

## **SIMPOSIOS**

### **S1. ALTERATIONS IN GABAERGIC TRANSMISSION IN HUMAN TEMPORAL LOBE EPILEPSY.**

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Center for Anatomy. Temporal lobe epilepsy (TLE) is arguably the most common seizure disorder. The cellular and molecular mechanisms underlying TLE in humans are poorly understood. Various data suggest that aberrant GABA<sub>A</sub> receptors may contribute to hyperexcitability (e.g. Brooks-Kayal et al. 1998). We investigated neuronal and synaptic properties of human neocortical tissue from epilepsy surgery to identify possible alterations in synaptic transmission contributing to hyperexcitability. Here we focus on two aspects, namely the mechanisms augmenting individual synaptic responses and the temporal properties of such events. Our data indicate two key changes of GABAergic transmission, firstly a depolarizing GABA<sub>A</sub> receptor-mediated inhibition in some of the neurons and secondly greatly reduced GABA<sub>B</sub> receptor-mediated events (see Deisz, 2003). We provide experimental evidence demonstrating that depolarizing GABA<sub>A</sub> responses account for the augmented synaptic potentials in human epileptogenic cortex and that the depolarizing action of GABA is due to impaired chloride extrusion mechanisms. The reduced function or density of presynaptic GABA<sub>B</sub> receptors inferred from our experiments contributes to repetitive interictal activity. The implications of these alterations of GABAergic transmission will be discussed in the context of pharmacoresistance and spread of focal activity.

Brooks-Kayal, A.R., et al. (1998). *Nature Medicine* 4, 1166-1172.

Deisz, R.A. (2003). *Novartis Foundation Symposium* 243, 186-206.

### **S2. INTRACELLULAR REGULATION OF INHIBITORY RECEPTORS IN THE CENTRAL NERVOUS SYSTEM.**

Luis G. Aguayo, Gonzalo Yevenes and Gustavo Moraga-Cid. Department of Physiology. University of Concepcion, Chile. Inhibitory glycine receptors (GlyRs) play a critical role in neuronal excitability in the mammalian brain stem and spinal cord. Receptor activation following the binding of glycine to the extracellular domain leads to channel opening and to a subsequent increase in Cl<sup>-</sup> current. This membrane phenomenon effectively reduces the excitability and firing mechanisms of neurons associated to sensory information, motor control and respiration. The glycine-activated Cl<sup>-</sup> current is potentiated by ethanol and general anesthetics leading to an enhancement of inhibition in mammalian central neurons. A line of research that intends to explain the mechanisms by which these allosteric modulators affect the GlyR has been the search for molecular sites that allow their binding within the GlyR. For instance, mutations in TM2 within the  $\alpha$ 1 GlyR was reported to change the sensitivity to ethanol and general anesthetics. Additionally, we are examining the hypothesis that activation of G proteins modulates the sensitivity of GlyR to low concentrations of ethanol and

general anesthetics. Examination of the large intracellular loop connecting the TM<sub>3-4</sub> within the glycine receptor reveals the presence of a cluster of basic amino acids in the NH<sub>2</sub> terminus, which could potentially be the region for G protein interaction. We have mutated this receptor region and examined the effect of G protein activation on GlyR function. The data shows that the mutations affected the sensitivity of GlyRs to intracellular GTP- $\gamma$ -S indicating that this cluster of positive amino acids is critical for the modulation of the receptor by G proteins. The mutated GlyR (5A) was not affected by ethanol at concentrations as high as 200 mM, thus indicating that this basic amino acid cluster is an important determinant for ethanol modulation. On the other hand, the sensitivity of the receptor to propofol (50  $\mu$ M) was unchanged suggesting that the site is selective for ethanol induced modulation. Supported by NIAAA 15150 and Fondecyt 1020475.

**S3. CYTOSKELETAL MOTORS AND ADAPTORS FOR GABA(B) RECEPTORS IN NEURONS.** Andrés Couve. Neurotransmitter receptors are precisely located at synaptic or extrasynaptic sites. The regulation of neurotransmitter receptor trafficking, including assembly, transport, insertion, anchoring, recycling and degradation is essential to maintain and modify the efficacy of synaptic transmission. GABA, the main inhibitory neurotransmitter in the central nervous system, activates ionotropic GABA(A) and metabotropic GABA(B) receptors (GABA<sub>B</sub>Rs). The mechanisms that regulate the trafficking of GABA(B)Rs remain poorly understood and the proteins that link GABA(B)Rs to the cytoskeleton have not been described. We studied aspects of GABA(B)R trafficking in dendrites and axons of cultured hippocampal neurons combining confocal microscopy, image analysis and a new quantitative colocalization procedure. We complemented these studies with live cell imaging in neurons and biochemical analyses in the brain. Contrary to our expectations, the GABA(B)R subunits displayed high abundance of intracellular monomers in dendrites and axons, despite significant colocalization at the plasma membrane. Surprisingly, the prevalence of monomers was independent of synaptogenesis. We conclude from these experiments that GABA(B)Rs subunits are transported individually in neurons. We also explored the association of GABA(B)Rs and the interacting protein Marlin-1 to the cytoskeleton. GABA(B)Rs and Marlin-1 associated robustly with the molecular motor kinesin-I. More importantly, a kinesin-I mutant severely impaired receptor transport. We conclude that Marlin-1 and kinesin-1 link GABA(B)Rs to the tubulin cytoskeleton in neurons. (Fondecyt 1071001, ICM-P04-068-F).

**S4. ROLE OF GABAERGIC INHIBITION IN MATURATION AND PLASTICITY OF CEREBRAL CORTICES.** Bernardo Morales. Laboratory of Neurosciences, Department of Biology, University of Santiago de Chile. In the visual cortex, the experience induces profound functional and structural modifications in the cortical circuits. An example of this cortical plasticity is the shift in ocular dominance as consequence of monocular deprivation. That connectivity modification occurs during early postnatal life and during a brief period of time called critical period. LTP and LTD have been proposed as a model of synaptic plasticity in the visual cortex. Many of the basic properties of synaptic plasticity during the critical period have been described in detail. However the cellular and molecular mechanisms that initiate and eventually end the critical period are still controversial. Data of recent studies in our and other

laboratories, using whole cell recording and field excitatory postsynaptic potentials (fEPSP) techniques in visual cortex slices, suggest that maturation of the GABAergic circuits is an important factor determining the critical period for synaptic plasticity. We found that the IPSCs increased during the development in a way that is correlated with the time window of plasticity in the visual cortex. Interestingly this effect was found to depend on visual experience since raising rats in the dark inhibited the increase of the GABAergic postsynaptic currents.

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#### **S5. REPEATED COCAINE ADMINISTRATION ALTERS D2 DOPAMINERGIC MODULATION IN NUCLEUS ACCUMBENS CORE.**

La administraci3n repetida de coca3na modifica la modulaci3n dopamin3rgica D2 en n3cleo accumbens core. Perez Mariela<sup>1</sup> and Hu Xiuti<sup>2</sup>. <sup>1</sup> Departamento de Farmacolog3a, Facultad de Ciencias Qu3micas Universidad Nacional de C3rdoba, and <sup>2</sup>Dept. of Pharmacology, Rush Medical Center, Chicago, IL. The nucleus accumbens (NAc) is an important forebrain region involved in sensitization, withdrawal effects, and self-administration of cocaine. Dopamine (DA) regulates activity of medium spiny neurons in the NAc. Both DA D1Rs and D2Rs are thought to play important roles in the development of cocaine addiction. Our recent studies have demonstrated that repeated cocaine administration decreases intrinsic excitability in NAc neurons of drug-withdrawn rats. The decreased excitability is characterized by a reduction in whole-cell voltage-sensitive Na<sup>+</sup> currents and high-voltage activated (HVA-) N- and R-type Ca<sup>2+</sup> currents (I<sub>Ca</sub>), along with enhanced voltage-gated K<sup>+</sup> currents. However, although the reduced I<sub>Ca</sub> in cocaine-withdrawn NAc neurons appears to be primarily modulated by enhanced activity of the D1R/PKA/PP1 pathway via indirect dephosphorylation of non-L-type Ca<sup>2+</sup> channels, D2R modulation of Ca<sup>2+</sup> channel function in these cells is not clearly understood. In the present study, we performed whole-cell current-clamp recordings in brain slices to determine whether chronic cocaine exposure alters D2R modulation of Ca<sup>2+</sup> plateau potential in NAc MSN located in the core region. Rats received saline or repeated cocaine administration for 5 consecutive days followed by either a short- or long-term withdrawal. In saline-withdrawn rats, D2R stimulation suppressed HVA-Ca<sup>2+</sup> potentials in NAc neurons in a dose-dependent manner. This inhibitory effect of D2Rs on Ca<sup>2+</sup> potentials was also mimicked by calcineurin and blocked by inhibition of calcineurin activity. However, the D2R-mediated inhibition in Ca<sup>2+</sup> potentials was almost abolished after repeated cocaine administration with either a short- or long-term withdrawal. These results indicate that D2R stimulation suppresses HVA-Ca<sup>2+</sup> potentials by activating calcineurin that dephosphorylates Ca<sup>2+</sup> channels; repeated cocaine administration diminishes this D2R effect on inhibiting Ca<sup>2+</sup> potentials by decreasing calcineurin function; and chronic cocaine-induced maladaptations in D2R function could last for at least three weeks of withdraw.

#### **S6. HETEROMERIC ADENOSINE RECEPTORS: A DEVICE TO REGULATE NEUROTRANSMITTER RELEASE AND CAFFEINE TOLERANCE.**

Francisco Ciruela. Departament de Bioqu3mica i Biologia Molecular, Facultat de Biologia, Universitat de Barcelona, 08028 Barcelona, Spain.. Since 1990 it has been proved that dimers are the basic functional form of nearly all G-protein-coupled receptors (GPCRs) and that homo- and heteromerization may play a key role in the proper receptor

maturation and trafficking to the plasma membrane. Notwithstanding, homo- and heteromerization of GPCR has become a matter of debate especially in searching for the precise physiological meaning of this phenomenon. This presentation focuses on how heteromerization of adenosine A<sub>1</sub> and A<sub>2A</sub> receptors, which are coupled to apparently opposite signalling pathways, allows adenosine to exert a fine-tuning modulation of striatal glutamatergic neurotransmission, providing a switch mechanism by which low and high concentrations of adenosine inhibit and stimulate, respectively, glutamate release. Furthermore, it is also shown that A<sub>1</sub>R-A<sub>2A</sub>R heteromers constitute a unique target for caffeine and that chronic caffeine treatment leads to modifications in the function of the A<sub>1</sub>R-A<sub>2A</sub>R heteromer that could underlie the strong tolerance to caffeine's psychomotor effects. Ref. Ciruela et al. Presynaptic control of striatal glutamatergic neurotransmission by adenosine A<sub>1</sub>-A<sub>2A</sub> receptor heteromers. *J Neurosci.* 2006, 26(7):2080-7

**S7. INACTIVATION OF THE INTEROCEPTIVE INSULA DISRUPTS DRUG CRAVING AND MALAISE INDUCED BY LITHIUM.** “La inactivación de la insula interoceptiva previene el craving por droga y el malestar inducido por litio”. Marco Contreras Abarca. Departamento de Ciencias Fisiológicas, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. Addiction profoundly alters motivational circuits so that drugs become powerful reinforcers of behavior. The interoceptive system continuously updates homeostatic and emotional information as key elements in motivational decisions. We tested the idea that interoceptive information is essential in drug craving and in the behavioral signs of malaise. We inactivated the primary interoceptive cortex in amphetamine experienced rats which prevented the urge to seek amphetamine in a place preference task. Interoceptive insula inactivation also blunted the signs of malaise induced by acute lithium administration. Drug-seeking and malaise both induced Fos expression in the insula. We conclude that the insular cortex is a key structure in the perception of bodily needs that provides direction to motivated behaviors. Financed by Fondecyt 1060476 and Iniciativa Científica Milenio.

**S8. No hay resumen**

**S9. No hay resumen**

**S10. No hay resumen**

**S11. No hay resumen**

**S12. PERSISTENCE OF LONG-TERM MEMORY STORAGE REQUIRES A LATE PROTEIN SYNTHESIS- AND BDNF-DEPENDENT PHASE IN THE HIPPOCAMPUS.** La persistencia de la memoria de largo término requiere de una fase tardía dependiente de síntesis proteica y BDNF en el hipocampo. Pedro Bekinschtein<sup>1</sup>, Martín Cammarota<sup>1,3</sup>, Lionel Müller Igaz<sup>1,4</sup>, Lia R. M. Bevilaqua<sup>3</sup>, Iván Izquierdo<sup>3</sup> and Jorge H. Medina<sup>1,2,1</sup>. Instituto de Biología Celular y Neurociencias, <sup>2</sup> Departamento de Fisiología, Facultad de Medicina, UBA, Buenos Aires, Argentina. <sup>3</sup> Centro de Memoria, Instituto de Pesquisas Biomédicas, PUCRS, Porto Alegre, Brazil. <sup>4</sup> Current Address for Lionel Müller Igaz Center for Neurodegenerative Disease Research, University of Pennsylvania, School of Medicine, Philadelphia, USA. Persistence is the most

characteristic attribute of long-term memory (LTM). To understand LTM, we must understand how memory traces persist over time despite the short-lived nature and rapid turnover of their molecular substrates. It is widely accepted that LTM formation is dependent upon hippocampal *de novo* protein synthesis and Brain-Derived Neurotrophic Factor (BDNF) signaling during or early after acquisition. Here we show that 12 h after acquisition of a one-trial associative learning, a novel protein synthesis and BDNF-dependent phase in the rat hippocampus is critical for the persistence of LTM storage. Our findings indicate that a delayed stabilization phase is specifically required for the maintenance, but not the formation, of the memory trace. We propose that memory formation and memory persistence share some of the same molecular mechanisms and that recurrent rounds of consolidation-like events take place in the hippocampus for maintenance of the memory trace.

