis buffer. Samples were separated by SDS/Page electrophoresis, blotted onto nitrocellulose membranes, and probed with antibodies against Erk 1/2, pErk 1/2, smad2/3, pSmad2/3 and β Tubuline I,II. We observed that TGF β 1 stimulation of hippocampal slices of young rats increased the expression of pErk1/2 compared to the controls. In contrast, no differences were found in the expression of pErk1/2 in control and hippocampal cultures of aging rats exposed to TGF β 1. Preliminary results indicate that changes in activation of Smad2/3 pathway is not related to changes in the synaptic response induced by hypoxia. In conclusion, these results suggest that Erk-MAP Kinase signaling could be related to TGF β 1-dependent changes of the synaptic efficacy

in response to hypoxia in hippocampal neurons of young rats. The lack of effect of TGF β 1 treatment on the response to hypoxia and expression of pErk1/2 in aging rats could reflect the high expression of endogenous TGF β 1 in aging rats. Supported by Fondecyt # 1040832 and 1090353

P16. BDNF/nitric oxide induced neuroprotection in cortical neurons following excitotoxicity.

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El hipocampo es una estructura vulnerable frente a insultos cerebrales como la excitotoxicidad (muerte neuronal por estimulación excesiva de receptores glutamatérgicos tipo NMDA), lo que está asociado con patologías neurodegenerativas como las epilepsias, caracterizadas por hiperactividad neuronal. La estimulación prolongada del receptor tipo NMDA, acoplado a la óxido nítrico sintasa neuronal (nNOS), lleva a la producción de óxido nítrico (NO) lo que induce muerte neuronal. Sin embargo, la corteza cerebral es resistente a tales insultos excitotóxicos. Postulamos que existen mecanismos adaptativos frente a insultos excitotóxicos en neuronas corticales pero no hipocámpales dependientes de factores neurotróficos protectores, como BDNF. Para poner a prueba esta hipótesis, medimos la sobrevida de neuronas corticales e hipocámpales en cultivo primario, estimulados por dos días con el antagonista Gabaérgico bicuculina 10 μ M para inducir una condición "epiléptica" (hiperactividad prolongada). Luego los cultivos fueron estimulados por 1 hora con NMDA 30 μ M y se midió la viabilidad neuronal por el test de exclusión de Azul de Tripán. Se aplicaron inhibidores de BDNF, NOS y receptores glutamatérgicos para evaluar su participación en el daño excitotóxico. Encontramos que las neuronas corticales son resistentes a la estimulación con NMDA, lo que depende de BDNF y NO. En cambio, la sobrevida de neuronas hipocámpales disminuye a un 30%, lo que está mediado por NO, sugiriendo un papel antagónico en cultivos corticales vs hipocámpales. Para medir la producción de NO, las neuronas se cargaron con el colorante fluorescente 4-amino-5-methylamino-20,70-difluorofluoresceína (DAF-FM). BDNF estimuló la producción de NO en neuronas corticales pero no hipocámpales mientras que NMDA indujo mayor síntesis de NO en neuronas hipocámpales que en corticales.

Este estudio muestra que BDNF tiene un papel neuroprotector frente a un insulto excitotóxico en neuronas corticales pero no hipocámpales a través

de NO, cuyo efecto biológico dependería de la vía de señalización implicada en su síntesis. Financiado por Anillo ACT09-2006 Proyecto Anillo ACT09-2006 (UW)

P17. IGF-I strongly stimulates proliferation in the caudal region of the rat hypothalamus.

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El hipocampo y la zona subventricular del estriado de los ventrículos laterales son consideradas las dos regiones neurogénicas por excelencia del SNC adulto de mamíferos. Existen otras áreas, como la región del tercer ventrículo, que aunque presentan células progenitoras con capacidad de división, tienen una tasa de proliferación bastante baja en condiciones basales. Tras la infusión intracerebroventricular de IGF-I durante 7 días y la administración intraperitoneal de BrdU en los 3 últimos días, se observa un aumento, mantenido en el tiempo, en el número de células BrdU positivas en toda la región periventricular y parenquimal del tercer ventrículo, siendo este aumento significativamente mayor en la región ventro-medial de la pared del tercer ventrículo. Estudios de doble marcaje con anticuerpos y microscopía electrónica de transmisión muestran la existencia de al menos dos tipos de células periventriculares proliferantes: astrocitos subependimarios y tanicitos. Nunca observamos células ependimarias multiciliadas positivas a los marcadores de proliferación empleados (BrdU y timidina tritiada). Algunos astrocitos subependimarios presentan prolongaciones apicales que contactan con el LCR. Estos contactos, así como el número de células proliferantes, son más abundantes en la zona de solapamiento, donde también se aprecian numerosos laberintos de membrana basal perivascular. Las neuronas son el tipo celular más abundante producido (54%). Además de astrocitos y tanicitos, también se observa producción de microglia, oligodendrocitos y células endoteliales en respuesta a IGF-I. Nuestros resultados nos llevan a concluir que la zona de solapamiento de la región hipotalámica se comporta como un nicho neurogénico sensible a factores como IGF-I. Financiado por el Ministerio de Educación y Ciencia (BFU06-11754) y el Proyecto de Excelencia de la Junta de Andalucía (P07-CVI-03079).

P18. MECANISMOS MOLECULARES DE LA INACTIVACIÓN DE CANALES DE CALCIO CAV2.3

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The Voltage-dependent ion channels undergo inactivation following activation. In the sodium and potassium channels, the molecular determinants that govern the mechanism of inactivation involve pore blocking by a cytoplasmic particle. In calcium channels, the consensus model is that the intracellular loop joining the first two homology domain of the pore forming subunit (loop I-II) is part of inactivation gate or hinged-lid. To investigated key features of this model, we expressed the neuronal calcium channel CaV2.3 in *Xenopus* oocytes and recorded ionic and gating currents using the cut-open voltage-clamp technique. Ionic current were recorded in either Ba²⁺ or Ca²⁺. Here we report two key features that are consistent with a hinge lead mechanisms. 1) During sustained depolarization, inactivation follows a single exponential time course without changes in the deactivation kinetic (tail current) and it is slowed if depolarization is interrupted repeatedly to force the channel to visit closed states indicating that it proceeds from the open-state or nearby closed states 2) It lead to 50% immobilization of gating-charges indicating that it prevent the channel gate from closing and 3) Recovery from inactivation is faster with large concentration of Ba²⁺ or with Ca²⁺, suggesting that when the pore contains a divalent ion, it repels the inactivation particle. Supported by ACT-46 and FONDECYT 1980635 to AN and a CONICYT Fellowship to GC

P19. Wnt-5a modulates functional GABAA-R expression in the neuronal surface

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Los receptores GABAA (GABAA-Rs) median la inhibición sináptica rápida y son el principal receptor inhibitorio en el sistema nervioso central (SNC), jugando un papel esencial en el control de la excitabilidad neuronal. Se ha determinado que la vía de señalización Wnt tiene un papel fundamental en la estructuración y función de las sinapsis del SNC adulto. Sin embargo, se desconoce el detalle de los mecanismos celulares y moleculares implicados.

Previamente, hemos demostrado que los ligandos Wnt pueden modular las sinapsis excitatorias, remodelando las regiones pre y postsinápticas. En el presente trabajo investigamos si esta vía de señalización, modula también la transmisión sináptica inhibitoria, enfocándonos en la regulación de la expresión de los GABAA-Rs en la superficie neuronal. Observamos que el ligando Wnt-5a provoca un aumento de la expresión y mantención de los GABAA-Rs en la membrana postsináptica, lo que induce un aumento de la amplitud de las corrientes inhibitorias postsinápticas (IPSC). Además, Wnt-5a induce un aumento en el reciclaje de los GABAA-Rs, sin afectar su endocitosis. Estos resultados indican que Wnt-5a modula la sinapsis inhibitoria regulando el reciclaje de los GABAA-Rs, dándole a la vía de señalización Wnt un papel central en la modulación de la sinapsis GABAérgica. Financiado por Centro CARE N°PFB12/2007, FONDAF N°13980001 y MIFAB (N.C.I), DIPUV N°46/2007 (M.F.), Beca Predoctoral CONICYT (L.C.)

P20. Chronic intermittent hypoxia increase Interleukin-1B expression in the rat carotid body.

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La exposición de animales a ciclos cortos de hipoxia intermitente crónica (CIH), similar al patrón de hipoxia-reoxigenación que presentan pacientes con apnea obstructiva del sueño (OSA) produce hipertensión. La CIH potencia las respuestas quimiosensoriales del cuerpo carotídeo (CC), lo que contribuye a aumentar el tono simpático. Estudios realizados en pacientes con OSA han reportado aumentos en los niveles plasmáticos de citoquinas pro-inflamatorias. Dado que la interleuquina-1B (IL-1B) se expresa en el CC y produce excitación quimiosensorial, es posible esperar que esté aumentada en el CC de ratas sometidas a CIH. En ratas sometidas a CIH (5-6% O₂, 12 veces/hora por 8 horas) durante 21 días, registramos la frecuencia de descarga quimiosensorial en el nervio carotídeo en respuesta a varios niveles de oxígeno (PO₂ 30-640 Torr por 15-60 s) y estudiamos la expresión de IL-1B en el CC mediante técnicas inmunohistoquímicas. La exposición de ratas a la CIH, aumentó la descarga quimiosensorial carotídea en normoxia, y potenció las respuestas quimiosensoriales a la hipoxia aguda (p < 0.05, Anova 2 vías). Además, la CIH aumentó la expresión de IL-1B en los CC de ratas expuestas a CIH. El aumento de inmunorreactividad de IL-1B se correlacionó con el aumento de la respuesta quimiosensorial a la hipoxia aguda en los animales

sometidos a CIH. Los resultados sugieren participación de IL-1B en la potenciación de los quimiorreceptores del CC, lo que podría contribuir al desarrollo de la hipertensión en animales expuestos a CIH. FONDECYT 1070584

P21. Copper modulates neuronal excitability in stratum lacunosum moleculare of rat hippocampus

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Copper has been detected differentially in certain regions of the hippocampus like the stratum lacunosum moleculare (slm). A proposed role for copper is that of an endogenous modulator of excitability. If copper is endogenously released in the slm, activity on this layer would be sensitive to exogenously added copper or to the presence of chelators. Synaptic transmission and spiking activity was recorded in this layer in response to different stimulating protocols. After recording in control conditions copper (100 nM or 4-30 μ M) was added. The gabaergic inhibitor Picrotoxin (PTX) was employed for the experiments with 100 nM copper. The chelator bathocuproine sulfonate (BCS) and the reducing agent DTT were employed in attempt to reverse the copper effects. Copper 4 and 30 μ M increased spike frequency and copper 100 nM decreased it, in the presence of PTX the effect of 100 nM copper was highly dependent of the initial activity. Copper 30 μ M decreased synaptic transmission; the effect was reversed by DTT but not BCS. When a stimulating pulse of 10 Hz was applied, BCS markedly decreased the spike frequency with no effect on synaptic transmission. Our results allow us to propose: 1) copper is probably endogenously released at slm, 2) there are neuronal clusters with different copper sensitivities and 3) some of the effects of exogenous copper can be explained by redox modulation. Because the output of the hippocampus is affected by the activity at slm, it is important to understand the modulator role of endogenous copper. Fondecyt 1080670

P22. Age dependent localization of copper in rat hippocampus.