### **S13. SPATIAL MEMORY TRAINING IN RATS STIMULATES RyR3, DMT1 AND PKM $\zeta$ EXPRESSION.**

El entrenamiento espacial aumenta en ratas la expresión del transportador de hierro DMT1, RyR3 Y PKM $\zeta$ . <sup>1</sup>Haeger, P, <sup>1</sup>Adasme, T., <sup>1</sup>Carrasco M.A., <sup>2</sup>Núñez, M.T. & <sup>1</sup>Hidalgo, C. <sup>1</sup>FONDAP-CEMC, F. Medicina, <sup>2</sup>F. Ciencias, U. de Chile. Spatial memory has been associated with the hippocampus in mammalian brain. The contributions of Brain Derived Neurotrophic factor (BDNF) and calcium to the acquisition and consolidation stages of spatial memory, and a role for protein kinase M $\zeta$  (PKM $\zeta$ ) in consolidation have been reported. Late-LTP studies indicate that hippocampal postsynaptic calcium signals arising from N-methyl-D-aspartate (NMDA) receptor activation are amplified by calcium-induced calcium release (CICR) through ryanodine receptors (RyR). Previous work from our group has shown that iron-generated hydroxyl radical promotes RyR-mediated CICR in hippocampal cells in primary culture. Our present aim is to study the expression of 3 proteins - RyR, the iron transporter DMT1, and PKM $\zeta$  - in the hippocampus of rats trained in a Morris water maze (MWM), and in hippocampal cells in primary culture stimulated with BDNF. Rats were trained for spatial memory (5d, 2d rest, 2d platform free) in the MWM and the hippocampus was dissected 6 h after the last behavioral task. Ryanodine (4  $\mu$ g) or vehicle was injected intra-CA1 24 h before initiating training. Hippocampal cells in primary culture were incubated with 50 ng/ml BDNF for 6 h. Tissue or cell cultures samples were prepared for immunofluorescence or RT-PCR experiments. The hippocampus of trained rats showed increased expression of RyR3 protein and of DMT1 and PKM $\zeta$  mRNAs. Intra-CA1 injection of ryanodine increased the acquisition rate of the spatial memory task. Parallel experiments showed that hippocampal cells incubated with BDNF displayed increased expression of RyR3 protein and of DMT1 and PKM $\zeta$  mRNAs; 1 h pre-incubation with 50  $\mu$ M ryanodine prevented this increase in mRNA levels. Our results suggest that training in a spatial memory task promotes the expression of key proteins involved in calcium signal generation, iron uptake and spatial memory consolidation. Results from *in silico* analysis indicate that RyR, DMT1 and PKM $\zeta$  promoter genes have calcium-dependent response elements. Thus, RyR-mediated CICR, which is also activated by iron, may contribute to enhance RyR, DMT1 and PKM $\zeta$  expression in the hippocampus of spatially trained rats. Supported by FONDECYT-Post-Doc 3070035, CEMC-FONDAP 15010006, Millennium Institute P05-001F, and FONDECYT 1060177.

**S14. TRPM7 ION CHANNEL CONDUCTANCE IS REQUIRED FOR THE FUSION OF ACETYLCHOLINE- CONTAINING VESICLES WITH THE PLASMA MEMBRANE.** La fusión de vesículas que contienen acetilcolina requiere de la conductancia del canal de iones TRPM7. Sebastian Brauchi, Grigory Krapivinsky, Luba Krapivinsky and David E. Clapham Howard Hughes Medical Institute, Cardiology Children's Hospital Boston, Harvard Medical School TRPM7 is a channel/kinase of the transient receptor potential family. Previously we showed that this protein is in the membranes of acetylcholine (ACh)-secreting synaptic vesicles of sympathetic neurons, forms a molecular complex with proteins of the vesicular fusion machinery, and is critical for stimulated neurotransmitter release (Krapivinsky et al. 2006). It was unclear whether TRPM7 was required for synaptic vesicle fusion with synaptic membranes, or rather, affected neurotransmitter content of the vesicle. To monitor single vesicle fusion events, we constructed a pH-sensitive GFP (pHluorin) fused with the vesicular ACh transporter (VAcHT). Vesicle fusion with the plasma membrane exposes pHluorin to pH 7.5 solution, substantially enhancing pHluorin fluorescence. PC-12 cells containing small synaptic-like vesicles (SSLV) loaded with ACh were monitored by total internal reflection fluorescence (TIRF) microscopy. Here we show that endogenous and expressed TRPM7 and VAcHT-pHluorin were co-localized to SSLVs. VAcHT-pHluorin-transfected cells exhibited spontaneous transient point flashes of light, reflecting single vesicle fusion; the frequency of these events was increased by cell depolarization. TRPM7 knockdown by the siRNA, or abolishing channel activity by expression of the dominant negative TRPM7 (dnTRPM7) pore mutant, prevented spontaneous and voltage-stimulated vesicle fusion events. In contrast, expression of a TRPM7 kinase-dead mutant did not affect vesicle fusion frequency. Fluorescence decay time remained unchanged in siRNA and dnTRPM7-transfected cells. We conclude that TRPM7's conductance across the vesicle membrane is required for the vesicle fusion process in these cells. This work was supported by Howard Hughes Medical Institute and Pew Programs in the Biomedical Sciences.

**S15. DURATION AND DELAY TUNING IN THE INFERIOR COLLICULUS OF THE LONG-CONSTANT FREQUENCY BAT *Pteronotus parnellii*.** Silvio Macías<sup>1</sup>, Emanuel C. Mora<sup>1</sup> and Manfred Kössl<sup>2</sup>. <sup>1</sup> Departamento de Biología Animal y Humana, Facultad de Biología, Universidad de La Habana, La Habana, Cuba. <sup>2</sup> Instituto de Biología Celular y Neurociencias, Universidad de Frankfurt/Main, Frankfurt/Main, Alemania. Neurons measuring time in the auditory system of bats can respond selectively to specific delays between call and echo or to certain sound durations, and were described at and above the level of the inferior colliculus. Delay-tuned neurons have been studied in species of FM bats (bats with frequency modulated echolocation calls) and CF bats (bats with constant frequency echolocation calls), and recognize target range. Duration-selective neurons, however, have been studied only in certain species of FM bats and recognize species-specific calls. For both types of neurons the mechanism underlying the measurement of time has been modeled using inhibitory and excitatory neuronal inputs with specific temporal relations. In addition, studies on duration- and delay-tuning have been conducted independently on each of two species of FM bats, in which similar operational time ranges (1 - 20 ms) have been found. It is thus still unclear whether the same neuron could selectively respond to certain durations and delays and whether same mechanisms are involved. CF bats are a unique biological

model to clarify these issues. We found duration-sensitive neurons in the central nucleus of the IC of the long CF-bat *Pteronotus parnellii* with a tuning preference to 12-21 ms which correspond to the duration of echolocation calls. A subpopulation of the neurons was also sensitive to echo-call delays in the same time range. We also found a large population of delay-tuned neurons that responded to FM-FM combinations, as they have not been described for the IC previously. Currently, we are studying duration tuning and FM-FM sensitivity and to investigate in detail neurons that are both duration- and delay-tuned.

## COMUNICACIONES LIBRES

**C1. POSTNATAL EPENDYMOGENESIS ¿AN UNDERESTIMATED PHENOMENON?.** Ependimogénesis postnatal: ¿un fenómeno subestimado?. Luis Federico Batiz<sup>1</sup>, Antonio J. Jiménez<sup>2</sup>, César D. Toledo<sup>1</sup>, José Manuel Pérez-Figares<sup>2</sup>, Esteban M. Rodríguez<sup>1</sup>. <sup>1</sup> Instituto Anatomía, Histología y Patología. Fac. Medicina. Universidad Austral de Chile. <sup>2</sup> Depto. Biología Celular. Universidad de Málaga, Spain. [federicobatiz@uach.cl](mailto:federicobatiz@uach.cl) Background. The *hyh* (hydrocephalus with hop gait) mouse carries a point mutation in alpha-SNAP protein and develops inherited hydrocephalus. Mutant mice are born with moderate hydrocephalus and a patent Sylvius aqueduct (SA) but during the first postnatal week, SA obliterates and a severe hydrocephalus, characterized by enormous expansions of the dorsal walls of the third ventricle (3Vd) and the SA (SA), develops. Interestingly, neither of these dilated cavities presents spontaneous ventriculostomies and both structures remain lined by ependymal cells, regardless the age of the mouse or the severity of hydrocephalus. What are the cellular mechanisms occurring at ventricular walls that allow such enormous dilations without ventriculostomies nor ependymal loss? How can ependymal surface increase when ventricles enlarge? Material and Methods. Brains of wild type (non-hydrocephalic) and mutant (hydrocephalic) *hyh* mice were studied by light and scanning electron microscopy at various age intervals (P1 to P30). Proliferative activity, especially at the ventricular walls, was studied by PCNA (proliferative cell nuclear antigen) immunohistochemistry, and 5'-Bromo-2-deoxyUridine (BrdU) labelling. **Results.** In wild-type mice no BrdU-labelled or PCNA-positive cells were observed in the ependyma of the ventral walls of SA and third ventricle. However, proliferative cells were found in two discrete ependymal regions of the 3Vd and the SA. Here, proliferative activity continued at least during three weeks after birth and showed a distinct temporal and spatial profile. The localization, cytology and immunocytochemical properties indicate that both regions originate ependymal cells postnatally. Interestingly, in mutant (hydrocephalic) *hyh* mice, this phenomenon increased several folds and is related to the enlargement of the surface of these ventricular walls. **Conclusions.** 1. In non-hydrocephalic animals all ependymal cells lining the floor of the aqueduct are born during the fetal life; however, in the dorsal wall of the aqueduct and the roof of the third ventricle ependymogenesis continues during postnatal life. 2. In mutant mice, the hydrocephalic process triggers a dramatic increase of proliferative activity in these two ventricular regions letting them to expand without any disruption and, probably, allowing a longer survival. 3. In the cerebral aqueduct there are various ependymal lineages: one of them detaches, other proliferates while another neither detaches nor proliferates. Since all these ependymal populations are exposed to the same environment, their differential response to the hydrocephalic status

can best be explained by their distinct genetic programme. Supported by Fondecyt 1030265/1070241 to EMR, CONICYT and DID-UACH D-2005-12 to LFB, FIS PI030756/PI060423, Spain to JMPF.

**C2. SVCT2 VENTRICULAR POLARIZATION IN RADIAL GLIA. IMMUNOHISTOCHEMISTRY AND *IN UTERO* ELECTROPORATION ANALYSIS.** Polarización ventricular de SVCT2 en la glia radial. Análisis inmunohistoquímico y por electroporación *in utero*. Carmen Silva-Alvarez, Pedro Cisternas, Katterine Salazar, Sean Liour<sup>#</sup>, Francisco Nualart. Research Center for the Study of the Nervous System: Cell Biology and Biomedical Applications. Department of Cell Biology, Concepcion University, Chile. <sup>#</sup>Developmental Neurobiology Program, Institute of Molecular Medicine and Genetics (IMMG), Medical College of Georgia (MCG), Augusta, Georgia, USA. Due to its role as a precursor cell for neurons and glia, radial glia is considered the main stem cell of the Central Nervous System (CNS). Radial glia cells have an elongated morphology and takes contact with the meningeal surface and cerebrospinal fluid (CSF). Furthermore, this cell guides cortical neuron migration and later transforms into an astrocyte. At the present, the factors that modulate radial glia differentiation are unknown; however, this process could be stimulated by vitamin C. Vitamin C (ascorbic acid) uptake into adult CNS cells is mediated by the SVCT2 transporter; however, there is no evidence about the presence of this transporter during brain development. Accordingly, we have defined the expression and localization of SVCT2 in radial glia during brain cortical development (E11, E13, E15, E17 and E19 stages). SVCT2 mRNA was detected in the periventricular area of radial glial cells and in cortical neurons by RT-PCR and *in situ* hybridization. Additionally, we demonstrated SVCT2 polarization in the ventricular edge of radial glia by immunohistochemical studies. Furthermore, a similar distribution of SVCT2 was observed in human embryonic brain. In order to study SVCT2 over-expression during CNS development *in vivo*, we performed *in utero* electroporation using the pEYFP-N1/hSVCT2wt plasmid construct. We confirmed that SVCT2 is preferentially polarized to the periventricular area of the brain, suggesting that vitamin C is incorporated to the radial glia from the CSF. Supported by Grant ACT-02.

**C3. CHRONIC STRESS AFFECTS THE GROOMING AND FEAR CONDITIONING IN RATS. IMPLICATIONS IN THE DEVELOPMENT OF SOME DEPRESSIVE SYMPTOMS.** (El estrés crónico afecta el grooming y el condicionamiento al miedo en ratas. Implicaciones en el desarrollo de algunos síntomas depresivos). Dagnino A.<sup>1,2</sup>, Muñoz P.<sup>1</sup>, Terreros G.<sup>2</sup>, Wyneken U.<sup>3</sup>, Díaz-Véliz G.<sup>4</sup>, Aboitiz F.<sup>2</sup>. <sup>1</sup>Laboratorio de Neurociencia Conductual y Neurobiología, Facultad de Medicina, Universidad Católica del Norte. <sup>2</sup>Centro de Estudios Neurobiológicos, Facultad de Medicina, Pontificia Universidad Católica de Chile. <sup>3</sup>Laboratorio de Neurociencia, Facultad de Medicina, Universidad de los Andes, <sup>4</sup>Programa de Farmacología, Facultad de Medicina, Universidad de Chile. Previously we found stress impair the auditory system and decrease the level of fear threshold activation in the rat brain. In this study we analyze whether these alterations affect to complex behaviors such as grooming and fear conditioning. We found the increased freezing induced by chronic stress throughout fear conditioning did not relate to the acquisition of an aversive memory in the rat brain, the footshock used during fear conditioning by itself

can increase the freezing in both control and stressed rats. In addition, chronic stress decreased the time and number of grooming, and the auditory unconditioned stimuli ( $\geq 90$  dB) increased selectively the number of grooming interruptions in the stressed rats. Our results suggest that the auditory conditioned stimuli ( $\leq 80$  dB) became processed in part as auditory unconditioned stimuli in the stressed animals. Comparable alterations could be induced by the psychosocial stress in humans and have a role in the development of depressive disorders. Acknowledgements: This work was supported by Anillo de Ciencia y Tecnología ACT09-2006 and DGIP 540101-10301217-Universidad Católica del Norte grants (to A. D.) and Núcleo Milenio de Neurociencias Integradas grant (to F. A.).

**C4. CHANGES OF THE EPSP WAVEFORM REGULATE THE TEMPORAL WINDOW FOR SPIKE TIMING DEPENDENT PLASTICITY.** CAMBIOS EN LA FORMA DE ONDA DEL “EPSP” REGULA LA VENTANA TEMPORAL DEL “SPIKE TIMING DEPENDENT PLASTICITY”. Marco Fuenzalida<sup>1,2</sup>, David Fernández de Sevilla<sup>1</sup> y Washington Buño<sup>1</sup> 1- Instituto Cajal, Madrid, España, 2-Centro de Neurobiología y Plasticidad del Desarrollo, Departamento Fisiología Facultad de Ciencias, Universidad de Valparaiso, Chile. Using spike-timing dependent plasticity (STDP) protocols that consist in pairing an EPSP and postsynaptic back propagating action potential (BPA) we investigated the contribution of the changes in EPSP waveform induced by the slow Ca<sup>2+</sup>-dependent after hyperpolarization (sAHP) in the regulation of long term potentiation (LTP). The ‘temporal window’ between Schaffer collateral EPSP and back propagating action potentials in CA1 pyramidal neurons required to induce LTP was narrowed by a reduction of the amplitude and decay time constant of the EPSP that could be reversed with cyclothiazide. The EPSP changes were caused by the increased conductance induced by activation of the sAHP. Therefore the EPSP waveform and its regulation by the sAHP are central in determining the duration of the temporal window for STDP, thus providing a possible dynamic regulatory mechanism for the encoding of cognitive processes. Patrocinio: Manuel Roncagliolo Pastene. Fuentes de financiamiento: Proyectos Dr. W. Buño: Dirección General de Investigación Científica y Tecnológica, Ministerio de Ciencia y Tecnología (BFI2002-01107 y BFU2005-07486) y Comunidad Autónoma de Madrid (GR/SAL/0877/2004) España.

**C5. THE ORPHAN RECEPTOR NURR1 ACTIVATES THE TRANSCRIPTION OF THE TYROSINE HYDROXYLASE AND C-RET GENE PROMOTERS: EXPLORING THE *IN VIVO* BINDING OF NURR1 TO DIVERSE GENOMICS ELEMENTS.** El receptor huérfano Nurr1 activa la transcripción de los promotores de Tirosina Hidroxilasa y c-Ret: explorando la unión *in vivo* de Nurr1 a distintos elementos génicos. Galleguillos, Danny & Andrés, María Estela. Dpto. de Biología Celular y Molecular, Fac. de Cs. Biológicas, P. Universidad Católica de Chile. email: [degalleg@puc.cl](mailto:degalleg@puc.cl). The orphan nuclear receptor Nurr1 is critical for the generation of dopaminergic neurons of the Substantia Nigra (SN) and the Ventral Tegmental Area. Functioning of Nurr1 has been associated with the maintenance of the midbrain dopaminergic phenotype in the adult brain and it has also been suggested that Nurr1 could participate in adaptive responses to damage. Studies in the Nurr1 knock out mice have shown that Nurr1 is necessary for the expression of Tyrosine Hydroxylase (TH)

and of the GDNF receptor c-Ret in SN. However, the role of Nurr1 in the adult dopaminergic system remains elusive. Rat TH promoter harbors four NGFI-B response elements (NBRE). Nurr1 can activate the transcription of the rat TH gene promoter in various cell lines, yet, it is still unclear the mechanism and which NBRE elements of the TH promoter are bound by Nurr1 *in vivo*. By using chromatin immunoprecipitation (ChIP) and Real Time PCR we have determined that Nurr1 is bound to two different regions in the rat TH promoter in the SN obtained from adult rats. On the other hand, sequence analysis of the rat c-Ret promoter reveals the presence of two NBRE containing regions. By means of ChIP assays we show that Nurr1 is bound to one of these regions in rat SN. This result suggests that Nurr1 indeed could participate in the transcription of the c-Ret gene. To explore this possibility we study the effect of Nurr1 over the activity of the human c-Ret promoter. Using different cell lines we show that Nurr1 induces the activation of the human c-ret promoter in a cell type specific- and dose-dependent manner. This effect requires the AF2 domain of Nurr1. Using transient transfection of serial deletions of the c-Ret promoter in a human neuroblastoma cell line we show that Nurr1 activity does not require the presence of any NBRE in the human c-Ret promoter. These results suggest that Nurr1 enhances the human and rat c-Ret promoter activity through different mechanisms.

Funded by FONDECYT N° 107-0349

#### **C6. PARS TUBERALIS-SPECIFIC CELLS SECRETE TUBERALIN II UNDER THE INFLUENCE OF MELATONIN.**

Las células específicas de la pars tuberalis secretan tuberalina II bajo la influencia de melatonina. Guerra M, Vásquez P, Toranzo D, Rodríguez S, Carvajal A, Blázquez JL, Pelaez B, Pastor F, Rodríguez EM. Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral de Chile. Valdivia, Chile

Departamento de Anatomía e Histología Humana, Facultad de Medicina, Universidad de Salamanca, Salamanca, España. The rat pars tuberalis (PT) of the adenohypophysis is formed by three populations of cells: cells secreting PD hormones (LH, TSH), PT-specific cells and follicular cells. The actual cellular organization of the PT, the functional significance of its cells populations as well as its spatial relationship with the median eminence, is not known. The present investigation has dealt with the fine structural organization and immunocytochemical properties of the PT of control and pinealectomized rats. Antibodies against the following compounds were used: tuberalin II, beta-TSH, beta-LH, alpha-subunit common to TSH, LH and FSH, melatonin receptors I and II. Results: (i) PT-specific secretory cells are embedded in a network of intercellular channels. The channels are in open communication with the subarachnoid cerebrospinal fluid. Each secretory cell projects a single (9+0) cilium into these channels. Follicular cells are endowed with a rich tubular and vesicular system (smooth endoplasmic reticulum?) and devoid of secretory granules. Beta 1 tanycytes of the median eminence establish close contact with the PT-specific cells localized laterally. (ii) PT-specific cells react with anti-tuberalin II, anti-alpha-subunit and anti-TSH (iii) PT-specific cells and tanycytes display immunoreactive MEL II receptor. (iv) After 1 and 2 weeks of pinealectomy PT-specific cells have a hypertrophied Golgi complex, a reduced number of secretory granules, a large expansion of the intercellular channels and numerous granules undergoing exocytosis. (v) Pinealectomy resulted in a drastic reduction of tuberalin II immunoreactivity but TSH immunoreactivity did not change.

Conclusions: 1. PT-specific cells secrete tuberalin II and alpha-subunit and express MEL II receptors. 2. Secretion of tuberalin II by PT-specific cells would increase after

pinealectomy. 3. Pineal melatonin would reach the PT-specific cells via the CSF filling the intercellular channels of the PT. Supported by DID-UACH S-2006/13 (MG) and Fondecyt 1070241 (EMR)

**C7. SAPORIN-OREXIN-B LESIONS IN THE LATERAL HYPOTHALAMUS OF THE RAT: RAPID EYE MOVEMENT (REM) SLEEP REBOUND AFTER SELECTIVE REM SLEEP DEPRIVATION.** Lesiones neurotóxicas en el hipotálamo lateral orexinérgico mediante el conjugado saporina-orexinaB: homeostasis del sueño de Movimientos Oculares Rápidos. Francisco Ibáñez<sup>1</sup>, Sebastián López<sup>1</sup>, Fernando Torrealba<sup>2</sup>, Ennio Vivaldi<sup>1</sup>, Adrián Ocampo-Garcés<sup>1,3</sup>. <sup>1</sup> Programa de Fisiología y Biofísica, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile. <sup>2</sup>Departamento de Fisiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, <sup>3</sup>Dep. Neurología y Neurocirugía, Hospital Clínico Universidad de Chile. The lateral hypothalamic orexinergic system is involved in the control of the mammalian sleep-wake cycle, favoring the stabilization of wakefulness. Lesions in the orexinergic system are associated to a deep disorganization of the architecture of sleep cycle, particularly the temporal profile of REM sleep. Here we explore the REM sleep homeostatic rebound after selective REM sleep deprivation placed at rest and active phases in saporin-orexin-B lesioned rats under skeleton photoperiod (SP). Male Sprague-Dawley rats were bilaterally injected with 200 ng (0.5 ul) of saporin-orexin-B conjugate in the lateral hypothalamus. After three weeks of recovery, polygraphic recordings started under a 12:12 LD (lights-on at 8:00 AM) for three days, and then transferred to a SP (20 minute light pulses at local time 8:00-8:20 and 19:40-20:00 hours). “Rest phase” and “active phase” correspond to the 8:00 to 19:59 and 19:00 to 7:59 hour intervals respectively. Three hours of selective REM sleep deprivation were performed during rest and active phases in non-consecutive days under SP. Lesions were evaluated by counting hypothalamic orexin-A immunoreactive neurons. Lesions that affected more than 30% of orexinergic neurons tend to abolish REM sleep rebound. Our results suggest that the hypothalamic orexinergic system is involved in executive mechanisms of REM sleep homeostatic response in the rat. FONDECYT 1061089

**C8. ENHANCEMENT OF DELTA BAND (1-4 Hz) POWER SPECTRUM DURING NON-REM SLEEP INTERFERES WITH LONG-TERM REM SLEEP HOMEOSTASIS IN THE RAT.** El aumento del poder espectral de la banda Delta del sueño No-MOR interfiere en la homeostasis de largo plazo del sueño MOR. Sebastián López<sup>1</sup>, Francisco Ibáñez<sup>1</sup>, Enzo Brunetti<sup>1</sup> and Adrián Ocampo-Garcés<sup>1,2</sup>. <sup>1</sup> Programa de Fisiología y Biofísica, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile. <sup>2</sup>Dep. Neurología y Neurocirugía, Hospital Clínico Universidad de Chile. Delta band (1-4 Hz) power spectrum observed in the EEG at the beginning of a Non-REM (NREM) sleep episode is function of the time accumulated in wakefulness. It decays exponentially during the NREM sleep episode, suggesting that NREM sleep expression is under the control of an hourglass type sleep homeostat, whose output is somehow related to thalamo-cortical circuits involved in cortical synchronization. It has been described that the enhancement of NREM sleep EEG delta power obtained after total sleep deprivation interferes with REM sleep expression. Here we explore the REM sleep rebound occurring under high and low delta power conditions. An intermittent

REM sleep deprivation protocol (IRD) was applied after two hours of Total or REM-selective sleep deprivation (TSD and RSD respectively). Ten chronically implanted rats under a 12:12 light:dark cycle were subjected after baseline days to: a) 2T2I protocol: 2 hours TSD followed by 2 hours of IRD; (b) 2R2I protocol: 2 hours of RSD followed by 2 hours of IRD. IRD consisted in the alternation, for four successive times, of 10 minutes of spontaneous sleep (REM sleep permission window), and 20 minutes of selective REM sleep deprivation. Protocols sessions were started at hour 4 after lights-on. Delta power was estimated by means of Fast Fourier Transformation of the EEG signal. Delta power measured after two hours of total sleep deprivation is 40% higher than after 2 hours of selective REM sleep deprivation. Consistently, REM sleep rebound is delayed in 2T2I respect to 2R2I. The rate of NREM to REM sleep transitions is not affected by factor protocol whereas the REM sleep episodes obtained during IRD of 2R2I are notoriously longer. Our results suggest that delta power enhancement during NREM sleep interferes with long term REM sleep homeostasis (consolidation of REM sleep episodes) but not with short-term REM sleep homeostasis (NREM to REM sleep transitions rate). FONDECYT 1061089

**C9. Ih IN PERIPHERAL COLD TERMORRECEPTORS: ELETROPHYSIOLOGICAL CHARACTERIZATION, MOLECULAR IDENTITY AND PHYSIOLOGICAL ROLE.** Ih en termorreceptores periféricos sensibles a frío: caracterización electrofisiológica, identidad molecular y papel fisiológico. Patricio Orio<sup>1,2</sup>, Tansy Donovan-Rodríguez<sup>2</sup>, Carlos Belmonte<sup>2</sup> y Félix Viana<sup>2,1</sup>. Centro de Neurociencia de Valparaíso, Universidad de Valparaíso, Chile and <sup>2</sup>Instituto de Neurociencias de Alicante, Alicante, España. Hyperpolarization-activated currents (Ih) in excitable cells are functionally diverse and mediated by the expression of different combinations of hyperpolarization-activated, cyclic-nucleotide-gated (HCN) channel subunits. We investigated the expression and functional properties of Ih in cold-sensitive (CS) trigeminal ganglion neurons. All CS neurons expressed a hyperpolarization-activated inward current fully blocked by HCN channel blockers such as Cs<sup>+</sup> (3mM) and ZD7288 (20 μM). The kinetic properties and activation curve of Ih in CS neurons were very similar to those recorded in HEK293 cells after expression of heteromeric HCN1/HCN2 channels. Like other Ih-expressing neurons, CS neurons show a strong sub-threshold resonance in the 5-7 Hz range which completely disappears upon HCN channel blockade. However, blockade of Ih did not abolish the electrical response to a cold or menthol stimulus. The effect of Ih blockade was also studied in CS nerve endings from the guinea pig cornea. CS nerve endings show a spontaneous activity of 5-8 nerve terminal impulses (NTIs) per second, with an increase in frequency (up to 30 s<sup>-1</sup>) upon cooling or exposure to menthol. After addition of ZD7288 (50 μM), the spontaneous NTIs were progressively reduced and even disappeared, however the response to cooling was maintained. These results show that Ih in trigeminal CS neurons is sustained by heteromeric HCN1/HCN2 channels, and suggest that Ih participates in the membrane potential oscillations that originate the spontaneous activity of cold-sensitive nerve terminals. However, Ih does not seem to have a role in the transduction of the cold stimulus.