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Cobre es un elemento esencial en el desarrollo y funcionamiento del sistema nervioso y se lo ha propuesto como agente neuromodulador. Una de las estructuras que presenta concentraciones considerables de cobre es el hipocampo; sin

embargo no existen estudios detallados de su localización dentro de éste. Por otra parte, nuestros estudios electrofisiológicos muestran un posible rol modulador endógeno en sub-regiones del hipocampo. Una técnica utilizada en la detección de metales es la Tinción de Timm, esta técnica permite el análisis semi-cuantitativo de imágenes. Para diferenciar de modo específico el cobre presente, se agrega un paso extra al protocolo habitual, lavando las rebanadas con ácido, lo que elimina hierro y zinc del tejido antes de la tinción, permitiendo una marca exclusiva para cobre. Encontramos cobre en el soma de las células piramidales del cornu ammonis (CA), en las células granulares del giro dentado y en el stratum lacunosum moleculare. Las estimaciones que permite la técnica, indican distribución sectorizada y cambios de esta distribución dependientes de la edad entre 17 y 60 días. Financiamiento: Fondecyt 1080670.

P23. Nicotinic receptors of amacrine cells in the rat retina

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In the mammalian retina, acetylcholine, released from "starburst" amacrine cells, play important roles during development and adulthood. Immunohistochemical studies have suggested that several classes of retinal cells have nicotinic receptors, but only in ganglion cells its existence has been electrophysiologically demonstrated. The purpose of this work is to determine the presence of functional nicotinic receptors on morphological and physiologically classified amacrine cells of the adult rat retina. Using whole cell Patch Clamp in retina slices, we have found that in a heterogeneous population of amacrine cells (n=78/126), nicotinic agonists evoke inward currents insensitive to synaptic blockade (cobalt 1 mM, n=18). These inward currents are mainly blocked by the nicotinic antagonists mecamylamine (2 μ M, n=43) and erysodine (20 μ M, n=28), but are insensitive to the α 7 antagonist methyllycaconitine (20 nM, n=22), suggesting the presence of heteromeric nicotinic receptors in amacrine cells. We have also seen that nicotinic agonists can induce inhibitory postsynaptic currents in bipolar (n=9), ganglion (n=10) and amacrine cells (n=42), and are therefore capable of modulate intercellular communication at the inner plexiform layer. In despite of this, the response of amacrine cells to a single flash of light is unaffected by nicotinic blockers (n=68), suggesting that nicotinic receptors of amacrine cells could be involved in more complex functions of retina signal processing, like direction selectivity, which is known to depend in

a cholinergic circuit. Proyecto anillo ACT-45; Financiamiento de apoyo a la realización de tesis doctoral, Conicyt

P24. NEUROGENESIS IN THE DENTATE GYRUS AND SUBVENTRICULAR ZONE AFTER ASPHYXIA IS MODULATED BY FGF-2 AND MAPK/ERK PATHWAY

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Adult neurogenesis is considered an active process occurring throughout life in specific neurogenic regions, including the dentate gyrus (DG) of the hippocampus and the subventricular zone (SVZ). Previous studies have shown that perinatal asphyxia induces delayed neurogenesis, an increase of FGF-2 levels and activation of the MAPK/ERK pathway in the hippocampus of the rat. In the present study, we investigated the role of FGF-2 and MAPK/ERK pathway in neurogenesis observed in the DG and SVZ cultures. Organotypic cultures were obtained from control (basal condition) and asphyxia-exposed rats at P 2-3. At DIV 15, the cultures were treated with the cell proliferation marker 5-bromo-2'deoxyuridine (BrdU, 10 μ M) and saline or exogenous FGF-2 (50 nM) or PD173074 (a specific FGFR1-inhibitor, 100 nM) or U0126 (an ERK-pathway inhibitor, 10 μ M). After 48 h, cultures were fixed with formalin solution for assaying cell proliferation and neuronal phenotype with antibodies against BrdU and microtubule associated protein-2 (MAP-2) respectively. Cell counting using an epi-fluorescence inverted microscope and confocal microscopy showed that FGF-2 increased neuronal proliferation compared with saline in cultures from both control and asphyxia-exposed rats. Treatments with FGFR1 and ERK inhibitors prevented this effect under both conditions. These results suggest that FGF-2 modulates neurogenesis in the DG and SVZ cultures from control and asphyxia-exposed rats through MAPK/ERK pathway activation. FONDECYT 11070192, 1080447, 1095021, CONICYT/DAAD.

P25. The inhibition of lateral septum nucleus increase histamine in freely moving rats

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The lateral septum (LS) evaluates the affective relevance of incoming sensory input from the hippocampus and conveys this information on to hypothalamic areas that control the expression of goal-directed behaviors important to survival. The tuberomammillary nucleus (TMN) of hypothalamus,

the only source of brain histamine, is one of the principal target of the LS, and is implicated in reward and motivation. The histamine is important in arousal and alertness, the presentation of tasty food triggers a seeking behavior and activates histaminergic neurons. We have proposed that LS could modify the activity of histaminergic neurons, modifying histamine release through GABAergic inputs. The reduction in GABAergic input to the TMN should increase vigilance. We made polysomnographic recording to correlate the increase in histamine levels with waking and using microdialysis we measured extracellular histamine and GABA levels in the LS and ventral hypothalamus while modifying LS activity using muscimol (3.5 μ M). We found that muscimol into LS increased histamine levels in LS, increased the wake state and searching behavior. Furthermore, in these rats we found a low activity in LS neurons measured with c-fos, indicating that muscimol indeed decreased LS neuronal activity. The LS controls mood and motivation, TMN is important in motivation too, therefore we propose that the LS controls TMN activity and wake state to maintain arousal at levels appropriate for the current behavior. Supported by Fondecyt grant # 1060476 and Millennium nucleus grant P06/008-F.

P26. How amphetamine derivatives bind to the serotonin transporter. A molecular simulation study

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Serotonin transporter (SERT) is the target of several psychoactive drugs including antidepressant, entactogenic and anorectic drugs. Amphetamine derivatives such as 3,4-methylenedioxymethamphetamine (MDMA) or 4-methylthioamphetamine (MTA) are potent serotonin releasing agents and it is widely accepted that this effect is initially mediated through a direct interaction of the drugs with SERT. Nevertheless, this interaction remains poorly understood at a molecular level. Then, structural information about SERT interactions with amphetamine derivatives is relevant for the understanding of the mechanism of action of these compounds and also for future drug design. In the present work, the binding mode of three amphetamine derivatives MDMA, MTA and naphthylisopropylamine (PAL-287), the later being

an amphetamine derivative in which the benzene ring has been replaced by a naphthyl moiety, was studied using a molecular simulation approach. For this purpose, docking experiments were carried out on a homology model of SERT generated using the LeuTAA crystal structure as template. Analysis of the binding poses suggests that all compounds bind in the same region as the endogenous ligand serotonin, but elicit different interactions with specific amino acid residues of the transporter. These results give preliminary insights about the structural basis associated to substrate uptake by SERT. FONDECYT grants 1090037, 1085051, 11085002; PBCT PDA-23; ICM P05-001-F, DICYT-USACH.

P27. The beta 1 adrenoceptor agonist dobutamine improves long-term synaptic potentiation in the frontal cortex of normal and malnourished rats

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There is evidence suggesting that beta adrenoceptors are involved in neuroplastic processes in the central nervous system, including long-term potentiation (LTP). Non-selective beta adrenergic antagonists are known to inhibit LTP in hippocampus and cortex slices, ex vivo, in a model of hidden prenatal protein malnutrition. In contrast, unspecific beta adrenergic agonists increase LTP in normal rats. In this work, the effects of dobutamine (1.5 mg/kg i.p.), a specific beta 1 adrenergic agonist, over LTP evoked in vivo by tetanizing shocks in the frontal cortex of rats were studied. Control rats were fed with standard chow diet (casein 25%) ad-libitum and compared with malnourished rats (casein 8%). Dobutamine improved LTP by 50% in control rats and by 80% in malnourished rats. Malnourished rats did not evoke LTP in the absence of dobutamine, suggesting that beta 1 adrenoceptors may not become sufficiently activated in the model of hidden prenatal protein malnutrition. The results suggest that activation of beta 1 adrenoceptors is involved in neuroplastic processes at frontal cortex and that they are required to improve LTP in normal and malnourished conditions.

Fondecyt 1070028

P28. EFFECT OF REPEATED ADMINISTRATION OF AMPHETAMINE ON DOPAMINE RELEASE IN THE MESOCORTICOLIMBIC PATHWAY

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Addiction to drugs of abuse is a pathology with a strong compulsive component concerning to drug seeking and a loss of behavioral flexibility. The mesocorticolimbic system is a dopaminergic pathway implicated in the development of the addictive behaviour. The animal model of locomotor sensitization after repeated administration of amphetamine is a behavioral paradigm that resembles the pathological process of addiction. However, the repeated administration of amphetamine is not enough to develop locomotor sensitization, and there is a wide between-subjects variation in the behavioral response to this treatment. We hypothesize that a differential modification of the mesocorticolimbic dopaminergic pathway underlie this complex locomotor behaviour observed in rats after repeated amphetamine administration. To test our hypothesis, rats were injected once daily with amphetamine (1.5 mg/Kg ip) or saline for 5 days. The expression of sensitization was carried out 2 days later. Microdialysis experiments assessing extracellular dopamine levels in the medial Pre-Frontal Cortex (mPFC) were carried out one day after expression of sensitization (Basal levels: 0.73 +/- 0.14 fm/uL; K+ 70 mM: 5.83 fm/uL). Preliminary results show a decrease in DA extracellular levels in the mPFC of amphetamine-sensitized rats after expression of sensitization. Proyecto Fondecyt de Inicio N° 11075068 Núcleo Milenio N P06/008-F

P29. Neurodegeneration in Niemann-Pick type C Disease.