**C10. SVCT2 AND GLUT1 ARE INVOLVED IN THE UPTAKE OF VITAMIN C TO THE CENTRAL NERVOUS SYSTEM.** (SVCT2 y GLUT1 están involucrados en

*el transporte de vitamina C al sistema nervioso central*). Viviana Ulloa\*, María de los Angeles García, Karin Reinicke, Fernando Pérez, Francisco Nualart. Research Center for the Study of the Nervous System: Cell Biology and Biomedical Applications. Department of Cell Biology, Concepcion University, Chile. Vitamin C is incorporated from the plasma to the Central Nervous System through the choroid plexus cells. It has been postulated that the basolateral membranes of choroid plexus cells (contacting the blood vessels), are involved in the uptake of the reduced form of vitamin C, ascorbic acid (AA) using the sodium vitamin C transporter (SVCT2). In addition, it has been demonstrated that the oxidized form of the vitamin C, dehydroascorbic acid (DHA), is incorporated to the cells through the facilitative glucose transporters (GLUTs). Using immunohistochemistry and confocal microscopy analysis we demonstrated the normal basolateral co-localization of SVCT2 and GLUT1 in mouse choroid plexus explants. The expression of these transporters was confirmed by RT-PCR and Western blot. Additionally, we determined the kinetic parameters of GLUT1 and SVCT2 in cells isolated from human choroid plexus papilloma. We defined an affinity constant of 1.5 mM for GLUT1 (DHA uptake) and 30  $\mu$ M for SVCT2 (AA uptake). Finally, to demonstrate the DHA uptake associated to GLUT1, we performed choroid plexus cells and PMA-activated human neutrophils co-cultures. We detected a marked increase in vitamin C uptake by the choroid plexus cells that is associated to the superoxide generation and vitamin C oxidation (*Bystander effect*). Choroid plexus cell use two different transporters to increase intracellular concentration of vitamin C. This mechanism is amplified during neutrophils infiltration (inflammation) or in choroid plexus tumours.. Grant Ring ACT-02.

**C11. HALONITROCYTISINE DERIVATIVES: THE INTERACTION OF C-3 AND C-5 SUBSTITUENTS IN MODULATING AFFINITY AND EFFICACY AT CENTRAL NICOTINIC  $\alpha$ 4 $\beta$ 2 RECEPTORS.** Derivados halonitrados de citisina: la interacción de los substituyentes en C-3 y C-5 en la modulación de la afinidad y la eficacia en receptores nicotínicos neuronales  $\alpha$ 4 $\beta$ 2. Patricio Sáez-Briones,<sup>1</sup> Marco Rebolledo-Fuentes,<sup>2</sup> Mirko Moroni,<sup>3</sup> Anna Carbone,<sup>3</sup> Bruce K. Cassels<sup>2</sup> & Isabel Bermúdez<sup>3</sup> <sup>1</sup> Facultad de Ciencias Médicas, Universidad de Santiago de Chile. <sup>2</sup> Facultad de Ciencias, Universidad de Chile. <sup>3</sup> School of Life Sciences, Oxford Brookes University (UK). Because of their role in the modulation of dopamine release in the mesolimbic dopaminergic pathway,  $\alpha$ 4 $\beta$ 2 nicotinic acetylcholine receptors are believed to represent a major therapeutic target for the rational design of drugs for novel smoking cessation therapies. (-)-Cytisine, an alkaloid of the quinolizidine family, has been used as a lead compound in the development of new molecules with affinity and potency at neuronal nicotinic acetylcholine receptors. Among these, mono-halogenated cytisine derivatives at positions C-3 and C-5 have been characterized as highly selective and potent agonists at  $\alpha$ 4 $\beta$ 2 receptors. Moreover, it has been demonstrated that substitution at C-3 seems to be critical for the modulation of affinity and selectivity at the receptor binding site. On the other hand, the pharmacological characterization of 3-nitrocytisine indicated that the incorporation of a polar group at C-3 produced a low-efficacy derivative only at  $\alpha$ 4 $\beta$ 2 receptors, without altering affinity. In order to explore the effect of nitration on the affinity and efficacy of C-3 and C-5-halogenated cytisine derivatives, we have now synthesized 5-nitrocytisine and two halonitro derivatives and characterized them pharmacologically at heterologously expressed  $\alpha$ 4 $\beta$ 2 and  $\alpha$ 7 receptors. Nitration at C-5 diminishes affinity at  $\alpha$ 4 $\beta$ 2 and  $\alpha$ 7 receptors as compared to 5-Br-cytisine. It also abolishes efficacy at  $\alpha$ 4 $\beta$ 2 receptors, a similar effect to that

observed with the 5-bromo compound. 3-Bromo-5-nitrocytisine is a partial agonist of very low efficacy, whereas 5-bromo-3-nitrocytisine is a low potency antagonist. The coexistence of a bromine and a nitro group at C-3 or C-5 seems to produce a similar pharmacological profile to that of 3,5-dibromocytisine, although some differences in efficacy were observed after nitration at C-5. Our results are in agreement with a secondary role of C-5 in the modulation of both affinity and efficacy at  $\alpha 4\beta 2$  receptors and indicates that the chemical nature of the substituent at C-3 may determine the effects on both affinity and efficacy, regardless of the substitution at C-5.

**C12. REVERSAL OF SYNAPTIC MEMORY BY  $Ca^{2+}$ /CALMODULIN-DEPENDENT PROTEIN KINASE II (CaMKII) INHIBITOR.** Reversión de la memoria sináptica por un inhibidor de CAMKII. Magdalena Sanhueza<sup>§</sup>, Charmian C. McIntyre\*, and John E. Lisman\*. <sup>§</sup>Depto de Biología, Fac. de Ciencias e Inst. Milenio ICDB, Universidad de Chile, and \*Biology Department and Volen Center for Complex Systems, Brandeis University, Waltham, MA, USA. Long-term potentiation (LTP) is an activity-dependent strengthening of synapses that is thought to underlie memory storage. CaMKII has been a leading candidate as a memory molecule because it is persistently activated after LTP induction and can enhance transmission. Furthermore, a mutation that blocks persistent activation blocks LTP and forms of learning. However, direct evidence for a role of the kinase in maintaining synaptic strength has been lacking. Here we show that a newly developed non-competitive inhibitor of CaMKII strongly reduces synaptic transmission in the CA1 region of the hippocampal slice. This occurs through both presynaptic and postsynaptic action. To study the role of CaMKII in the maintenance of LTP, inhibitor was applied after LTP induction and then removed. Inhibition occurred in both LTP and control pathways, but only partially recovered. The non-recovering component was due primarily to a postsynaptic change. To test whether nonrecovery was due to a persistent reversal of LTP, we first saturated LTP and then transiently applied inhibitor. This procedure allowed additional LTP to be induced, indicating a reversal of an LTP maintenance mechanism. This is the first procedure that can reverse LTP by chemical means and suggests that a component of synaptic memory is due to CaMKII. The procedure also enhanced the LTP that could be induced in the control pathway, consistent with the idea that CaMKII is involved in controlling basal synaptic strength, perhaps as a result of LTP that occurred in vivo. Work supported by The Pew Latin American Fellows Program for the Biomedical Sciences (MS) and NIH Grants R01 NS050944 and R01 NS027337.

**C13. EFFECT OF ERK PATHWAY ON STRUCTURAL PLASTICITY ASSOCIATED WITH THE NR2B SUBUNIT IN VENTRAL SPINAL CORD NEURONS.** Efecto de la vía ERK sobre la plasticidad estructural asociada a la subunidad NR2B en neuronas ventrales de medula espinal. Fernando J Sepúlveda<sup>1</sup>, Fernando Bustos<sup>1</sup>, Manuel Lagos<sup>1</sup>, Carlos Opazo<sup>2</sup>, Luis Aguayo<sup>2</sup>, Martin Montecino<sup>3</sup> and Brigitte van Zundert<sup>1</sup>. <sup>1</sup>Dept Physiopathology, <sup>2</sup>Dept Physiosiology, <sup>3</sup>Dept Biochem. and Mol. Biol. Faculty of Biological sciences, University of Concepción. Functional and structural plasticity is drastically reduced in the central nervous system during development. The precise molecular mechanisms of how the NMDA receptors (NMDARs) regulate structural plasticity can not be established because of the expressing of the tri-heteromers NR1NR2ANR2B. To circumvent this dilemma we used ventral spinal cord neurons (VSCNs) in which the expression of the NR2A and

NR2B subunits is suppressed. Using electrophysiological and immunocytochemical analyses we demonstrate that overexpression of either NR2A or NR2B results in functional synaptic NR1NR2A and NR1NR2B receptors, respectively. Interestingly, we found that overexpression of the NR2B subunit, but not NR2A, significantly increased dendritic outgrowth and branching in these VSCNs. Similarly, we found that overexpression of RNAi-NR2B, but not RNAi-NR2A (Kim et al., Neuron, 2005), drastically decreased structural plasticity in hippocampal neurons. Next, we started to investigate through which possible mechanism the NR2B subunit allows dynamic dendritic changes. We focused on the ERK signaling cascade because it is a key regulatory pathway of neuronal plasticity and can be activated by RasGRF1, which interacts specifically with the NR2B subunit. For this we overexpressed binding-dominants of NR2B (NR2B-BD) and RasGRF1 (RasGRF1-BD), known to specifically impede the association between NR2B and RasGRF1 (Krapavinsky et al, 2003). Analysis of VSCNs overexpressing the NR2B subunit showed that the ERK phosphorylation was reduced when either NR2B-BD or RasGRF1-BD was co-expressed. Interestingly, we also found that VSCNs overexpressing NR2B-BD or RasGRF1-BD display decreased dendritic outgrowth and branching. Together, these results suggest that structural plasticity associated with the NR2B subunit is dependent on the ERK pathway. Funded by Fundación Andes N° C-14060/25, DIUC 206.034.009-1.0 and Fondecyt 1070494.

**C14. NGF-induced ADAM17-mediated shedding of p75 by TrkA activation; a key step regulating p75 neurotrophin signaling?.** NGF induce el procesamiento de p75 por ADAM17 a través de la activación de TrkA; un factor clave para la regulación de la señalización mediada por p75?. María Soledad Urra\*, Edgardo Allende\*, Wim Annaert# and Francisca C Bronfman\*. \*Center for Cellular Regulation and Pathology (FONDAP), Faculty of Biological Sciences. Physiology Department, Catholic University of Chile. #Membrane Trafficking Laboratory, Center for Human Genetics, KULeuven/VIB4, Belgium. The p75 neurotrophin receptor (p75) is involved in several aspects of neurotrophin signaling including neuronal death and axonal elongation. p75 promotes neurotrophin-induced neuronal survival and differentiation by forming a heteromeric co-receptor complex with the TrkA receptor. These differential cellular responses are partly due to the association of p75 with different signaling complexes formed by association with different co-receptors and ligands. Moreover, p75 becomes internalized and retrogradely transported in endosomes. Another aspect involved in p75 signaling is the p75 proteolytic processing. p75 receptor was recently identified as a new substrate for Presenilin1 (PS1) mediated  $\gamma$ -secretase cleavage. p75 is first cleaved by a metalloprotease generating a membrane associated carboxy-terminal fragment (p75-CTF) followed by  $\gamma$ -secretase cleavage releasing an intracellular fragment (p75-ICD). We have demonstrated that TrkA activation by nerve growth factor (NGF) regulates the metalloprotease-mediated shedding of p75 generating the p75-CTF fragment, which is internalized into recycling endosomes where  $\gamma$ -secretase-mediated cleavage occurs. The metalloprotease-mediated shedding of p75 is the key step regulating the production of p75-ICD but the mechanism by how neurotrophins regulate this process and the subcellular location of the shedding event is still unknown. We compared cells where NGF induces p75 shedding with hippocampal neurons, where p75 shedding is not regulated by

neurotrophins. We detected a different ADAM17 expression profile between PC12 cells and rat hippocampal neurons. By using interference RNA (iRNA) for ADAM17, we determined that ADAM17 is the principal sheddase participating in the NGF-induced p75 processing. Preliminary subcellular fractionation experiments indicate that ADAM17 is localized in endosomes. Furthermore, we also found  $\alpha$ -secretase activity for p75 receptor. These results demonstrate that ADAM17 is a crucial mediator of p75 processing and that NGF tightly coordinates the ectodomain shedding and the endocytic trafficking of the receptor, two key steps regulating the p75 neurotrophic signaling. (FONDECYT 1040799 (FCB), FONDAPE Center for Biomedicine (FCB), DIPUC (SU) and Flemish-Chilean International Agreement (FCB, WA) for financial support).

#### **C15. CHANGES IN THE PROTEIN COMPOSITION OF CEREBROSPINAL FLUID FROM HYDROCEPHALIC RATS AND HUMAN PATIENTS.**

Cambios en la composición de proteínas del líquido cefalorraquídeo en ratas hidrocefálicas y pacientes humanos). K Vío, CA Jaramillo, RI Muñoz, EM Rodríguez. Instituto de Histología y Patología. Facultad de Medicina. Universidad Austral de Chile. There is a significant body of evidence indicating that the cerebrospinal fluid (CSF) carries vital signal molecules to the germinal epithelium of the developing brain cortex. Rat CSF collected at E19 has the highest capacity to induce neuronal proliferation, as compared with CSF obtained at other developmental periods, indicating that the CSF composition changes during development (Miyama et al. 2005). Furthermore, the CSF of hydrocephalic HTx rats inhibits neuronal proliferation and migration; the compounds responsible for such an effect have not yet been identified. The present investigation was designed to analyse the proteins present in the CSF of hydrocephalic and non-hydrocephalic HTx rats by two-dimension electrophoresis and immunoblotting using antibodies against candidate proteins to be altered in the hydrocephalic state, namely, the secretory proteins of the subcommissural organ (SCO), reelin and L1 CAM. CSF was collected from cisterna magna and lateral ventricle of embryos, newborns and adult rats. Silver nitrate-stained 2D gels revealed: (i) a set of proteins that were more concentrated in the hydrocephalic CSF; (ii) a set of proteins only present in the hydrocephalic CSF; (iii) a group of proteins that were missing in the hydrocephalic CSF. The immunoblot analysis revealed: (1) There were qualitative and quantitative changes in the CSF throughout development; (2) in the ventricular CSF there were abnormal forms of SCO-proteins whereas in the cisternal CSF these proteins were largely reduced in concentration or missing. (3) The CSF concentration of reelin and L1 CAM in the hydrocephalic CSF was several folds higher than that of the normal CSF. A similar analysis of human CSF collected from non-hydrocephalic neonates and hydrocephalic human foetuses and newborns is in progress. The hydrocephalic CSF displayed abnormal forms of SCO-proteins and a higher concentration of reelin. Conclusions: 1. The protein composition of CSF changes during normal brain development. 2. During development and adulthood the CSF proteins of hydrocephalic rats undergo significant changes. 3. Some of these proteins have been identified and are good candidates to be involved in the abnormal development the brain cortex occurring in the HTx rats. Supported by FONDECYT 1070241. BICENTENARIO IPA 004. DID S-200672/S-200674

**C16. METHYLPHENIDATE DOES NOT RELIEVE EFFECTS OF D1/D5 RECEPTOR BLOCKADE IN THE CUE NAVIGATION TASK OF THE MORRIS WATER MAZE IN RATS.** Metilfenidato no alivia efectos de bloqueo de receptores D1/D5 en la tarea de navegación al blanco en el Laberinto de Morris en ratas. Marc L. Zeise<sup>1\*</sup>, Sergio Espinoza<sup>1</sup>, Adolfo González<sup>1</sup>, Fernanda S. Cerda<sup>1</sup>, Natalia Alarcón<sup>1</sup>, Carol Donoso<sup>1</sup>, Bernardo Morales<sup>2</sup>. <sup>1</sup>School of Psychology, Faculty of Humanities, University of Santiago de Chile; <sup>2</sup>Dept. of Biology, Faculty of Chemistry and Biology, University of Santiago de Chile, Methylphenidate use in the treatment of mainly young humans has increased dramatically and is still on the rise in Chile as well as worldwide. We investigated its effects on spatial orientation using the established method of the Morris Water Maze. As we have reported recently (Zeise et al., 2007), methylphenidate (1mg/kg) improves cue navigation in the Morris Water Maze while not affecting place navigation. The D1/D5 receptor blocker SCH 23390 (0.5 mg/kg) strongly slowed the latencies in getting to the visible platform target. Methylphenidate (1 mg/kg) is unable to alleviate that effect. Rather, as preliminary results suggest, it is exacerbating SCH 23390 action. These results are compatible with the view that the effect reported earlier is due to D1/D5 receptor mediated mechanisms. Methylphenidate may further augment the imbalance in dopamine receptor activation, especially the D1/D2 ratio. Zeise ML, Espinoza S, González A, Cerda FS, Nacarate J, Yáñez CG, Morales B (2007) Methylphenidate improves cue navigation in the Morris water maze in rats. NeuroReport, 18 1059-1062

## PANELES

**P01. RELEASE OF ENDOGENOUS BDNF FROM CA1-CA2 REGION OF THE RAT HIPPOCAMPUS : STUDIES “IN VIVO” WITH MICRODIALYSIS TECHNIQUES COUPLED TO ELISA IMMUNOASSAYS.** (Liberación de BDNF endógeno desde la region CA1-CA2 de hipocampo de rata: estudios “in vivo” mediante técnicas de microdiálisis acoplada a inmunoensayos de ELISA) Jorge Abarca, Eduardo Riquelme, Cristian León, and Gonzalo Bustos. Lab. of Biochemical Pharmacology, Dept. of Cell and Molecular Biology, Pontificia Universidad Católica de Chile, Santiago. A functional coupling between N-methyl-D-aspartate (NMDA) receptor activation and brain –derived neurotrophic factor ( BDNF ) expression seems to play an important role in synaptic plasticity and cell survival occurring in limbic areas of the brain . However , such functional coupling has never been demonstrated in vivo in the adult animal . We now report the use of microdialysis techniques coupled to ELISA immunoassays in order to validate the in vivo release of endogenous BDNF and its eventual regulation by NMDA receptors in CA1-CA2 hippocampal region of the adult rat. Reverse dialysis perfusion with NMDA (500 uM) in the presence of bicuculline (100 uM) (antagonist of GABA-A receptor) , generated a transient increase on endogenous BDNF release from CA1-CA2 hippocampal region . The addition of Ifenprodil (100 uM)(antagonist of NR2B-NMDA extrasynaptic receptors) converted the transient BDNF response to NMDA into a sustained release of BDNF from CA1-CA2 region . This sustained effect on BDNF release was completely blocked by the co-perfusion with MK-801 (antagonist of NMDA synaptic receptors). Finally , reverse dialysis perfusion with K<sup>+</sup> (70mM) , also evoked a sustained release of BDNF from CA1-CA2 , when the experiments were conducted in the presence of Ifenprodil . To the

best of our knowledge , this is the first demonstration of an *in vivo* release of BDNF from the adult hippocampus and that such release may be regulated by NMDA receptor activation . Our results also suggest that “synaptic “ and “extrasynaptic” NMDA receptor may have opposing effects on BDNF release in the hippocampus . Synaptic NMDA receptors may be implicated in the activation of such release whereas extrasynaptic NMDA receptors could exert a negative control upon BDNF release in the hippocampus. ( Supported by FONDECYT –Chile 105-0981 and Millenium Nucleus on “ Stress and Addiction”, Mideplan , Chile)

**P02. PRESENCE OF TRP AND TRPL CHANNELS IN THE SYNAPTIC TERMINAL OF *Drosophila* photoreceptors.** (Presencia de canales TRP y TRPL en el terminal sináptico de fotorreceptores de *Drosophila*). Guadalupe Astorga, Ricardo Delgado and Juan Bacigalupo. Dept. Biología, Fac. Ciencias e Instituto Milenio de Dinámica Celular y Biotecnología, Universidad de Chile. The first TRP channels were discovered in *Drosophila* photoreceptors. The TRP and TRPL channels present in specialized phototransduction membranes (rhabdomers) have been widely studied. Nevertheless, they are not confined to the rhabdomeral membrane. In this work we show the presence of both, TRP and TRPL channels in the synaptic terminals of *Drosophila* photoreceptors. We used specific monoclonal antibodies against TRP and TRPL in *Drosophila* brain slices. Both channels were detected in the photoreceptor membrane, axon and synaptic terminals. To confirm that these channels were located in the synaptic terminals, we used two different approaches. We applied a monoclonal antibody against histamine, the neurotransmitter released by the photoreceptor synaptic terminal in the first optic neuropile, the lamina. We also used the fluorescent dye FM1-43, which fluoresce when bound to membranes to label synaptic vesicles. We induced massive exocytosis of the synaptic vesicles at the terminal with high extracellular  $K^+$ . After wash, the only remaining dye was bound to the endocytosed vesicles. Histamine and synaptic vesicles were located in the outermost part of the lamina, together with TRP and TRPL. This observation is in agreement with previous reports showing the same location for other synaptic elements such as the histamine receptor and the synaptic vesicle marker, synaptotagmine. Anillos Ciencia y Tecnología ACT-45, P. Bicentenario CONICYT; MIDEPLAN ICM-P05-001-F y JS Guggenheim Fellowship (JB).

**P03. COMPARATIVE ANALYSIS OF AUTOMATIC AND VISUAL SLEEP SCORING IN A RAT MODEL.** (Análisis comparativo de diagnóstico automático y visual de estados del sueño en un modelo de rata). Barra R., Araya C., Estrada J., Brunnetti E., Bassi A., Vivaldi E. Laboratorio de Sueño y Cronobiología. Facultad de Medicina, Universidad de Chile. Sleep studies must assign every consecutive epoch of predefined duration (typically 30 sec. in human and 5 sec. in rats) to one of the 3 states of the sleep-wake cycle (CSV); wake (W), no rapid-eyes movement sleep (N) or rapid-eye movement sleep (REM). This process known as “sleep state scoring” is canonically performed through visual inspection by a expert. In the rat, the algorithm for state diagnosis are: high electromyogram (EMG) indicate active wakefulness, low EEG means N if there are abundance of delta or sigma waves, R when there is abundance of hippocampal theta waves and restful wakefulness in absence of either sleep-indicative activities. Computerized analysis of the signals allows for prolonged and continuous

automated scoring and a quantitative and deeper analysis of the EEG. We have developed a system based in the analysis of the frequency domain for the characterization of the brain and muscular activity. The data were recorded for the EEG and EMG continuously at 250 Hz. The signals are divided into a sequence of segments of 500 samples (2 seconds). An spectrogram consisting of the power spectrum of each segment is created for the whole signal. This spectrum is characterized with four activity variables: delta, theta, sigma and EMG, calculated for the adequate integration for every activity. The four activity variables are combined to produce a 2D projection using a principal components analysis (PCA). The 2D projection of the activities normally forms three clusters that are represented by their centroids. The distance to those centroids is used as scoring criteria. Like example of the sensibility and specificity of our method we describe the data corresponding to the scoring of the analysis of the EEG and EMG of four days of registry of a rat compared to the visual diagnostic developed by an expert. The results show that the sensitivity ranges from 89.44 – 95.36 for REM and 93.73 – 96.83 for NREM and the specificity ranges from 98.35 – 98.79 for REM and 85.39-94.88 for NREM. It was develop further modifications at the assignation criteria of automatic diagnostic in order to diminish the discrepancy the automatic and human diagnostic. Key words: Sleep, EEG, automatic diagnostic, Frequency domain analysis. Fondecyt project 1060250

**P04. NEURONAL ACTIVATION IN PRE-BÖTZINGER NUCLEUS IN RESPONSE TO HYPERCAPNIA AND THERMAL STRESS. ACTIVACIÓN NEURONAL EN EL NÚCLEO PREBÖTZINGER EN RESPUESTA A HIPERCAPNIA Y ESTRÉS TÉRMICO .** E. Bravo, C. Ordenes, J. Eugenin, I. Llona,. Laboratory of Neural Systems, Biology Department, USACH, Alameda 3363, Chile. Respiratory rhythm is generated by brainstem neurons located in the pre-Bötzinger nucleus (preBötC). The preBötC receives sensorial input from chemoreceptors and thermoreceptors. We demonstrated the presence of somatostatin (SS) neurons in pre-BötC suggesting that SS participates in the generation and/or control of breathing. In this work, we studied if the respiratory response to hypercapnia and thermal stress is related to the activation of SS neurons in preBötC. We evaluated ventilatory parameters in newborn mice by pletismography. Thermal stress was achieved by exposure to 34 or 36 °C. Hypercapnia was produced by inhalation of 10% CO<sub>2</sub>. Neuronal activation of SS neurons was investigated by immunocytochemistry for c-Fos. Ventilation increased in response to both thermal stress and hypercapnia. Depending on the age, the increase in ventilation reflected changes in frequency or tidal volume. The ventilatory response to hypercapnia was of higher (230%) than the response to thermal stress (30%). Hypercapnia produces a significant increase (3 times) of activated neurons in the preBötC nucleus, but not SS neurons. On the other hand, thermal stress did not change significantly the activation of preBötC neurons. In conclusion our results suggest that SS neurons in the preBötC did not participate in the respiratory response to hypercapnia and thermal stress in neonatal mice.