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Niemann-Pick type C (NPC) disease is a fatal autosomal recessive disorder characterized by the accumulation of free cholesterol and glycosphingolipids in the endosomal-lysosomal system. NPC patients suffer a markedly progressive neuronal loss, mainly of cerebellar Purkinje neurons. Our previous results show that the pro-apoptotic c-Abl/p73 system is activated in the cerebellum of

NPC mice and colocalize with active caspase-3 in Purkinje cells ongoing cellular death. The purpose of this study was to analyze the relevance of the c-Abl/p73 signalling and its link with the neurodegeneration in other cerebral nuclei. The brains of NPC mice showed significantly higher number of c-Abl and p73 positive neurons than the brains of wild type mice. The increase in the c-Abl and p73 positive neurons was observed in inferior olive and supramammillary nucleus. c-Abl/p73 signal co-localized with caspase-3 active. Our results support the contribution of c-Abl/p73 signalling in the neurodegeneration of other brain structures, besides the cerebellum, that could be related with some characteristic symptoms of NPC disease. However, we also observed brain nuclei that show a progressive neuronal loss, without an increase of the c-Abl/p73 signalling, suggesting that there are other apoptotic pathways involved in the NPC pathology. Our results show that the c-Abl/p73 signalling module is involved in the neuronal loss in NPC brain suggesting that a possible treatment with c-Abl inhibitors can be useful in delaying progressive neurodegeneration, nonetheless they also indicate that there are other apoptotic pathways involved in this disease to further study. This work is financially supported by The Ara Parseghian Medical Research Foundation for the NPC study and Fondecyt Grants # 1070622 and #1080221.

P30. RGS mRNA LEVELS ARE MODULATED BY UNILATERAL 6-OHDA-LESION OF ASCENDING MEDIAL FOREBRAIN BUNDLE IN RAT BRAIN.

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Several neuropsychiatric disorders, such as Parkinson's disease, schizophrenia, and drug addiction (psychostimulants and opioids) share a common dopaminergic pathway in the brain. Molecular changes in dopamine-innervating regions can lead to behavioural dysfunctions. One plausible target for the dysfunction is the Regulators of G Protein Signalling (RGS) family, which modulates the G protein signalling associated to D1 and D2 dopamine receptors in relevant dopaminergic pathways, which have important roles for cognitive, motor and rewarding functions. This work investigated the modulation of the expression of some relevant members of the RGS family, such as

RGS2, RGS4 and RGS9-2, in de-afferented dopamine regions following a unilateral 6-OHDA lesion. 6-OHDA (4µl of 2 µg/µl solution) was stereotactically injected into the left ascending medial forebrain bundle in rat brain when rats had body weight of ~150g. The lesion was functionally evaluated by apomorphine (0.05 mg/kg, s.c.)-induced rotational behaviour, 10 days after the lesion. Rats turning >10 contralateral turns/min were selected for further experiments. Seven days after the apomorphine evaluation, the rats were killed for sampling brain tissue (left and right neostriatum, nucleus accumbens, substantia nigra and ventral tegmental area), and processed for RGS2, RGS4 and RGS9-2 mRNA evaluation using RT-PCR. The 6-OHDA lesion produced significant changes in the expression of RGS2, RGS4 and RGS9-2 mRNA in the de-afferented compared to the non de-afferented hemisphere. The changes observed in RGS mRNA following a unilateral 6-OHDA lesion suggest neuroadaptive mechanisms compensating for the damage produced by the lesion, for stabilising and/or recovery of DA homeostasis. This work is funded by FONDECYT (No 1080447, 11070192, 1095021). Manuel A. Gutiérrez-Hernández is supported by a PhD fellowship granted by CONICYT-Chile (No 21080079). We are grateful for the excellent assistance of Mr J. Santibañez, MS C. Almeyda and MS AM Mendez.

P31. Expresión of Protein Kinase M z in Addiction

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The drug addiction is a chronic disease mainly characterized by the drug compulsive seeking beyond the individual control. All abuse drugs activate the mesocorticolimbic circuitry, form by dopaminergic neurons of the ventral tegmental area (VTA) which projects to Nucleus Accumbens and Prefrontal cortex among others. The repeated activation of this circuitry modified the synaptic plasticity in these areas. In particular, the plasticity in the VTA is required for the behavioral changes induced by addictive drugs. It has been suggested that there is a common molecular and cellular mechanism among the different forms of plasticity. It has been proved that an PKC isoform, PKMz, is both, necessary and sufficient for Long Term Potentiation (a form of plasticity) and also, its activity is required for maintenance of long term associative memories. However, until date, its role in drug induced plasticity has not been shown. The main

goal of this work is to determinate the role of PKMz in the drug-induced plasticity in VTA. To do that, we repeatedly treated rats with amphetamine (1.5 mg/kg i.p) we evaluated changes in PKMz mRNA levels in VTA. We found an increase in the PKMz mRNA levels in presence of amphetamine. This result suggests the participation of PKMz in the amphetamine sensitization. Next, we will inhibit the activity of PKMz directly in VTA to test the participation of this kinase in the drug-induced associative memory. With this project we are trying to add understanding to the function of PKMz and also its possible role in addiction. MSI N° P06/008-F and FONDECYT 1070340

P32. Repetitive fluoxetine treatment induces changes in astrocyte metabolism and morphology in the rat telencephalon

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Los fármacos antidepresivos (como la fluoxetina, flx) deben ser administrados por tiempos prolongados para lograr efectos terapéuticos, lo que se debería a la inducción de cambios plásticos en el Sistema Nervioso Central. En el laboratorio de Neurociencias hemos estudiado los cambios plásticos inducidos por flx en las sinapsis glutamatérgicas del telencéfalo de ratas. La naturaleza de estos cambios es desconocida, y para evitar sesgos realizamos un estudio proteómico de fracciones subcelulares enriquecidas en membranas intracelulares obtenidas de animales tratados por 2 y 4 semanas con flx. Encontramos 12 proteínas que cambian significativamente después del tratamiento. De ellas, el 25% está involucradas en la transducción de señales, 16,5% corresponde a enzimas que participan en el plegamiento de proteínas y un 33% corresponde a enzimas de la vía glicolítica, sugiriendo que flx regula el metabolismo celular. Otras proteínas encontradas participan en la fusión de membranas o forman parte del citoesqueleto. Entre las enzimas glicolíticas destaca la aldolasa C, una proteína presente exclusivamente en astrocitos en el telencéfalo, cuyos niveles cambian 700% en la fracción analizada. Para validar este resultado, se detectó aldolasa C por western blot e inmunohistoquímica. Encontramos que aldolasa C y GFAP aumentan en $108 \pm 0,5\%$ y $80 \pm 0,7$, respectivamente. Por IHQ pudimos comprobar que aldolasa C se expresa sólo en astrocitos, cuyo número aumenta en la región CA1 del hipocampo, observándose cambios morfológicos que aún están en estudio. Nuestros resultados indican, por primera vez, que los cambios plásticos inducidos por flx incluyen regulación de astrocitos.

El significado terapéutico de este fenómeno debe ser estudiado en el futuro. Anillo ACT09-2006(para UW) Conicyt (para MS)

P33. Variable threshold of cold-sensitive neurons is determined by a balance between TRPM8 and Kv1 potassium channels.

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Cold thermoreceptor fibers show variable sensitivities to temperature. However, the molecular determinants of their threshold differences and the contribution of high threshold thermoreceptors to noxious cold detection were unknown. Recently we have shown that such differences correlate with the relative expression of IKD, a current dependent on Shaker-like Kv1 channels that acts as an excitability brake, and ITRPM8, a cold-activated excitatory current. Neurons responding to small temperature changes have high functional expression of TRPM8 and low expression of IKD. In contrast, neurons activated by low temperatures that presumably cause sensations of unpleasant cold or pain have a lower expression of TRPM8 and a prominent IKD. Otherwise, both subpopulations have nearly identical membrane and firing properties, suggesting that they belong to the same neuronal pool. Blockade of IKD shifts the threshold of cold-sensitive neurons to higher temperatures and augments cold-evoked nocifensive responses in mice. Similar behavioural effects of IKD blockade were observed in TRPA1(-/-) mice. Moreover, only a small percentage of trigeminal cold-sensitive neurons were activated by TRPA1 agonists, suggesting that TRPA1 does not play a major role in the detection of low temperatures by uninjured somatic cold-specific thermosensory neurons under physiological conditions. Our findings suggest that innocuous cooling sensations and cold discomfort are signalled by specific low- and high-threshold cold thermoreceptor neurons, differing primarily in their relative expression of two ion channels having antagonistic effects on neuronal excitability.

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P34. Hierarchy of the Interoceptive Insular Cortices

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We suggested that the interoceptive system is organized in a way similar to exteroceptive sensory systems, so that the insular cortex is at the top of the hierarchy, much like the occipital cortex is the top structure of the visual system. The present work established a hierarchical organization of the different insular cortices using a detailed comparison with the hierarchy of the visual cortices, using connectional criteria like the connections of insular cortices with the the Reticular Thalamic Nucleus (TRN) and the pattern of cortico-cortical connections, and citoarchitectonic criteria. The boundaries of the interoceptive insular cortex have not been described in detail and the criteria for distinguishing between primary and secondary interoceptive insular cortex have not been fully implemented. The aim of this study was to use the criteria of the visual system to determine the hierarchy and the anatomical boundaries of the interoceptive cortex. The methods used were anterograde (BDA) and retrograde (CTB) axonal tracers injected in different insular cortices using the stereotaxic coordinates of Swanson's atlas. The results indicate that the thalamic relay nucleus of the anterior insula corresponds to parvocellular Ventroposteromedial while for the posterior region receives from the parvocellular Ventroposterolateral thalamic nucleus. The TRN presented in connection with its inner tier above the anterior interoceptive insular cortex and with the posterior region and later in the boundaries between the two areas provides an intermediate disposition. The projections from the posterior to the anterior insula involves mainly terminals in layer 4 in the other direction extends from Layer 1, 2 / 3, 5, 6 and very mildly to layer 4.. Conclusions: insular cortex like the visual system, presents a hierarchical pattern where the posterior insula corresponds to the primary, and the anterior insula to the secondary interoceptive cortex. Financed by Núcleo Milenio P06/008-F.

P35. Analysis of feeding behavior in animals after glucokinase glial knockdown

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Se ha demostrado que la enzima glucoquinasa (GK) es fundamental para el mecanismo sensor de

glucosa, en células β -pancreáticas. Recientemente, hemos demostrado que esta enzima se localiza en el hipotálamo específicamente en la glia periventricular (tanicitos). Estas células se encuentran en estrecha relación con neuronas del hipotálamo ventromedial, denominado centro de la saciedad. De esta forma, inhibimos in vivo la expresión de glucoquinasa en la glia periventricular para evaluar cambios en el comportamiento alimenticio. Se utilizó un adenovirus-GFP (Ad-GFP) para estandarizar la inyección estereotáxica cerebral y determinar la capacidad del virus para infectar la glia periventricular en ratas, reconocida usando anti-vimentina. Posteriormente se inyectó el Ad-GK-siRNA para inhibir la expresión de GK. Se midió la ingesta alimenticia durante los primeros tres días y los siguientes se evaluaron respuestas de saciedad en ciclos de ayuno (24h) y alimentación. Además, se evaluó la expresión de GK mediante qRT-PCR e inmunohistoquímica. La co-localización con vimentina demostró que los tanicitos α y $\beta 1$ fueron rápidamente infectados (12h) con Ad-GFP. La mayor expresión de GFP se logró después de 24h. La infección con Ad-GK-siRNA produjo una disminución de la expresión de GK. Cuando se realizaron los ciclos de ayuno y alimentación, se observó que los animales inyectados con Ad-GK-siRNA presentaron un retardo marcado en la saciedad que es restablecido después de 2 ciclos. El mecanismo sensor de glucosa cerebral, mediado por la expresión de GK en los tanicitos, puede estar asociada a la regulación de la saciedad. Proyecto Anillo ACT-02.

P36. Psychophysics of visual perceptual learning using Kanji characters

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El aprendizaje perceptual (AP) es la mejora del desempeño en una tarea perceptual, seguido a la práctica, y es una manifestación de plasticidad del cerebro adulto. El AP ha sido estudiado en diversos sistemas sensoriales. En el visual, se han descrito los cambios que producen diferentes atributos físicos de estímulos simples. En situaciones naturales el AP involucra estímulos más complejos y con relevancia ecológica. Por esto, hemos desarrollado una tarea de discriminación "igual/diferente" de 30 caracteres Kanji de 17 y 18 trazos que resulta en aprendizaje perceptual. Se entrenó a 12 sujetos por 2 semanas (9 días) y se midió el número de respuestas correctas como también el tiempo de reacción. En el día 10 se estimuló con 15 caracteres conocidos y 15

caracteres nuevos. A pesar de que los sujetos no habían sido enfrentados a los estímulos nuevos, su desempeño en la tarea fue aproximadamente 70% del máximo logrado con los caracteres conocidos, lo que refleja en parte, AP. Como control se entrenó a 8 sujetos con otros estímulos (tablero de ajedrez) con un similar grado de dificultad. En la sesión 10 fueron evaluados con caracteres de la categoría Kanji. Con este análisis, se separaron los diferentes componentes del aprendizaje: AP, procedural (e.g. memoria de trabajo, rotación mental y aprendizaje motor) y de memoria explícita. Conicyt, Milenio P04068F.

P37. Short term-high fat but not regular diet feeding in young adult mice determines dietary preference for high fat diet.

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Obesity is currently a pandemic worldwide due at least in part to consumption of highly caloric diets. Several studies have shown that chronic high fat diet consumption may lead to obesity and related disorders clustered as the metabolic syndrome. In this work we explored the effects of short-term consumption of a high fat diet on dietary preferences. Five weeks-old ICR male mice were subjected to either high fat (60% kcal in fat) or low fat diet (10% kcal in fat) ad libitum during 18 days. No differences either in energy intake, body weight, blood glucose levels or anxiety-like behavior were observed between groups. Thereafter, a diet preference test was performed during five days. Mice formerly subjected to low fat diet showed a subtle preference for high fat diet (53% in energy intake) if any, whereas mice formerly subjected to high fat diet strongly preferred it (75% in energy intake) over low fat diet. This observation suggests that short-term exposure to high fat diet in young adult mice is enough to determine dietary preferences and it happens before any metabolic disturbances occur. The results –if extrapolated to humans– might be relevant in order to prevent further alterations leading to obesity and metabolic syndrome. The physiologic mechanisms involved in high fat diet-induced dietary preference are unknown up to our knowledge. MHIRT-FOGARTY to AB FONDECYT #1090230 to CM

P38. Glutamate stimulates vitamin C recycling in the astrocyte-neuron interaction

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La vitamina C es reciclada en el cerebro debido a una interacción metabólica entre la glia y la neurona. El astrocito captaría la vitamina C oxidada o dehidroascórbico (DHA) a través del transportador GLUT1 y la reduciría intracelularmente para generar ácido ascórbico (AA), el cual sería liberado al espacio extracelular. El AA sería captado por las neuronas para aumentar su defensa antioxidativa. En este contexto, glutamato genera un micro ambiente oxidante que aumenta los niveles de DHA extracelular, sin embargo se desconoce cuál es el efecto de glutamato en el reciclaje de la vitamina C a nivel de neuronas y astrocitos. En este trabajo analizamos el efecto de glutamato en la captación y reducción intracelular de DHA, utilizando como modelo astrocitos mantenidos en cultivo por 15 y 30 días y además, cultivos primarios de neuronas. Se utilizaron técnicas inmunocitoquímicas, RT-PCR y Western blot, para confirmar la expresión de GLUT1 y GLUT3 (transportadores de DHA) en astrocitos y neuronas en cultivo. Se analizó mediante estudios de transporte la incorporación de DHA en ambos tipos celulares. Determinamos por medio de HPLC que glutamato estimula la reducción intracelular de DHA en forma diferencial en astrocitos GLUT1+ de 15 y 30 DIV. Paralelamente, observamos que las neuronas GLUT1 y GLUT3+ presentan una menor capacidad reductora, la cual aumenta sólo en presencia de altas concentraciones de glutamato. En este estudio determinamos que glutamato está directamente involucrado en el reciclamiento de vitamina C en el SNC, el cual se realiza preferentemente en los astrocitos.

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P39. Neuroactive compounds present in extracts of a Chilean seaweed: possible application for the treatment of neurodegenerative diseases

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The seaweed fishery is mainly focused on the production of carrageenin, agar and alginates. These compounds, due to their gelling, water retention and emulsification properties are widely used in the nourishing, cosmetic and biotechnological industry. Nevertheless, in the last years, an emergent area has been the development of new drugs from molecules present in seaweed extracts. Particularly in Chile, the pharmacological screening of bioactive compounds from seaweed is a feasible form to take advantage of the biodiversity of our seaweed resource, with potential to increase the productivity and to improve the health, besides recovering and protecting the environment. In our laboratory we have obtained from a Chilean seaweed (protected identity) a methanolic-extract that displays anticholinesterase activity and a aqueous-extract capable of potentiating the activity of the enzyme acylpeptide hydrolase, recently described as a beta-amyloid degrading enzyme. In addition, the methanolic extract displays properties for the enhancement of synaptic plasticity in hippocampal slices. These results indicate that different molecules present in this macroalgae could have a therapeutic effect in neurodegenerative pathologies. The future step is to validate these activities in animal models in vivo. Support by: Programa Bicentenario en Ciencia y Tecnología PSD11 and INNOVA CORFO.

P40. SVCT2 is expressed in type C neurogenic progenitors.

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SVCT2 corresponde a un co-transportador descrito en el sistema nervioso central, el cual, sería fundamental para el ingreso de vitamina C al líquido cefalorraquídeo y al parénquima cerebral. Estudios in vitro han demostrado que vitamina C promueve la diferenciación de neural-stem-cells embrionarias, sin embargo, se desconoce su efecto en el nicho neurogénico post-natal, área que contacta el líquido cefalorraquídeo y que presenta altas concentraciones de vitamina C. El objetivo de este trabajo es analizar la expresión del transportador de vitamina C SVCT2 a nivel del nicho neurogénico adulto. Se utilizaron técnicas de co-localización por inmunofluorescencia e hibridación in situ (HIS) en cortes de cerebro de rata y ratón post-natal. El mRNA fue localizado en la pared ventricular y región subventricular del nicho neurogénico. Además, se observó en la corriente de migración rostral. El análisis inmunohistoquímico se realizó utilizando

anticuerpos para neuronas (anti-tubulina bIII), endimocitos (anti-S100), y células del nicho neurogénico (anti-nestina, anti-GFAP). De esta forma, demostramos la localización de SVCT2 en células BrdU-positivas y GFAP-negativas, observadas en la región subventricular del nicho neurogénico. Este estudio sugiere que SVCT2 se expresaría preferentemente en progenitores tipo C, de esta forma vitamina C sería activamente incorporada a células progenitoras en el nicho neurogénico postnatal. Financiado por Proyecto Anillo ACT-02.

P41. Functional evaluation of potential crosstalk between SUMOylation and phosphorylation of Nurr1.

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SUMOylation is a post-translational modification that involves the covalent attachment of SUMO peptide in target lysine of protein. This modification has become important in the regulation of transcriptional activity, and also in the stability and subcellular localization of numerous transcription factors. Nurr1 is a transcription factor essential for the development and maintenance of midbrain dopaminergic neurons. This factor belongs to orphan receptor subfamily. In other words, Nurr1 does not have a known ligand and it is constitutively active as transcription factor. In our lab, we identified PIASg, SUMO-E3 ligase, as a repressor of Nurr1 transcriptional activity; allowing us to hypothesize that Nurr1 is regulated by SUMOylation. Indeed, by using SUMOsp and SUMOplot databases, we identified potential SUMO sites in Nurr1. Site-directed mutagenesis of K91 and K74 to arginine increased Nurr1-dependent transcriptional activity, as tested in transient transfection assays using 3xNBRE-tk-luciferase reporter. Since it has been reported that phosphorylation and SUMOylation are regulatory processes that work in coordination in several transcription factors, we also mutated serine 68 in the AF1 of Nurr1. Changing S68 by alanine decreased Nurr1 transcriptional activity, while a significant increase was observed by replacing S68 by glutamic acid. None of the point mutations modified Nurr1 expression neither its localization, suggesting that SUMOylation as well as phosphorylation directly regulate Nurr1 transcriptional activity. We have also observed that ERK2 or its constitutive active form increase the transcriptional activity of Nurr1 in transient transfections assays. In summary, our results suggest that Nurr1 transcriptional activity is

regulated by SUMOylation and phosphorylation. Supported by FONDECYT N° 1070349 and Núcleo Milenio N° P06/008-F.