**P05. EARLY SENSORY-MOTOR STIMULATION AS A COMPENSATING FACTOR FOR DELETERIOUS EFFECTS INDUCED BY PRENATAL MALNUTRITION ON VISUO-SPATIAL MEMORY. (Poliestimulación sensorio-motora temprana como factor compensador del efecto deletéreo inducido por la**

malnutrición prenatal en la memoria visuo-espacial). <sup>1</sup>Burgos H., <sup>1</sup>Jofré C., <sup>1</sup>Martínez J., <sup>1</sup>Reyes L., <sup>1</sup>Rodríguez S., <sup>1</sup>Zapata C., <sup>1</sup>Gaete., P., <sup>1</sup>Núñez K., <sup>1</sup>Meza K., <sup>2</sup>Fernández V., <sup>3</sup>Soto-Moyano R., <sup>3</sup>Pérez, H., <sup>4</sup>Hernández A. <sup>1</sup>Universidad de las Américas, Facultad de Ciencias Jurídicas y Sociales. <sup>2</sup>Centro de Educación Montessori. <sup>3</sup>Universidad de Chile, INTA. <sup>4</sup>Universidad de Santiago de Chile, Facultad de Química y Biología, Laboratorio de Biología y Química. Malnutrition causes overt and hidden deleterious effects on the central nervous system of living organisms. Partial substitution of dietary proteins by carbohydrates and fats leads to the so-called “hidden malnutrition”, especially when substitution is made during the gestational period. Among other disturbances, rat pups born from mothers fed on a hypoproteic/isocaloric diet (8% casein) show alterations of noradrenergic systems in the neocortex and hippocampal formation, as well as deficits in visuo-spatial memory performance. On the other hand, polysensory stimulation early in life produces favourable neuronal changes, such as greater dendritic arborization and expanded axonal fields, which may lead to improved behavioral/cognitive functions. In particular, memory has been shown to be favoured by anatomical and physiological plastic changes caused by early stimulation (axonal and dendritic reorganization, increased long-term potentiation, changes in the expression of some neurotransmitter receptors), but today is unknown if polysensory stimulation during early postnatal life could revert some memory deficits produced by hidden prenatal malnutrition. Twenty prenatally malnourished rats (from mothers fed 8% casein diet) were divided in two groups of 10 pups each: one group was submitted to sensory-motor stimulation starting from day 3 after birth, while the other (unstimulated) constituted the social control. Twenty eutrophic rats (from mothers fed 25% casein diet) submitted to similar stimulation conditions constituted the dietary controls. On postnatal day 60 the visuo-spatial performance of rats was evaluated in an eight-arm radial Olton maze, and the number of errors and the time required for solving the task measured. The results showed a significantly better visuo-spatial performance in stimulated subject with respect to social controls; also, stimulated nutritionally deprived animals showed significantly improved performance compared to non stimulated subjects, showing performance scores that did not significantly differ to those of eutrophic animals. The present data suggest that early poly-stimulation could exert a compensatory role on cognitive impairment caused by prenatal malnutrition in rat. Fondecyt 1070028. Proyecto UDLA 2006-2007

**P06. RELEASE OF NUCLEOTIDES AND NORADRENALINE BY METHYL PHENIDATE AND TRANSMURAL DEPOLARIZATION OF SYMPATHETIC NERVE ENDINGS.** Liberación de nucleótidos y noradrenalina inducidas por metilfenidato y estimulación eléctrica desde los terminales simpáticos. S. Cárdenas, M. Zeise, MV. Donoso, JP. Huidobro-Toro. Laboratorio de Nucleótidos, Centro Regulación Celular y Patología, Instituto MIFAB, Departamento Fisiología, Facultad Ciencias Biológicas, P. Universidad Católica de Chile, y Universidad de Santiago. To test the hypothesis that amphetamines predominantly release bio-amines from nerve endings, we compared the extracellular outflow of noradrenaline (NA) and co-stored purines including ATP, elicited by either 1mM methylphenidate or transmural electrical depolarization of the nerve endings. Method: Segments of thoracic aorta from adult Sprague Dawley rats were mounted in superfusion baths maintained with Tyrode buffer (37°C, bubbled 95% O<sub>2</sub>/5% CO<sub>2</sub>). The tissue perfusate media was collected each 30 s during 17 min. In the same sample we determined the NA, ATP and metabolites. NA was separated by HPLC and quantified electrochemically, while purines reacted with

chloroacetaldehyde and quantified by HPLC coupled to a fluorescence detector; results are expressed as pmol/mL. Results: Transmural electrical nerve ending depolarization (70 V, 1 ms, 16 Hz, during 4-min) induced the release of ATP and metabolites to the superfusate media. While the release of ATP amounted to  $39.7 \pm 14.1$ , the ADP was  $101 \pm 25.3$ , likely indicating an active tissue metabolism; total outflow purines amounted to  $282.9 \pm 75.5$  (n=8). The NA overflow was  $6.7 \pm 3.3$  (n=8). In contrast, the NA released to the superfusate following 1mM methylphenidate (5-min) was  $35.2 \pm 11.2$  (n=8), a value 6-fold larger than that observed with the electrical depolarization ( $p < 0.05$ ). The total purines released by the amphetamine was only  $64.2 \pm 22.1$ ; the ATP was  $26.7 \pm 14.8$  and ADP  $25.1 \pm 10.1$ . Conclusion: The present results demonstrate that electrical depolarization elicited an almost 35-fold ratio of tissue purines over NA, while this ratio was reduced to 1.8 after tissue incubation with this clinically-relevant amphetamine, favoring the notion that amphetamines mobilize predominately bio-amines from sympathetic nerve endings. Funded by FONDAP 13980001 and MIFAB P04-071-F.

**P07. NICOTINE-INDUCED DECREASE IN VENTILATORY RESPONSE TO HYPERCAPNIA IS NOT ASSOCIATED TO DAMAGE.** (Disminución de la respuesta ventilatoria a hipercapnia inducida por nicotina no se asocia a daño).

V. Cerpa<sup>2\*</sup>, E. Bravo<sup>1\*</sup>, I. Llona<sup>1</sup>, R.von Bernhardt<sup>2</sup> and J. Eugén<sup>1</sup>. <sup>1</sup> Universidad de Santiago de Chile, <sup>2</sup> P. Universidad Católica de Chile. Nicotine is the main candidate to be the link between maternal cigarette smoking during pregnancy and Sudden Infant Death Syndrome (SIDS). Infants who died from SIDS, showed several respiratory dysfunctions and reduced arousal responses induced by hypoxia and hypercarbia. We assess whether reduction in the responses to hypercarbia found in neonatal mice after being exposed to nicotine during intrauterine and perinatal development is associated to morphological signs of damage at the respiratory-related brainstem nuclei. Osmotic minipumps were implanted subcutaneously into 5-7 days CF1 pregnant mice to deliver saline (controls) or nicotine ditartrate  $60 \text{ mg Kg}^{-1} \text{ day}^{-1}$  (experimentals). P0-P4 neonates were placed in a temperature-controlled plethysmographic chamber and were allowed to breath air spontaneously during 10 minutes, followed by 10 minutes inhaling air enriched with 10% CO<sub>2</sub> (21% O<sub>2</sub>, N<sub>2</sub>-balanced). After 1 ½ hr the neonates were anesthetised with ether, perfused transcardially with 4% p-formaldehyde and 0.01% picric acid, their brains removed and fixed for 42 hr. Cryostat made sections were processed for immunohistochemistry anti-somatostatin (pre-Bötzing nuclei marker) and anti-glial fibrillary acidic protein (anti-GFAP, astrocyte marker). Other group of sections were stained with cresyl violet to evaluate the number of picnotic nuclei on the respiratory ventral group. We confirmed that P0-P3 nicotine exposed mouse neonates had a reduction in the responses in volume minute induced by inhalation of air enriched with 10% CO<sub>2</sub>. In P0 to P3 pups, no evident signs of astrogliosis secondary to neurodegeneration or necrosis, as well no sign of increase in the number of apoptotic neurons were observed on the pre-Bötzing nuclei and adjacent respiratory ventral group nuclei. Likewise, the number and shape of somatostatin positive neurons of the pre-Bötzing nuclei were undistinguishable from controls. Our results suggest that perinatal nicotine exposure reduced the response to hypercarbia not challenging the survival of respiratory-related neurons. Thus, nicotine seems to affect ventilation through more subtle changes in the development of neural network properties, likely affecting their synaptic interactions. Supported by FONDECYT 1060110.

**P08. IDENTIFICATION AND CHARACTERIZATION OF THE FACILITATORY AND INHIBITORY SITES FOR DIVALENT METALS IN THE P2X<sub>4</sub> RECEPTOR.** Identificación y caracterización de los sitios facilitador e inhibidor de modulación por metales divalentes en el receptor P2X<sub>4</sub>. Claudio Coddou, Claudio Acuña-Castillo, Paulina Bull y J. Pablo Huidobro-Toro. Centro de Regulación Celular y Patología J.V. Luco, Instituto Milenio de Biología Fundamental y Aplicada MIFAB, Departamento de Fisiología, Laboratorio de Nucleótidos, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. Based on the hypothesis that the P2X<sub>4</sub> receptor has at least two distinct and separate allosteric modulator sites that account for the inhibitory and facilitatory modulation of the receptor activity by zinc or copper, we aimed at identifying amino acid residues involved in trace metal interaction in the primary sequence of the receptor in the immediate linear vicinity of His-140, a residue previously identified as critical for the copper-induced ATP-evoked current inhibition. Mutants for conspicuous amino residues comprised in the extracellular domain region Thr<sup>123</sup>-Thr<sup>146</sup> were generated and the cDNAs for the wild-type and receptor mutants were expressed in *Xenopus laevis* oocytes and examined by the two-electrode technique. Cys-132, but not Cys-126, proved to be crucial for the zinc-induced potentiation of the receptor activity, moreover this mutant was inhibited by zinc with an IC<sub>50</sub> of 18.2 ± 10.1 μM, in contrast to wild-type and C126A who showed a biphasic potentiation curve for zinc. The C132A mutant conserved a wild-type phenotype for the copper modulation. The SH alkylating agent MTSET, decreased the zinc potentiation from 5.9 ± 0.4 to 3.1 ± 0.2-fold (p<0.01) in wild-type receptors confirming that free cysteines are responsible for zinc modulation. In contrast this treatment did not change ivermectin-potentiation nor copper-inhibition. Likewise, Asp-138, but not Asp-131 proved critical for copper but not zinc allosteric modulation; moreover, mutant D138A was 10-20-fold more reactive to zinc than the wild-type receptor, and the zinc concentration-response curve was a sigmoid instead of a biphasic curve. Residues Asp-129, Asp-131 and Thr-133 had minor roles in metal effects. We conclude that this region of the P2X<sub>4</sub> receptor has a pocket for trace metal coordination in its ectodomain. Funded by FONDAP 13980001 y MIFAB.

**P09. HYPOTHALMIC DISTRIBUTION OF MONOCARBOXYLATE TRANSPORTERS.** (Distribución hipotalámica de transportadores de monocarboxilatos). Cortés C.; Elizondo R.; Reinicke K.; Nualart F.; García MA. Research Center for the Study of the Nervous System: cell Biology and Biomedical applications. Department of Cellular Biology, University of Concepcion, Concepcion, Chile. ([ccortes@udec.cl](mailto:ccortes@udec.cl)). It has been demonstrated that the neuronal function is closely supported and regulated by glial elements. Examples of this functional association are seen in the development and migration of neurons, in the tripartite synapses, in the volume sensing mechanism and in the energetic metabolism (exchange of lactate). This way it has been proposed that the glial cells that are in contact with the neurons of the arquate nucleus can capture glucose and liberate lactate, a molecule capable of activating the neurons involved in the mechanism of response to glucose. Thus, the brain glucose sensing mechanism would be another mechanism involving the functional interaction between neuron and glia. To validate this mechanism it is necessary to demonstrate that both types of cells express lactate transporters. Using RT-PCR and Western blotting we show that MCT1, MCT2 and MCT4 are expressed in the hypothalamus of rat. To determine the cell type expressing these different isoforms of lactate transporters we analyzed their localization in the hypothalamic area by confocal

imaging. We determined that MCT1 is the main transporter present in this region showing the highest levels of reactivity in the ventricular area and in glial cells contacting the proximal part of the arquate nucleus. On the other hand MCT2 is localized mainly to the distal part of this nucleus suggesting a neuronal localization. Further MCT4 showed a more restricted immunoreactivity in this area being observed mostly at the distal part of the glial processes that contact the marginal glia. A newly developed primary culture of tanycytes was used to confirm the expression and function of MCTs. These determinations on isolated hypothalamic glia enabled us to determine that the only isoform participating in the capture of lactate is MCT1. This way the distribution of MCTs in the hypothalamic area could support the metabolic glia-neuron interaction of tanycytes proposed for the physiological mechanism of hypothalamic glucose sensing. Supported by Grant: Anillo ACT-02.

#### **P10. SUBTHRESHOLD SODIUM CURRENT UNDERLIES ESSENTIAL FUNCTIONAL SPECIALIZATIONS AT PRIMARY AUDITORY AFFERENTS.**

Una corriente de sodio subumbral confiere especializaciones funcionales esenciales a aferentes auditivas. Sebastian Curti<sup>1,2</sup>, Leonel Gomez<sup>3</sup>, Ruben Budelli<sup>3</sup> and Alberto E. Pereda<sup>2</sup>. <sup>1</sup>Departamento de Fisiología, Facultad de Medicina, Universidad de la República, Uruguay. <sup>2</sup>Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York. <sup>3</sup>Sección Biomatemática, Facultad de Ciencias, Universidad de la República, Uruguay. Auditory afferents are generally perceived as passive, timing-preserving, lines of communication. Contrasting this view, identifiable auditory afferents on the goldfish M-cell undergo potentiation of their synapses in response to high frequency bursts of activity. This property likely represents a mechanism of input sensitization as these afferents provide the Mauthner cells with essential information for the initiation of an escape response. Consistent with such specialization, we show here that these afferents exhibit an intrinsic ability to respond with bursts of high frequency firing to auditory stimuli and that property critically relies on the activation of a persistent sodium current. Further, this conductance is essential for the amplification of an electrical resonance that results from the interaction between an A-type potassium current and this afferent's passive membrane properties. Remarkably, the frequency preference of this resonance is consistent with both the goldfish's effective range of hearing and the firing frequencies required for synaptic facilitation, an obligatory requisite for the induction of activity-dependent changes. While the relationship between the biophysical properties of specific ion channels and neuronal encoding during sensory processing is often elusive, our data shows that the presence of the persistent sodium current allow these afferents to translate sounds of behaviorally relevant auditory signals into patterns of activity that match the requirements of their fast and highly modifiable synapses. The functional specializations of these neurons suggest that auditory afferents might be capable of more sophisticated contributions to auditory processing than has been generally recognized.