P42. Neural resonance within the theta range, in neurons of the olfactory amygdala

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Las neuronas piramidales de la capa II de la corteza olfatoria de la amígdala (ACo) de rata presentan oscilaciones intrínsecas del potencial de membrana, con frecuencias dentro del rango θ (2 – 12 Hz). La actividad θ se correlaciona con tareas de exploración y formación de memoria. Se ha propuesto que neuronas que presentan oscilaciones eléctricas intrínsecas podrían responder de manera preferencial a estímulos de frecuencia cercana a su frecuencia natural, fenómeno denominado resonancia neuronal. En este trabajo se analizó una posible “preferencia eléctrica de frecuencia” por parte de las neuronas de ACo, mediante el estudio de su impedancia de membrana. Se registraron 126 neuronas (potencial de reposo: $-57 \text{ mV} \pm 5.1 \text{ mV}$, promedio $\pm$ D.E.) en rebanadas coronales de cerebro de ratas Spague Dawley infantiles, en modalidad de célula completa (corriente controlada). Se aplicó una corriente sinusoidal de amplitud constante y frecuencia decreciente (15 – 0 Hz), registrándose el voltaje oscilatorio de salida. Usando la transformada de Fourier rápida, se obtuvieron los perfiles complejos de la impedancia. Los análisis de la amplitud y la fase de la impedancia mostraron que una fracción considerable de las neuronas (48%) presentó un perfil resonante, con una impedancia máxima en el rango de 2 – 6 Hz. Esta resonancia es una propiedad intrínseca de las neuronas. Mediante herramientas farmacológicas se determinó la participación de una corriente de Na^+ persistente y otra activada por hiperpolarización. Modelamiento in silico corroboró que ambas corrientes son suficientes para generar resonancia. MIDEPLAN ICM-P05-001-F FONDECYT 1080630 Anillos CyT ACT-45 Beca Conicyt para Estudiantes de Doctorado.

P43. Propranolol depresses complex learning in Skinner box and the expression of neurotrophin BDNF in the rat neocortex

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Propranolol is a beta adrenoceptor antagonist, commonly utilized in clinical hypertension. It depresses LTP in vitro and in vivo, together with memory. The aim of this study was to investigate the influence of beta adrenoceptor blockade on a complex learning in Skinner box (operant conditioning task sequentially involving omnidirectional lever displacement - stimulus light - press bar - pellet dispenser) and the expression of the neurotrophin BDNF (ELISA kit) in the rat neocortex, after three days of chronic propranolol treatment (Alzet osmotic pump). At day 3 after the beginning of propranolol administration, a decrement of the number of correct responses in the three-link concurrent-chain schedule of reinforcement was observed. Concomitantly, BDNF expression in the occipital cortex was diminished. The results suggest that propranolol exerts a deleterious effect on neuroplastic mechanisms underlying complex operant conditioning, probably related to deficits of BDNF and other nuclear signaling components involved in learning processes. Funded by grant Fondecyt 1070028 and a grant from the School of Psychology, University of the Americas.

P44. Ventricular-peritoneal shunt in mutant HT-x hydrocephalic rats triggers antibodies against CSF proteins

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Introducción: La hidrocefalia, alteración del sistema nervioso central (SNC), caracterizada por acumulación de líquido cefalorraquídeo (LCR) en el sistema ventricular. Niños hidrocefálicos presentan alteraciones en su SNC que no pueden revertirse mediante, la derivación del LCR al peritoneo (shunt) y la ventriculostomía. En condiciones normales existen proteínas que están presentes en el LCR y ausentes en el plasma; consideradas “LCR-específicas”. Hipótesis de trabajo: el shunt ventrículo-cisternal expone estas proteínas al sistema inmunológico gatillando la generación de anticuerpos contra ellas. Dicha respuesta inmune podría ser responsable del déficit neurológico postnatal asociado a la progresión y persistencia de la hidrocefalia. Objetivos: Implementar la técnica del shunt ventrículo-peritoneal en ratas HT-x, para un posterior estudio de detección de anticuerpos contra proteínas LCR-específicas. Metodología: Ratas mutante HT-x, que desarrollan hidrocefalia congénita, fueron operadas instalándose un shunt

ventrículo-peritoneal. Se obtuvo suero pre shunt y suero a los 15, 30, 45 y 60 días post shunt. Los sueros fueron utilizados como anticuerpo primario para realizar inmunoblot de LCR pre-shunt e inmunocitoquímica en cortes de SNC de rata. Para el inmunoblot se utilizó la técnica de quimioluminiscencia y para inmunocitoquímica el método biotina-estreptavidina. Resultados: Los sueros post-shunt reconocen en el LCR bandas inmunorreactivas en un rango de 19 a 60 kDa. Resultados preliminares de la identificación de estas proteínas y su distribución en SNC ha revelado que inmunorreaccionan con neuronas de Purkinje del cerebelo. Conclusión: El shunt ventrículo-peritoneal induce la producción de anticuerpos contra compuestos que están presentes en el LCR (¿proteínas LCR-específicas?) lo que podría explicar, en parte, la progresión de la patología a pesar del tratamiento. Financiado por PROYECTO FONDECYT N°1070241.

P45. Functional changes of connexin channels in an in vitro neurotrauma model.

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Introduction: Traumatic brain injury is a leading cause of morbidity and death. Propagation of secondary damage after the trauma represents an attractive opportunity for medical intervention. In astrocytes, like in other cells types, connexin channels are a critical gateway to enable damage propagation. In fact, cell death propagation via connexin channels is one of the main mechanisms for bystander damage. Aim: To establish an in vitro model of neurotrauma for studying the possible involvement of cellular communication by connexin channels post trauma. Trauma was performed by scraping astrocytes-enriched rat glial cell monolayers with a G 21 needle. We also studied the effect of 60 mM extracellular potassium (K⁺e) since high concentration of K⁺ has been measured in models of in vivo neurotrauma. Results: Needle scrape allowed a reproducible ~60 µm width injury. Three experimental regions were defined at different distances from the injury site. The region "centre" at ~100 µm, "middle" at ~800 µm, and "periphery" at ~1,400 µm, respectively. In the periphery region, trauma plus high K⁺e increased cell uptake of Ethidium (one way Anova: p<0.0001; Dunnett's: versus control p<0.01; n=10), showing an increment of cell permeability that was abolished by 200 µM

La3⁺ (lanthanum), a connexin hemichannel blocker (Bonferroni's: p<0.01; n=10). The gap-junction dependent cell coupling was estimated by assessing the intercellular transference to neighbouring cells of Lucifer yellow microinjected in a cell. No change was appreciated in the dye coupling incidence (% of showing dye transfer) or index (number of cells coupled to each positive cell) (n=4). Conclusions: Thus, hemichannels may be relevant for the propagation of injury signals from the site of trauma to peripheral regions.

P46. PHARMACOLOGICAL IN VIVO CHARACTERIZATION OF STRUCTURAL ANALOGUES OF MDMA ("ECSTASY") BASED ON COMPARATIVE BEHAVIORAL PROFILES IN RATS

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Un entactógeno es una sustancia psicoactiva capaz de inducir un estado alterado de conciencia en el ser humano que consiste en la inducción de sentimientos de aceptación del propio yo y bienestar general, así como un aumento de la empatía hacia las personas y el entorno. Este síndrome es diferente del inducido por drogas estimulantes o alucinógenas y no resulta de la combinación de estos 2 efectos, ampliamente conocidos para drogas de diverso origen. El entactógeno prototípico es MDMA (3,4-metilendioximetanfetamina), comúnmente llamado "éxtasis". Debido a su potencial psicoterapéutico, sus propiedades de co-ayudante en el manejo farmacológico de diversos cuadros neuropsiquiátricos y su condición de droga de abuso, la búsqueda de análogos con propiedades entactogénicas constituye un área de particular interés. En el presente trabajo hemos utilizado una combinación secuencial de protocolos conductuales en ratas (asociados a efectos psicomotores, de ansiogénesis/ansiolisis y de condicionamiento activo por evitación) para elaborar perfiles conductuales a partir de curvas dosis-respuesta (0.25 – 10 mg/Kg) que permiten distinguir entre los efectos agudos inducidos por MDMA, un análogo estructural estimulante (metanfetamina, MA) y un análogo estructural alucinógeno (2,5-

dimetoxi-4yodo-feniletilamina, DOI). A partir de este modelo, hemos evaluado una serie de 10 drogas homólogas que según reportes empíricos en seres humanos serían similares a MDMA. Cada droga fue sintetizada a través de rutas clásicas e inyectada i.p. 30 minutos antes de iniciar las mediciones. Nuestros resultados indican que algunas de ellas muestran perfiles compatibles con MDMA, mientras que ninguna de ellas es similar a DOI y/o MA. FONDECYT 1085051 e ICM P05-001-F.

P47. IRON AND AMYLOID β OLIGOMERS STIMULATE RYANODINE RECEPTOR - MEDIATED CALCIUM RELEASE AND MITOCHONDRIAL FISSION IN HIPPOCAMPAL NEURONS

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Iron and A β oligomers (A β Os) are centrally involved in Alzheimer's disease pathogenesis, and promote intracellular calcium rises that may affect mitochondrial dynamics. Mitochondrial fission induces neurodegenerative cascades and requires Dynamin-related protein 1 (Drp1), a GTPase that binds to mitochondrial outer membranes to generate small and round organelles. The goal of this work was to investigate changes in the mitochondrial network induced by iron and A β Os. We also investigated the role of calcium release mediated by ryanodine receptors (RyR) on iron or A β Os-induced mitochondrial fission. Methods: Primary rat hippocampal neurons were incubated with Fe-NTA (20-50 μ M) or A β Os (500 nM) for different times. A β Os morphology was determined by transmission electron microscopy. Ca²⁺ signals were analyzed by confocal microscopy in neurons preloaded with Fluo3-AM and exposed to Fe-NTA or A β Os. Changes in mitochondrial dynamics, detected with anti-mHsp70, were evaluated by morpho-topological and western blot analysis for Drp-1 in mitochondrial and cytoplasmic subcellular fractions. Results: Fe-NTA and A β Os induced intracellular Ca²⁺ rises, which were attenuated by pre-incubation of neurons with ryanodine [50 μ M] to inhibit RyR activity. Incubation for 24 h with Fe-NTA and A β Os increased the population of small at the expense of large mitochondria, suggesting increased fission. Moreover, Fe-NTA induced Drp-1 redistribution from the cytosolic to the mitochondrial subcellular fraction. Pre-incubation of neurons with ryanodine to inhibit RyR prevented the changes in mitochondrial morphology induced by Fe-NTA. Conclusions: Our

results strongly suggest that Fe-NTA and A β Os produce intracellular calcium rises, via stimulation of RyR-mediated calcium release, which induce fragmentation of the mitochondrial network. Fondep CEMC 15010006, FONDECYT 3085025

P48. Repetitive fluoxetine treatment induces changes of the subunit composition of AMPA and NMDA receptors in the rat telencephalon

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La fluoxetina (flx), un inhibidor selectivo de la recaptación de serotonina, es un fármaco antidepresivo ampliamente usado para tratar la depresión mayor. Flx, al igual que todos los fármacos antidepresivos, debe ser ingerido por semanas a meses, lo que sugiere que el efecto terapéutico se debe a cambios plásticos lentos en el SNC cuya naturaleza aún se desconoce. Basados en resultados previos del laboratorio, en el presente trabajo se investigó si flx induce cambios en las subunidades que conforman los receptores para L-glutamato, tipo NMDA (R-NMDA compuesto por subunidades NR1, NR2A, NR2B) y AMPA (R-AMPA compuesto por subunidades GluR1 y GluR2), en el telencéfalo de ratas. METODOLOGÍA: Se trataron ratas con flx por dos y cuatro semanas, se obtuvieron fracciones subcelulares enriquecidas en sinaptosomas y densidades postsinápticas (DPS), las que fueron evaluadas por Western blot, inmunoprecipitaciones e inmunofluorescencia. RESULTADOS: En homogenizados de telencéfalo, observamos aumentos en los niveles de la subunidades GluR1, GluR2, NR1 y NR2A, mientras que la subunidad NR2B no cambió. Para detectar posibles cambios sinápticos se evaluaron las subunidades en DPSs: Mientras NR1 y NR2A aumentaron, GluR2 no cambió y GluR1 disminuyó. Además, se encontró mayor colocalización de NR2A y GluR2, pero no en GluR1 en sinaptosomas glutamatérgicos y en co-inmunoprecipitaciones se pudo confirmar un aumento de NR2A en R-NMDA y GluR2 en R-AMPA. CONCLUSIÓN: El tratamiento crónico con flx induce cambios en la composición de subunidades que conforman R-NMDA y R-AMPA lo que formaría parte de los cambios plásticos requeridos para una mejoría terapéutica. Anillo ACT09-2006 (para UW) Conicyt (para MS).

P49. Neuroprotective effect of astrocytes on hippocampal cultures depends on its modulation of the activation of microglial cell by Ab.

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Introduction: Neuronal death in Alzheimer's disease can be secondary to A β toxicity or depend on glial cell-mediated inflammation. We have shown that astrocytes have a protective effect for A β neurotoxicity in vitro. Aim: Evaluate if astrocytes' protective effect depends on the modulation of microglial activation or on the production of neuroprotective factors. Methods: We generated conditioned media (CMs) from astrocyte cultures stimulated with pro-inflammatory molecules, 1 μ g/ml LPS+10 ng/ml IFN γ (LI), (CM-ASLI) or 2 μ M AB, which were used for culturing microglia exposed to A β ; recovering CMs from stimulated microglia (CM-MG(AB+ASLI)) and (CM-MG(AB+ASAB)). Hippocampal cultures were exposed to various combinations of CMs from glial cultures. Apoptosis was evaluated with the TUNEL assay. Cell types were identified with cell identity markers (β -Tubulin III, neurons; GFAP, astrocytes and Griffonia simplicifolia-lectin, microglia). Results: Hippocampal cultures exposed to the microglial CMs (CM-MGAB) showed a 2-fold increase in the number of apoptotic cells, both for neurons and non-neuronal cells ($p < 0.005$). Exposure to CM-MG(AB+ASLI) did not induce apoptosis resulting in cell death similar to that observed in control cultures. In contrast, hippocampal cells exposed to a 1:1 mixture CM-AS LI+CM-MGAB, showed the same 2-fold increase in apoptosis induced by activated microglia CMs (CM-MGAB). Conclusion: Microglia activated by A β secretes soluble factors capable of inducing hippocampal cells apoptosis. Activated astrocytes showed a neuroprotective effect that depended on its modulation of the activation of microglia by AB. Supported by grant FONDECYT 1090353

P50. Degu spectral-sensitivity functions derived from behavior discrimination test, rol ON and OFF channels.

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The retina carry visual signal in two parallel streams, from cone photoreceptor to the ganglion cells. These two pathways originate at the bipolar cell (BC) : one class becomes depolarized in response to light (ON-BC), other class hiperpolarized in response to light (OFF-BC). We study a rodent chilean named Octodon degus , it have two types of cones with

spectral sensibility near ultra violet (360 nm , S-cone) and near to green (506 nm, M-cone). In previous work in our laboratory , the spectral sensitivity of the ON response was studied by the electroretinogram (ERG) , these response are compatible with the input of two principal cones. However the spectral sensitivity of the OFF response are compatible whith only M-cone. In this work examined the contributions to the vision color of both visual pathways (ON and OFF) in a series of behavioral experiments.. Four Animals were trained to discriminate between 360-nm and white lights and between 500-nm and white lights . Then these animals were anaesthetized and APB intravitreal injections were supplied (blocks ON channel). The discrimination to light were evaluated. Our results shows that in first animal the discrimination to 360 nm lights decreased 27,7 % in order to control and 500 nm light decreased 20,5 % , in second animal the discrimination to 360 lights decreased 30,5 % reference to control and 500 light decreased 7 %. These results suggest that in the color pathway ON and OFF channels contribute unequally to the colors perception. CONICYT PBCT-ACT45. Soto CR as a Graduate Fellowship from MECESUP.

P51. Differential release of dopamine in ventral tegmental area and lateral septum of rats exposed to repeated stress by immobilization.

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Las experiencias estresantes producen cambios adaptativos que afectan la homeostasis corporal. Sin embargo, la exposición crónica o persistente a estos estímulos genera conductas incontrolables e impredecibles que pueden llevar al abuso de drogas adictivas en individuos vulnerables. El septum lateral (SL), es un núcleo cerebral involucrado en las respuestas conductuales al estrés que recibe aferencias nerviosas desde el área tegmental ventral (VTA). Estudios de nuestro laboratorio han mostrado que la administración sistémica e intra-VTA de morfina en ratas, genera un aumento de la liberación de dopamina (DA) en el SL que es dependiente de una desinhibición GABAérgica en el VTA. El objetivo de este trabajo fue estudiar el efecto de la exposición repetida a estrés por inmovilización sobre los niveles extracelulares de DA basales e inducidos por cocaína en VTA y SL. La cuantificación de DA se realizó a través de

microdiálisis cerebral y HPLC acoplado a detección electroquímica. Los resultados muestran que la exposición repetida a estrés no genera cambios en los niveles basales de DA en ambos núcleos. Además, muestran que una inyección aguda de cocaína (10 mg/Kg, ip) induce un aumento en los niveles extracelulares de DA en ambos núcleos. La exposición repetida a estrés generó una pérdida del efecto de cocaína sobre los niveles extracelulares de DA en VTA pero no en el SL. Estudios en curso sugieren que la ausencia del efecto de cocaína en el VTA de ratas previamente estresadas se debe a un aumento del control inhibitorio sobre las neuronas dopaminérgicas del VTA. Financiado por: ICM N° P06/008-F y Proyectos FONDECYT Postdoctoral N° 3095007 para RSZ.

P52. Involvement of aging and inflammation-dependent changes of TGF β -Smad3 signaling on microglial activation in a murine model of Alzheimer Disease.

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Aging is the main risk factor for Alzheimer Disease (AD). It is characterized by an increase in pro-inflammatory signaling that could be involved on glial function modulation and in cytotoxic activation of microglial cells. Our previous studies show that astrocytes modulate cytotoxicity of microglia through the secretion of the anti inflammatory cytokine TGF β . TGF β is increased in AD but the expression of Smad3, its main pathway effector, is reduced. Approach & Results: To study the role of TGF β Smad3 pathway in microglial cell activation, we evaluated nitrite secretion, scavenger receptor expression and phagocytosis in microglial cultures obtained from rat neonates exposed to various stimuli plus TGF β and different pathway inhibitors. 0.5 mg/kg LPS (i.p) induced a 4.1 $\pm$ 0.5 fold increase on nitrite secretion, which was down regulated to control levels by TGF β (2 ng/ml). Modulation was not affected by Smad3 inhibition. However, ERK and p38 inhibition by themselves decreased LPS-induced nitrite secretion, and TGF β potentiated the decrease in nitrite secretion. TGF β also increased SR-A expression by 2.4 $\pm$ 0.3-fold and A β phagocytosis by 51%, effects that were prevented by Smad3 inhibition. We also determined in 2- and 12-month old APP/PS1 mice treated with LPS, the expression of Smad3 in the hippocampus and the effect of Smad3 inhibition on phagocytosis by adult microglia. We observed that LPS induced an increase in Smad3 protein levels (1.58 $\pm$ 0.18-fold) in 2-month but not in 12-month old mice hippocampus.

Microglial cultures showed that LPS increased A β uptake in the WT and APP/PS1 2 month old mice. However, uptake was not induced in the 12-month old mice microglial cultures. TGF β also induced A β Fluo III uptake by 72% in WT and 57% in APP/PS1 in 2 month, but not in 12 month old mice. Increased phagocytosis was abolished by Smad3 inhibition. Chronic inflammatory stimulus (LPS) also increased hippocampal amyloid plaques and MHCII(+) microglia in APP/PS1 mice. Conclusion: TGF β Smad3 pathway is relevant for microglial cells regulation and it is modulated by aging and inflammatory condition. Fondecyt 1090353 and grant VRAID-Puente08 from the Pontificia Universidad Católica de Chile.

P53. Modulation of depressive behaviors by inactivation of the retrosplenial cortex.