**P11. COMPUTER SIMULATION OF ION CHANNEL NOISE IN SINGLE OLFACTORY CILIUM.** (Simulación computacional del ruido de los canales de iones de un cilio olfatorio aislado). P. Daire, R. Delgado, J. Bacigalupo, O. Alvarez and M. Sanhueza. Dpto. Biología, F. Ciencias e Inst. Milenio ICDB, U. de Chile. Odor transduction occurs in the cilia of olfactory receptor neurons (ORNs). It involves a cAMP cascade, where cAMP activates a cationic cyclic nucleotide-gated (CNG) channel, allowing the influx of  $\text{Ca}^{2+}$  that activates  $\text{Cl}^-$  channels ( $\text{Cl}_{\text{Ca}}$ ). The CNG channel is well known, but the nature of  $\text{Cl}_{\text{Ca}}$  remains controversial. Noise analysis (NA) of macroscopic  $\text{Cl}^-$  currents ( $I_{\text{Cl,Ca}}$ ) has been used to unravel the channel fundamental properties.  $I_{\text{Cl,Ca}}$  has been recorded from entire individual cilia drawn into a pipette and excised from the ORN. Conventional NA works properly when space-clamp is correctly attained. But a cilium behaves as a leaky cable. Larsson et al (1997) proposed a correction for NA to deal with this problem. This method gives a unit conductance ( $\gamma$ ) for the  $\text{Cl}_{\text{Ca}}$  from 0.8 to 1.6 pS. However, these values are 10-fold smaller than single- $\text{Cl}_{\text{Ca}}$  currents recorded by us directly from the cilia. Our aim was to examine whether Larsson method underestimated  $\gamma$ . To this end we developed a computer simulator of  $I_{\text{Cl,Ca}}$  and applied the modified NA to the simulated currents. We compare  $\gamma$  obtained by NA with the  $\gamma$  used as input to the simulator. We found that the computed mean  $I_{\text{Cl,Ca}}$  vs. variance relation deviates significantly from Larsson NA theory and  $\gamma$  is underestimated. We used the simulator to determine the origin of the discrepancies, and found that important aspects were omitted in theoretical construction of non space-clamp NA. It was not considered that membrane potential fluctuates according random channel gating. Current variations produce subtle changes of membrane potential, diminishing current variance. We also concluded improper correction for background cilium conductance could dramatically alter NA results. These distorting factors may cause up to 50% underestimation of  $\gamma$ . Therefore the origin of the difference in  $\gamma$  values obtained from single channel and macroscopic currents remains unclear. Our simulator may also constitute a powerful tool to study signal integration in other neuronal specializations, such as dendrites. Anillos Ciencia y Tecnología ACT-45, P. Bicentenario CONICYT; MIDEPLAN ICM-P05-001-F y JS Guggenheim Fellowship (JB).

**P12. ANALYSIS OF THE EXPRESSION AND LOCALIZATION OF THE GABAERGIC SYSTEM AND THE DIVERSE RECEPTORS OF GABA IN THE RETINA OF OCTODON DEGUS.** Estudio de la expresión y localización del sistema GABAérgico y de los diversos receptores de GABA en retina de *Octodon degus*. Luz Marina Delgado, Adrián Palacios y Oliver Schmachtenberg. Centro de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso.  $\gamma$ -Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the central nervous system. In the vertebrate retina, it participates in inhibitory processes that modulate the visual response, such as light adaptation and center-surround contrast. In addition, it has been associated with a neurotrophic role during the development of the retina. Here we characterized the expression and distribution of GABA, of the two isoforms of the GABA-synthesizing enzyme glutamic acid decarboxylase, and of two subtypes of GABA receptors, by

means of immunohistochemical techniques in retinas from adult and neonate *O. degus*. The results demonstrate well-defined and essentially conserved patterns of expression during the postnatal retinal development. However, a diminution of immunoreactivity in cell bodies located in the inner nuclear layer and the ganglion cell layer was evident in adult animals. This study demonstrates that in *O. degus*, as opposed to the rat, the GABAergic system is expressed already at the time of birth in different retinal layers, suggesting its immediate post-natal participation in the modulation of visual signals through synaptic inhibition. As the retina of *O. degus*, a rodent of diurnal-crepuscular habits, functionally represents a more similar model to human retina than nocturnal animals like the rat, these results might also be relevant to the understanding of signal processing in our visual system.

**P13. PUTATIVE UNITARY TRP AND TRPL CHANNEL RECORDINGS FROM RHABDOMERIC EXCISED PATCHES OF *DROSOPHILA* PHOTORECEPTORS.**

(Probables canales TRP y TRPL registrados en parches escindidos del rabdómero de los fotorreceptores de la *Drosophila*). R Delgado and J. Bacigalupo. Department of Biology, Faculty of Sciences and Millennium Institute for Cell Dynamics and Biotechnology, University of Chile. The molecular constituents of *Drosophila* phototransduction are confined to the microvilli of the photoreceptor rhabdomeres. The TRP and TRPL light-dependent channels were identified genetically several years ago and have been extensively studied by whole-cell recording. Over the years a myriad of different TRP channels have been discovered, many of which are involved in sensory transduction in vertebrate and invertebrates. However, the fruit fly TRP and TRPL channels have not been recorded yet in their native membrane. Moreover, the agonist that opens these channels remains unclear, representing a major open question of *Drosophila* phototransduction. The study of these channels at the unitary level has been precluded because they are inaccessible to a patch-clamp electrode in the ommatidium. We developed a procedure that exposed the rhabdomere of dissociated ommatidia, making them amenable to patch-clamp recording. We have identified two different channels in excised rhabdomeric patches. One of them has a ~70 pS conductance (under 0-divalent solutions), is 100 times more selective for  $\text{Ca}^{2+}$  over  $\text{Na}^{+}$  and is sensitive to divalents and  $\text{La}^{3+}$  block, closely resembling TRP. The other channel has ~80 pS, is cationic unselective and insensitive to divalents and  $\text{La}^{3+}$ , as TRPL. Both channels are opened by 5  $\mu\text{M}$  diacylglycerol (DAG), arachidonic acid (AAc) or linolenic acid (LNA), all previously proposed as possible agonists of the light-activated channels. Based on their properties and location, we propose that the rhabdomeric single-channel currents reported herein correspond to TRP and TRPL involved in *Drosophila* phototransduction. Our results confirmed that the agonist of these channels is a phosphoinositide. Supported by Fondecyt 1040772 (RD); Rings of Science and Technology ACT-45, Bicentennial Program CONICYT; MIDEPLAN ICM-P05-001-F and a JS Guggenheim Fellowship (JB).

**P14. COGNITIVE ECOLOGY OF THE MOTHER-PUP VOCAL RECOGNITION IN PINNIPEDS (*Arctocephalus philippii*), EARLY LEARNING AND MEMORY.**

(Ecología Cognitiva del reconocimiento vocal materno-infantil en pinnípedos (*Arctocephalus philippii*), aprendizaje temprano y memoria). Hernán A. Díaz M. Laboratorio de Neurociencias, Departamento de Biología, Universidad de Santiago de Chile. In mammals, frequency tuning plasticity in the auditory cortex has proven to be very sensitive to classical conditioning protocols performed in Guinea pigs. Results showed fast development of associative response to the frequency stimulus and long-term neural consolidation of the process (Galván & Weinberger, 2002). A very similar auditory conditioning situation can be found in the field in any fur seal's reproductive colonies where mother and pups learn to vocally recognize each other since the first minute after birth. It is a crucial moment in the life of any fur seal's mother-pup pair because around in a week female will abandon her pup to go to the sea for feeding. In Juan Fernandez fur seal (*Arctocephalus philippii*, Carnivora:Pinnipedia) this abandonment can last for 12 days or more (Francis & Boness, 1991), so the particular importance of this early stage of development in the process of learning and memory of the mutual vocalizations. When the mother comes back from her feeding trips, she must locate her pup by the vocal cues learned during the 5 days that she spent with her pup before leaving. The recognition and reencounter process usually occurs in a colony that can gather from hundred to thousand animals and that may be settled under different ecological condition. A similar learning condition happens with pups, except by the fact that they remain on land starving, searching continually for mother by vocalizing at the colony. Finally, after any feeding trip or temporal abandonment, mothers are who do the last decision about identity (Díaz, 1994), so one of the main aspects to achieve by pups during the first days of their life, is to be able to utter and remember a very identifiable vocalization, facilitating the recognition and encounter with its mother. In this work we want to connect this natural condition that we can call a field natural experiment, to trace down the reach of this early ontogenetic plasticity on vocal recognition under field condition. In previous work it has been showed that the vocalizations used for mother-pup recognition are highly identifiable based on the ratio between inter-individual and intra-individual variation of 10 vocal parameters extracted from pup's Female Attraction Call vocalizations (FAC). In this report we explore the possibility that contextual environmental features, especially during critical period after birth, must have any incidence in the way that nervous system extends plasticity and consolidation of structural changes related with vocal/auditory recognition. To test the hypothesis that environmental context in free ranging animals can modify the inter- and intra-individual variation of the vocal cues used for identification, we compared the potential for individual identity coding (PIC value) based on inter-/intra- variation comparison, for 10 vocal parameters extracted from female attraction call (FACs), coming from pups of *Arctocephalus philippii*, raised in two different ecological (environmental) conditions potentially affecting in different degree the probability to fail in mother-pup recognition and reencounter. Risky and non-risky places was evaluated in term of animal density, beach structure and background noise. The 10 vocal parameters where extracted using FFT (Fast Fourier Transform) from the time domain vocal (FAC) signal recorded on field (Alejandro Selkirk Island, 33°46'00"S, 80°47'00"W) coming from 10 individuals from each place, 10 vocalizations for individual. The results after comparing the two places under study, shows that the one with potential higher risk of failure on recognition consequently shows higher PIC values for 9 of the 10 parameters analyzed. The difference in the mean value considering all of the individuality indexes (PIC) coming from the 10 parameters

measured, is of 32%, favorable to the risky place. (Mean PIC value Risky Place = 1,946 v/s Mean PIC value Non-Risky Place = 1,477). Besides this difference we also found that the PIC value of a particular vocal parameter may also be affected by the environment/ecological context, which can act as a specific frequency filter and noise generator for sound propagation and detection. This extra features coming from the physical restriction of the traveling media upon specific sound frequency bands, impose new constraints to the plastic mother-pup vocal recognition system, plasticity that can also be traced down by studying the sound structure of the animal's vocalizations and the actual structural and environmental conditions in the field, which superimpose over a biological context of a very plastic early complementing neurobiology of auditory/vocal learning and memory in mammals. *Acknowledgments: Dr. Isabelle Charrier, Dr. Peter Hodum, Dr. John Francis & Smithsonian Institution, National Zoo, WA D.C. USA.*

**P15. THE RELEASE OF CGRP FROM PERIPHERAL SENSORY NEURONS IS NOT MODIFIED BY NUCLEOTIDES.** La liberación de CGRP de neuronas sensoriales periféricas no está regulada por nucleótidos. M. V. Donoso, R. Miranda, J.P. Huidobro-Toro. Laboratorio de Nucleótidos, Centro Regulación Celular y Patología, Instituto MIFAB, Departamento Fisiología, Facultad Ciencias Biológicas, P. Universidad Católica de Chile, Casilla 114-D, Santiago, Chile. Calcitonin gene related peptide (CGRP), is a sensory neuron transmitter together with SP and serotonin. We recently demonstrated that 3 nM CGRP inhibited the release of sympathetic co-transmitters ATP, NA and NPY, implying that CGRP modulates sympathetic transmission. To assess whether purines and/or nucleotides may inversely modulate the release of CGRP, we now elicited the release of CGRP and studied its pre-synaptic modulation by purines and nucleotides. Methods: Prostatic segments of adult Sprague Dawley rat vas deferens were mounted in superfusion baths and maintained with Tyrode buffer gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub> at 37°C. The release of CGRP to the perfusion media was elicited by transmural field stimulation (16 Hz, 60 V, 1ms for 15, 30 or 60-sec). Superfusion buffer was collected; CGRP was concentrated by Sep-Pak and quantified by RIA. Non-stimulated tissues served as controls. Results: Transmural electrical depolarization elicited the release of CGRP to the tissue buffer; the outflow of the peptide depended on the duration of the electrical tissue depolarization. Following 15, 30 or 60 sec of stimulation, the CGRP collected in the buffer was: 217±50 (n=6), 436±161 (n=3) and 496±64 fmol (n=11), respectively. Experiments conducted in buffer lacking external calcium, the 60-sec CGRP released was reduced to 21±7 fmol (n=3), indicating the need of this divalent cation for the release process. Non-stimulated tissues released 11±3 fmol CGRP (n=3). Pre-treatment with 45 µM α,β-methylene-ATP, a procedure reported to desensitize P2X<sub>1</sub> receptors, did not alter the 60-sec CGRP-induced release (627±136 fmol, n=6). Likewise, 100 nM adenosine, used as a ligand for presynaptic modulator adenosine receptors, did not modify significantly the peptide released (461±15 fmol CGRP, n=3). Conclusions: CGRP is released by exocytosis from the nerve endings of the prostatic segment of rat vas deferens. The mechanism of peptide release is apparently not regulated by a P2X receptor nor by adenosine A<sub>1</sub> or A<sub>2A</sub> receptors. Funded by FONDAF 13980001 and MIFAB Institute.

**P16. NEONATAL SENSORY DENERVATION INDUCED LONG-TERM CGRP PRE AND POST SYNAPTIC SYMPATHETIC PLASTICITY**

Denervación sensorial en ratas neonatales produce cambios en la sinapsis simpática de larga duración. M. V. Donoso, D. Hermosilla, J.P. Huidobro-Toro. Laboratorio de Nucleótidos, Centro Regulación Celular y Patología, Instituto MIFAB, Departamento Fisiología, Facultad Ciencias Biológicas, P. Universidad Católica de Chile, Casilla 114-D, Santiago, Chile. Calcitonin gene related peptide (CGRP), one of the transmitters of sensory neurons, inhibited in a concentration-dependent manner the release of noradrenaline (NA), adenosine 5' triphosphate (ATP) and neuropeptide tyrosine (NPY) from the rat vas deferens nerve endings. To examine the role of the sensory neuron on the sympathetic modulator role of CGRP, we denervated the sensory neurons from neonatal rats and examined whether chronic denervation elicited long-term synaptic plasticity. Methods: Sprague Dawley neonates were injected with two consecutive doses of 50 mg/kg capsaicin at day 2 and 6 of life; as controls, parallel sets of newborns were injected with vehicle (10% Tween 40, 10% ethanol in 80% saline). 2 months later, segments of the prostatic portion of the rat vas deferens were mounted in superfusion baths containing Tyrode buffer (37°C, gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub>) supplemented with 1 μM desipramine, at a flow of 2 ml/min. Transmitter release was elicited by electrical field transmural stimulation (16 Hz, 60 V, 1ms during 1-min). ATP, NA and NPY were quantified analytically from a same sample perfusion buffer aliquot. Results: Transmural electrical depolarization induced the release of ATP, NA and NPY; denervation did not modify the total outflow of these transmitters. Denervated tissues were resistant to the presynaptic action of 3 nM CGRP, while the peptide reduced transmitter release in tissues from vehicle treated animals. 3 nM CGRP reduced the ATP from 57±14 pmol (n= 6) to 21±7 pmol (n=4, p<0.05), NA from 69±12 (n=6) to 33±8 pmol (n= 4, p<0.05) and NPY from 32±7 to 12 ± 5 fmol, (n=5, p<0.05). In addition, denervated tissues were also resistant to the postjunctional action of CGRP, characterized by the inhibition of the motor responses elicited by both ATP and NA. Conclusions: Denervation of sensory nerves elicited a long-term adaptation to the CGRP responses; the mechanism(s) involved are being further pursued. Funded by FONDAPE 13980001 and MIFAB Institute.