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La depresión es una enfermedad neuropsiquiátrica caracterizada por síntomas diversos como alteraciones del ánimo, sueño, apetito, funciones cognitivas y ansiedad. Esta sintomatología refleja la participación de diferentes estructuras cerebrales que pueden modular conductas depresivas, como la corteza prelímbica, el hipocampo, núcleos límbicos subcorticales y eje hipotálamo-hipófisis. La corteza retroesplenial (RSC), ubicada en la zona posterior del giro cingulado, se conecta con el hipocampo y núcleos límbicos subcorticales. Datos obtenidos en nuestro laboratorio indican que el tratamiento con el antidepresivo Fluoxetina induce cambios morfológicos en la RSC que están asociados con una reorganización molecular, sugiriendo que esa zona es blanco de la medicación con antidepresivos. Nuestra hipótesis de trabajo es que la RSC modula conductas depresivas. Para probar esta hipótesis, se inactivó bilateralmente la RSC de ratas adultas usando un bloqueador reversible de canales de Na⁺. Para ello, se implantaron cánulas intracerebrales y se comprobó histológicamente que alcanzaban la RSC. Posteriormente se inyectaron 0.5 ml de bupivacaína 0.5% por lado, 10 minutos antes de realizar las pruebas conductuales. La ansiedad fue evaluada por el test de supresión de la ingesta en ambiente novedoso (NSFT), y la desesperanza aprendida se evaluó por el nado forzado (FST). Nuestros datos indican una disminución de la ansiedad (por el NSFT) y de desesperanza aprendida (por el FST) para ratas con la RSC inactivada. Nuestros resultados sugieren

que la RSC podría ser el blanco de nuevas estrategias terapéuticas para tratar desórdenes del ánimo. Financiado por: FAI MED-001-09 (FJR) y Anillo ACT09-2006 (UW)

P54. Alterations of the reelin, conducting a changes in the composition of cerebrospinal fluid hydrocephalic

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Introducción. Reelina es una proteína que en la etapa prenatal participa en la organización cortical y en postnatal en plasticidad sináptica. Considerando que la hidrocefalia cursa con daño cortical, nos propusimos analizar la expresión de reelina en animales mutantes con hidrocefalia congénita. Métodos. Se analizaron los niveles de reelina mediante inmunoblot de muestras de cerebro y líquido cefalorraquídeo (LCR) de ratas H-Tx normales e hidrocefálicas en E19, E20, PN7 y PN30. Resultados. Los niveles del precursor de reelina de 420 kDa y de sus formas procesadas de 320 y 180 kDa no muestran diferencias entre los cerebros hidrocefálicos y normales. En el LCR normal reelina tiene su máxima expresión en E19 y PN7, bajando en PN30. En el LCR hidrocefálico de cisterna magna (CM) de embriones E20 se identificaron los compuesto de 420, 320 y 180 kDa; el compuesto de 180 kDa se encontró aumentado respecto del control. En LCR de VL los compuestos de 420 y 320 kDa no fueron detectados. En PN30 los compuesto de 420 y 320 no fueron detectados en CM normal pero si en LCR de CM y VL hidrocefálico. A esta edad el compuesto de 180 kDa se mantuvo aumentado en el LCR de CM y de VL de ratas hidrocefálicas. Conclusiones. (i) El aumento de reelina en el LCR hidrocefálico, en especial del compuesto de 180 kDa, podría ser un biomarcador de hidrocefalia. (ii) Los cambios de reelina en el LCR hidrocefálico sugiere su participación en el proceso neurodegenerativo en la rata H-Tx. FONDECYT 1030265

P55. The role of c-Abl in the synaptotoxicity caused by A β oligomers.

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Alterations in synaptic function and structure are one of the early events in Alzheimer disease. Specifically, it has been showed that A β oligomers induced a decreased in dendritic spines, synaptic loss, plasticity alterations and cognitive damage. These changes are associated with a decrease in

the levels of PSD-95, NMDAR and Efrine receptor tyrosine kinase. However, the molecular mechanisms activated by A β oligomers leading to synaptic dysfunction and loss have not been completely explained. We described that A β fibers induce c-Abl activation. This kinase is present in both pre and post synaptic structures and the c-Abl inhibition cause a decrease in PSD-95 synaptic accumulation and synaptic contacts number. Then, we propose that c-Abl is involved in the synaptotoxicity induced by A β oligomers throught of regulation synaptic proteins such as PSD-95. We treated hippocampal neurons (15 DIV) with synthetic oligomers A β , or oligomers A β obtained from CHO 7PA2 cells culture media. (This cell line express the Indiana mutation in the amyloid precursor protein and secretes sub-nanomolar levels of low-n oligomers of A β). Our results showed that A β oligomers cause a PSD-95 clusters decrease in neurons, as has been previously described. Also we observed the kinase c-Abl was activation and increase c-Abl signal in dendritic spines induced by A β oligomers. Interestingly we observed that A β increase the interaction between c-Abl and PSD-95. Our results suggest that c-Abl could participates in the synaptotoxicity events caused by A β oligomers regulating the PSD-95 in synapses. FONDECYT 1080221.

P56. Frequency tuning of the effect of the contralateral olivocochlear reflex on ipsilateral cochlear sensitivity in the chinchilla

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Outer hair cells of the mammalian cochlea receive a significant efferent innervation through the medial olivocochlear (MOC) system, comprising crossed and uncrossed fibers that originate in the region of the superior olivary complex. Significant differences have been found among species in the distribution of crossed and uncrossed MOC fibers; with the highest ratio of crossed/uncrossed fibers (4:1) found in chinchilla among rodents. We have measured the effect in cochlear sensitivity of the activation of olivocochlear efferents, through acoustic contralateral stimulation (contralateral olivocochlear reflex) in 10 anesthetized adult chinchillas. VIIIth-nerve compound action potentials (CAP) were recorded with a round-window electrode in response to acoustic stimuli at different frequencies and intensities. Stimuli were presented in 3 consecutive series of 64 trials, first ipsilateral stimulation alone,

then contralateral stimulation (500 ms) followed (after 5-10 ms) by ipsilateral stimulation (15 ms) and finally ipsilateral stimulation alone. Contralateral acoustic stimulation produced amplitude reductions in CAPs elicited by ipsilateral tones at frequencies ranging from 1 to 9 kHz that reached up to 14 dB. In all cases ipsilateral CAP reductions caused by contralateral acoustic stimulation were frequency tuned to the ipsilateral stimulus frequency; that is, CAP reductions were maximal for contralateral stimulus frequencies close or equal to those of ipsilateral tones. Tetrodotoxin injected into the contralateral cochlea almost abolished the contralateral neural response, significantly decreasing the MOC effect on ipsilateral cochlear sensitivity. These results show the existence of a tonotopic distribution of efferent olivocochlear fibers that reach most of the extent of the chinchilla cochlea. Supported by FONDECYT 1080227 and Fundación Guillermo Puelma. Thanks to Paul Delano for valuable advice and to Fernando Vergara for technical assistance.

P57. Nanomolar Copper Modifies Intrinsic Excitability Components of CA1 Neurons.

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Copper is an essential ion for development and functioning of the mammalian brain. It has been proposed as an endogenous neuromodulator but its mechanisms of action have not been clearly established. We previously found that nanomolar copper increases the firing frequency of some pyramidal CA1 neurons in slices of rat hippocampus, apparently by changing some of the synaptic inputs to CA1 cells (extrinsic excitability). Here we studied the effect of this metal on the intrinsic electrical properties of interneurons and pyramidal CA1 neurons using voltage and current clamp recordings. Neurons recorded in control conditions fired at frequencies from 0 to 15 Hz with firing thresholds between -75 and -55 mV. When exposed to 100 nM copper these values increased to 0-20 Hz and -85 to -50 mV, respectively (n = 2). Furthermore, the amplitude of action potentials decreased by nearly 10 mV. After washing, changes were not reversed, suggesting that changes produced by this metal are redox-mediated.

In 4 of 8 neurons in which the outward current was measured in voltage clamp conditions, 100 nM copper generated an 8 mV leftward shift in the relative peak conductance curve. Under current clamp conditions, this change would translate into a higher amount of outward current, a possible explanation for the increase in firing frequency and

decreased on action potential amplitude triggered by copper.

These results suggest that one of the actions of copper is over certain components that regulate the intrinsic excitability of CA1 neurons. Beca CONICYT para estudios de Magister y proyecto FONDECYT 1080670.

P58. Nitric oxide amplifies the ERG amplitude of the dark-adapted retina

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Nitric oxide (NO) participates in retinal signal processing through stimulation of its receptor enzyme, soluble guanylyl cyclase (sGC). Under pathological conditions such as inflammation or ischemia, elevated NO concentrations may cause protein S-nitrosylation in photoreceptors and other retinal cells, possibly leading to cellular injury and apoptosis. To better understand the role of NO in ocular function and disease, we investigated the effects of exogenous NO, produced by the NO donors NOC-12, NOC-15 and SNP, on the flash ERG of the rat. We found that within a limited concentration range, NO amplified the ERG a- and b-wave, oscillatory potentials, and scotopic threshold potentials by up to 100%. This signal gain was highest under dark adaptation and also observed when photoreceptor-bipolar cell synapses were blocked by AP-4. Inhibition of sGC by ODQ or NS2028 did not prevent the effect, indicating that NO acted on rod phototransduction through a sGC-independent pathway. Our results suggest that elevated levels of ocular NO may alter photoreceptor function. Whether the observed increase of scotopic ERG amplitudes reflects a physiological role of NO in retinal dark adaptation remains to be investigated. Grants PBCT ACT45, FONDECYT 1090343.

P59. Voluntary exercise prevented the anxiogenic effect and plasma corticosterone increase induced by food restriction in rats

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Food restriction increases lifespan and ameliorates disease outcomes, leading to improvement of cognitive functions and reduction of cardiovascular

and neurodegenerative diseases risk factors. However, food restriction has shown to induce anxiogenic effects in rat behavior. In contrast, voluntary exercise can prevent stress-related mood disorders, such as depression and anxiety. In this work, we tested the possible anxiolytic effect of voluntary wheel running in a rat model of food restriction, together with assessing plasma corticosterone levels and expression of 5-HT_{1A} receptors in the dorsal raphe nucleus (DRN). At day 29 and during seven weeks, rats were separated into 4 groups: food restriction-sedentary, where the rats were given 60% of food consumed by controls; food ad libitum-wheel running; food restriction-wheel running; and food ad libitum-sedentary (control). At day 78, rats were tested for anxiety on an elevated plus maze and the number of entries and time spent in the open arms recorded. On day 80, blood was collected from a tail vein and corticosterone concentration measured by ELISA method. On day 82, the DRN was dissected out and subjected to Western blot for examining 5-HT_{1A} protein expression. Two-way ANOVA indicated that wheel running decreased anxiety behavior and plasma corticosterone level in food restricted but not in rats fed ad libitum. Voluntary exercise did not modify the 5-HT_{1A} expression in the DRN. Results showed anxiolytic effect of voluntary exercise on food restricted animals, suggesting that exercise may be useful as an intervention for anxiety on people that restrict its food intake. Fondecyt 1070028.

P60. Codistribución de GK y GGRP en glia hipotalámica. (GK and GGRP co-distribution in hypothalamic glial cells).

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Se ha demostrado que la enzima glucoquinasa (GK) es fundamental para el mecanismo sensor de glucosa en células beta-pancreáticas. En el hígado es inhibida por compartimentalización nuclear dirigida por la proteína reguladora de la glucoquinasa (GGRP). Recientemente, hemos demostrado que GK se localiza en el hipotálamo, específicamente en la glia periventricular (tanicitos). Sin embargo se desconoce su identidad molecular y su posible interacción con GGRP para regular su compartimentalización. Utilizamos ratas en condiciones de hipoglicemia, normoglicemia e hiperglicemia para analizar la localización de GK y GGRP en el hipotálamo, a través de inmunolocalización asociada a microscopía confocal. Comparamos estos resultados con tejido hepático de los mismos animales. Mediante RT-

PCR, se logró clonar y secuenciar la región codificante de GK y GGRP. Los estudios de inmunolocalización en normoglicemia evidenciaron que GK se localiza en el núcleo y en el citosol de los tanicitos, estos resultados se corroboraron a través de inmunoblots. En condiciones de hipoglicemia disminuye la reactividad a nivel nuclear. En condiciones de hiperglicemia GK y GGRP aumentan su localización nuclear en el tanicito, mostrando un comportamiento inverso que en el hígado. El clonamiento de GK y GGRP nos permitió determinar las secuencias codificantes expresadas en el hipotálamo y la obtención de herramientas moleculares que nos permitirán estudiar interacción entre ambas proteínas. Financiado por Proyecto Anillo ACT-02.

P61. Elucidation of the secretion pathway for corticotropin releasing hormone binding protein

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The corticotropin releasing hormone binding protein, CRH-BP, is a 37kDa glycoprotein that binds, with high affinity, CRH as well as Urocortin I, UcnI. The first function described for CRH-BP was the control of CRH levels within plasma during pregnancy. However, it has been described that CRH-BP is also expressed on various nucleus of the central nervous system. It is of special interest the expression of CRH-BP on dopaminergic neurons of the ventral tegmental area, VTA, important nucleus related with the reward circuit, and specially affected during addictive episodes. During the last years it has been described that CRH-BP may play an important role on the modulation of excitatory activity induced by the activation of CRH receptors, specially of the CRHR2 type, in dopaminergic neurons and glutamate nerve terminals of the VTA. The evidence obtained during last years has attracted the interest on CRH-BP, specially of its role modulating CRH. However, the secretion mode of CRH-BP has not been studied. The aim of this study is to determine the secretion mode for CRH-BP. The results show that CRH-BP colocalizes with secretogranin II, a marker of the regulated secretory pathway, when it is overexpressed in PC12 cells, a model of neuroendocrine cells, so as in rat fetal cortical cells. CRH-BP, overexpressed in PC12 cells, is secreted to the medium after an acute stimulus with barium. The present findings support the hypothesis that CRH-BP would have a much more complex role than just buffering CRH, as it behaves

as a regulated secreted protein. Núcleo Científico Milenio en estrés y adicción: P06/008-F Fondecyt 1070340.

P62. Estudio de los elementos reguladores de la expresión neuronal de Pomc nPE1 y nPE2 en ratones transgénicos

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Universidad Austral de Chile. CECS. Valdivia, Chile. El gen de proopiomelanocortina (Pomc) se expresa principalmente en la hipófisis y el cerebro (hipotálamo y NTS). Pomc codifica varios péptidos que incluyen a la hormona adenocorticotrófica (ACTH), las melanocortinas α -, β -, y γ -, y el potente analgésico opioide β -endorfina. Las neuronas hipotalámicas de Pomc son particularmente importantes en la regulación central de la ingesta alimentaria y el peso corporal. Hace pocos años demostramos que la expresión específica de Pomc en el hipotálamo está controlada por dos regiones altamente conservadas entre mamíferos, presentes río arriba del sitio de inicio de la transcripción a quienes llamamos nPE1 y nPE2. Para estudiar el rol de estos dos elementos evolutivamente no relacionados, generamos varias líneas de ratones transgénicos que expresan los marcadores fluorescentes EGFP o tdimer2 (tomato) bajo el control transcripcional de nPE1 o nPE2, respectivamente. Nuestros resultados demuestran que tanto nPE1 como nPE2 son capaces de dirigir la expresión de estos genes reporteros a las mismas neuronas hipotalámicas tanto en el desarrollo embrionario como en ratones adultos, sin embargo solo nPE2 sería responsable de la expresión de Pomc en el NTS. La caracterización de estos módulos regulatorios distales aporta una herramienta molecular que permite dirigir la expresión de cualquier gen de interés específicamente a neuronas hipotalámicas de Pomc.

P63. Neurotoxicity of lupus autoantibodies through amplification of NMDA receptor signaling and mitochondrial stress.

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Autoantibodies that bind neural targets have been increasingly implicated in the pathogenesis of neuropsychiatric systemic lupus erythematosus (NPSLE). We have previously characterized a class of autoantibodies from NPSLE patients that bind double-stranded DNA and cross-react with a

pentapeptide sequence present in the NR2A and NR2B subunits of the n-methyl-d-aspartate receptor (NMDAR). Upon entry into brain, these autoantibodies produce neuronal death and ensuing cognitive dysfunction; however, the mechanisms of these effects have not been described. Here we report that both mouse and human antibodies displaying affinity for NR2A and NR2B work as positive modulators for the NMDAR, increasing the size of evoked NMDAR potentials and promoting excitotoxicity through increased mitochondrial permeability transition (mPT). The antibody effects are dose-dependent, require direct NMDAR activation, and are inhibited by NMDAR antagonists. These studies reveal how NMDAR-reactive autoantibodies which trigger both synaptic and cell-wide alterations that can cause the neuropsychiatric symptoms observed in patients with systemic lupus erythematosus.

P64. Chronic desipramine treatment reverses both bed nucleus of the stria terminalis dendritic morphology changes and enhanced anxiety-like behavior induced by repeated immobilization stress.

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1Depto. Biología Celular y Molecular, 2Depto. Farmacia, P. Univ. Católica de Chile, Santiago, Chile Repeated immobilization stress (RIS) has been shown to induce dendritic atrophy in the ventral lateral bed nucleus stria terminalis (vBNST), dendritic hypertrophy in the dorsal lateral BNST and reduced open arms activity in the elevated plus-maze. These effects persist after a 21-day stress-free period. Desipramine (DMI) administration during the stress period prevents both RIS-induced dendritic morphology changes of BNST neurons and enhanced anxiety. To study whether these changes can be reversed by chronic treatment with (DMI), a group of rats was subjected to RIS consisted of a daily session of immobilization (15 days, 2 h/day). On day 16, a group of stressed rats and its respective naïve controls received DMI (10 mg/kg, once a day), and another group of RIS and naïve rats received vehicle (once a day) during the next 3 weeks. On day 36, anxiety-like behavior was assessed by elevated plus maze (EPM). The animals were sacrificed on day 37 and their brains were subject to Rapid Golgi protocol. The results showed that chronic DMI administration induces opposite effects on anxiety-like behavior, reverses the long-lasting anxiety induced by RIS and induces anxiety-like- behavior in naïve rats. DMI administration also reverses the RIS-induced dendritic morphology changes of BNST neurons, but induces dendritic atrophy in the vBNST in naïve

rats. Thus, is it tempting to suggest that changes in dendritic morphology of lateral BNST and noradrenergic system underlie the effects of RIS. Grant/Other Support: FONDECYT 1070369 Grant/Other Support: Millennium Scientific Initiative Grant N° P06/008-F

P65. Characterization of the vitamin C function on a stem cell model

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Durante el último período del desarrollo del SNC embrionario es posible detectar altas concentraciones de vitamina C en el cerebro fetal, seguido de una brusca caída durante el período perinatal. Estos cambios se correlacionan con la neurogénesis y gliogénesis, respectivamente. Debido a que ácido ascórbico, la forma reducida de la vitamina C es incorporada al cerebro mediante el transportador dependiente de sodio SVCT2, se postula que los cambios en su expresión regularían la incorporación de vitamina C y jugarían una función en la promoción de la diferenciación neuronal. Para estudiar este fenómeno se obtuvieron neural stem cells, a partir de cortezas cerebrales de ratas embrionarias (E16-E18), y aquellas formadoras de neuroesferas, fueron caracterizadas analizándose la expresión y localización de SVCT2 por inmunocitoquímica, Western blotting y RT-PCR. Se observó que estas células expresan el transportador en altas cantidades, demostrando ser un marcador adecuado para células de fenotipo troncal. Para realizar análisis funcionales y de diferenciación neuronal se utilizó un modelo de células precursoras, la línea celular C17.2, obteniéndose que ésta expresa marcadores troncales y experimenta diferenciación espontánea a neurona. La expresión de SVCT2, sin embargo resulta ser muy baja en todos los estadios analizados, sin experimentar cambios notorios al suplementar los cultivos con vitamina C. Al analizar la funcionalidad del transportador, se encontró interesantemente que pese a expresar bajas cantidades de SVCT2, estas células transportan activamente vitamina C proceso inhibido por quercetina. Nuestros datos sugieren la presencia de un transportador diferente a SVCT2 en células troncales inmortalizadas. Financiado por Proyecto Anillo ACT-02.

P66. Long-term exposure to sub-acute doses of dichlorvos elicits an improvement in synaptic plasticity that is specifically correlated with acylpeptide hydrolase inhibition.

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Recently we have reported that short-term exposure of rat hippocampal slices to acute doses of the organophosphate molecule dichlorvos inhibits the enzyme acylpeptide hydrolase (ACPH) at a specific window of dosage and exposure time. A higher dose of dichlorvos or prolonged exposures causes the inhibition of both enzymes: acylpeptide hydrolase and acetylcholinesterase. 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 $\alpha 7$ nicotinic receptors. ACPH is an enzyme belonging 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. At present, the physiological role of ACPH in neuronal tissue is unknown, however, it has been reported that ACPH can degrade the α -amyloid peptide (A β) in vitro. Furthermore, we have found that brain ACPH activity decreases with age, therefore, the study of this enzyme as a possible target for improvement of neurocognitive functions declined by aging and/or neurodegenerative diseases is our interest. In agreement with our previous findings, here we report that long-term exposure (4 weeks) of male Sprague – Dawley rats (three weeks old) daily exposed to sub-acute doses of dichlorvos via sub-cutaneous injection elicits an enhancement of LTP that is correlated with acylpeptide hydrolase -and not with acetylcholinesterase- inhibition confirming in vivo the effect that we have described in vitro. Support by: Programa Bicentenario en Ciencia y Tecnología PSD11, DGIP from Universidad Católica del Norte and INNOVA - Corfo

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