**P17. LESION OF THE TUBEROMAMMILLARY NUCLEUS DECREASES ANTICIPATORY EVENTS INDUCED BY RESTRICTED FEEDING IN RATS.**

Lesiones del núcleo tuberomamilar disminuye los eventos anticipatorios en ratas entrenadas por la comida. Farías P, Recabarren MP, Valdés JL, Serón-Ferré MJ, and Torrealba F. Dep Cs. Fisiológicas, Fac. Ciencias Biológicas. P. Univ. Católica de Chile, Santiago, Chile. Under scheduled feeding animals anticipate feeding time with increased locomotor activity and body temperature called food anticipatory activity (FAA). This FAA persists even without suprachiasmatic nucleus (SCN), the master clock in mammals, indicating the presence of a food entrainable oscillator. The tuberomammillary nucleus (TMN) is the only source of brain histamine; this nucleus participates in alert, arousal and can be key brain region in motivated behavior as feeding. TMN belongs to the ascending arousal system. We have demonstrated that TMN is the first active arousal nucleus (immunoreactivity for c-fos) when fasting rats are enticed with food. To evaluate if TMN participates in FAA we lesioned the TMN with immunotoxin Orexin B-SAP and measured locomotor activity and body temperature in anticipation to feeding time. In addition we evaluated the changes in the

expression rPer2 in rostral brain structures and TMN around the time of restricted feeding. Rats with bilateral TMN lesions (mostly restricted to the ventral TMN) showed no significant increase in locomotor activity and body temperature compared with control rats. We observed lower expression of rPer2 in TMN and all structures studied during anticipatory hour in restricted feeding compared with circadian control, including infralimbic cortex, the principal afferent to TMN. rPer2 increased after eating in the infralimbic area and TMN relative to anticipation time. TMN was the only structure with increased rPer2 postprandial expression relative to the circadian control, indicating that TMN neurons are very sensitive to changes in redox-state related with eating. In contrast, rPer2 decreased in the SCN after feeding. We conclude that histaminergic neurons are very important to express the FAA, they changed rPer2 according to redox state and so they are a candidate to be the food entrainable oscillator or a down stream effector. Support by Grant Fondecyt # 1060476 and Fondecyt # 2010137.

**P18. EXCITABILITY OF CA1 NEURONS IS DIFFERENTIALLY MODULATED BY COPPER IN THE NANO AND MICROMOLAR RANGE.** (La

excitabilidad de neuronas CA1 es modulada diferencialmente por cobre en el rango nano y micromolar). <sup>+</sup>G. Fernandez, <sup>\*</sup>C. Maureira, <sup>+</sup>M. Sanhueza, <sup>+</sup>C. Vergara. <sup>+</sup>Biology Dept. Faculty of Sciences, U. of Chile; <sup>\*</sup>Neurobiology Dept., Yale University School of Medicine, New Haven, CT, USA. Copper is an essential ion for the metabolism of every aerobic cell but it also plays an important role in neuronal physiology as evidenced by the neurological symptoms of Menkes and Wilson patients that have decreased or increased brain copper levels respectively. In some neurons in the CNS copper is stored in synaptic vesicles and is released during normal activity. The hippocampus contains high levels of copper, nevertheless, the exact distribution of copper containing terminals is not known. Using hippocampal brain slices from 18-21 days old rats and standard stimulating and recording protocols we started a characterization of the effect of exogenous copper over the excitable properties of CA1 neurons (Soc for Neuroscience Abst 432.9 D67, 2006). We found an increase or a decrease of excitability when the slices were exposed to nano or micromolar copper. Interestingly, not all CA1 cells are equally affected by copper. Since the activity of CA1 neurons depends to great extent on the activity of CA3 cells and these cells have recurrent excitatory connections among them it seemed important to assess the effects of copper over CA1 neurons devoid of their CA3 connections. After removing CA3, we performed extracellular recordings from CA1 cells in slices exposed to 30-100 nM or 3-30  $\mu$ M copper. Additions of nanomolar copper triggered an increase on the basal firing frequency close to 200%, without effect of synaptic transmission. On the other hand, micromolar copper caused a diminution of  $\sim$  35% in the basal frequency accompanied with a 20% diminution on synaptic transmission. To separate the copper effect on synaptic transmission versus its effect on voltage dependent activity, some slices were pre-incubated for 20 minutes with a cocktail of blockers of excitatory and inhibitory transmission. Under these conditions, nanomolar copper did not trigger a change in basal firing frequency, but with micromolar copper we observed an increase of  $\sim$  4 times in the basal firing rate. These observations confirm that copper is a neuromodulator and that it is possible that the endogenous copper levels achieved during normal synaptic activity could affect the CA1 cells firing pattern. Funding: Fondecyt 1040681, Enlace DI 2007.

**P19. SPONTANEOUS BETA OSCILLATIONS IN PRIMARY VISUAL CORTEX IN THE ABSENCE OF VISUAL STIMULATION.** (Oscilaciones beta espontáneas en la corteza visual primaria en ausencia de estimulación visual). Flores FJ, Ossandón JP, Babul C, Maldonado PE. Programa de Fisiología y Biofísica Facultad de Medicina, Universidad de Chile. Several models of neural coding are based on temporal correlations in spiking patterns (Rieke et al, 1998). However, how these correlations are achieved still remains debatable. One hypothesis proposes the presence of underlying, spontaneous oscillations which acts as a pacemaker and increase the probability of temporal correlations (Fries, 2005). If such is the case, we hypothesized that this spontaneous oscillatory modes should be reflected in firing patterns of single neurons during periods of stimulus absence, in order to quickly entrain the system at the time of stimulus arrival. In order to examine this prediction, we trained two monkeys to hold the gaze on a small yellow dot in an otherwise blank screen, while simultaneously recording single unit activity and local field potentials (LFP) in the primary visual cortex (V1). In this way, receptive fields of V1 neurons, which are 5°-10° around the point of gaze, are always falling in a blank portion of the screen. Eye movements were recorded with the scleral search coil technique. Single units and LFPs were recorded with an array of 6 independent tetrodes. Through spectral and coherence analysis (Jarvis & Mitra, 2001) we examined the oscillatory patterns of both the spikes and the LFP. We found that approximately 50% (34/63) neurons indeed exhibit oscillatory modes in the beta band and gamma bands (8-47 Hz). Usually this oscillatory behavior was related to the conspicuous presence of bursting activity. In addition, all LFPs exhibited a strong oscillatory component in a narrow range (8-19 Hz). However, when we assessed spike-field coherence, we only find one single instance of significant coherence, out of 63 spike-field pairs. We conclude that spontaneous oscillations in the absence of stimulation are found in primary visual cortex under the conditions used in this experiment, both in single neurons and LFP, but this oscillatory modes are not coherent. Most probably, coherent activity is task or stimulus-related. These results suggest that oscillatory modes exist in the absence of stimulation, providing a mechanism to quickly entrain the system in a coherent state of activity at the time of the stimulus arrival.

**P20. EFFECT OF REPEATED ADMINISTRATION OF THE SELECTIVE KAPPA-OPIOID RECEPTOR AGONIST U-69593 ON THE ACTIVITY OF MESOCORTICOLIMBIC SYSTEM AFTER ACUTE ADMINISTRATION OF AMPHETAMINE: PRELIMINARY STUDIES IN THE MEDIAL PREFRONTAL CORTEX.** (Efecto de la administración repetida del agonista opioide kappa U-69593 sobre la actividad del sistema mesocorticolímbico después de la administración aguda de anfetamina: estudios preliminares en la corteza pre-frontal medial). Fuentealba, J.A.<sup>1</sup>, Gysling, K<sup>2</sup>, Andrés, M.E.<sup>2</sup>; <sup>1</sup>Dept. of Pharm., <sup>2</sup>Cell & Mol. Biol, Catholic Univ. Chile, Santiago, Chile. Acute administration of kappa opioid agonists decreases both dopamine extracellular levels in the mesocorticolimbic system (medial prefrontal cortex and nucleus accumbens) and locomotor activity. Opposing to its acute effects, repeated administration of kappa opioid agonists potentiate both induced locomotor activity and stimulated dopamine release in the nucleus accumbens. Since kappa opioids have been considered as potential treatments for stimulant dependence, their long-term effects on are of a major concern. The present study was undertaken to investigate the *in vivo* effect of repeated administration of the kappa opioid agonist U-69593 on dopamine extracellular levels in the nucleus accumbens and

medial prefrontal cortex after an acute injection of amphetamine (0.6 mg/Kg, i.p). Moreover, the horizontal locomotor activity was study. Rats were injected once daily with U-69593 or vehicle for four days. Microdialysis studies and the locomotor activity experiments were conducted one day later. Microdialysis studies revealed that pre-exposure to U-69593 had no effect on basal dopamine levels but significantly augmented amphetamine-induced dopamine extracellular levels (U-69593/amphetamine  $19.24 \pm 5.08$  fmol/ $\mu$ l; vehicle/amphetamine  $7.26 \pm 3.02$  fmol/ $\mu$ l). Accordingly, amphetamine-induced locomotor activity was also significantly potentiated in U-69593 pre-exposed rats (U-69593/amphetamine  $63.43 \pm 6.23$ ; vehicle/amphetamine  $42.87 \pm 5.20$ ; total crossover in 50 minutes). These results suggest that long-term effect of kappa opioids results in dopaminergic facilitation in the dopaminergic mesolimbic system accompanied by sensitization of the amphetamine-induced increase in locomotor activity. Microdialysis study in the medial prefrontal cortex is carried out to address the question if repeated administration of kappa opioids modifies in the same fashion mesolimbic and mesocortical pathways. Funded by Nucleus Millenium "Stress and addiction" and VRAID, PUC

**P21. CONSTITUTIVE EXPRESSION OF CONNEXIN43 HINDERS NEURONAL DIFFERENTIATION IN P19 CELL LINE.** (La expresión constitutiva de la conexina43 impide la diferenciación de neuronas en las células P19). <sup>1</sup>Gatica C., <sup>2</sup>Figuroa V., <sup>2</sup>Martínez, A.D. and <sup>1</sup>Sáez J.C. <sup>1</sup>Departamento de Ciencias Fisiológicas. Pontificia Univ. Católica de Chile, Santiago, and <sup>2</sup>Centro de Neurociencias de Valparaíso, Universidad de Valparaíso, Valparaíso. Chile. P19 cells, a multipotent embryonal carcinoma cell line, differentiate to neurons and macroglia after treatment of cell aggregates with retinoic acid (RA). Undifferentiated P19 cells express functional gap junction channels and connexin43 (Cx43), a protein subunit of these channels. Cx43 has a long cotoplasmatic C-terminus which can be phosphorylated by different protein kinases affecting the cell-cell communication. RA treatment reduces Cx43 levels and gap junctional communication. Our first objective was to investigate the effect of constitutive Cx43 expression (P19 cells permanently transfected with Cx43 cDNA, P19-Cx43) on levels of neuronal differentiation markers. Functional gap junctions were evaluated using the dye coupling technique (Lucifer yellow, LY). Levels and cellular distribution of proteins were evaluated by immunoblotting and immunofluorescence, respectively. After 48 h of 0.5  $\mu$ M RA treatment both P19 and P19-Cx43 cells expressed Nestin, an early neuronal differentiation marker, but 2 days later only a fraction of P19, but not P19-Cx43 cells, acquired a neuronal morphology and expressed the neuronal marker NeuN. Both Cx43 levels and dye coupling were absent in RA-treated P19 but present in P19-Cx43 cells. Then we studied if constitutive Cx43 expression, truncated in the amino acid 251, (P19-Cx43-CT251) changes the expression pattern of NeuN evaluated by confocal immunofluorescence. P19 cells that expressed Cx43-CT251 neither acquired neuronal morphology nor expressed NeuN. These results suggest that Cx43, possible by its channel function rather than its C-terminal mediated signal transducer activity impedes the RA-induced neuronal differentiation process in P19 cells. This work was partially supported by FONDECYT grants 1070592 (to J.C.S.) and Anillo ACT-46 (to A.D.M.).

**P22. GLYCOSYLATION OF SHAKER K<sup>+</sup> CHANNELS FAVORS ITS INCORPORATION TO THE PLASMA MEMBRANE OF XENOPUS LAEVIS OOCYTES.** La glicosilación de los canales de potasio tipo *Shaker* favorece su incorporación a la membrana plasmática de los ovocitos de *Xenopus laevis*. Gayol S., Naranjo D. and Neely A. Centro de Neurociencia de Valparaíso. Universidad de Valparaíso. Voltage gated potassium (K<sup>+</sup>) channels make up a large family of membrane proteins that play important physiological roles such as the repolarization of the action potential. The *Shaker* K<sup>+</sup> channel is particularly well suited for studies of ion channel biogenesis and protein quality control because they express well in a variety of systems, including *Xenopus laevis* oocytes. The wild-type protein folds and assembles into tetramers in the endoplasmic reticulum and is then efficiently transported to the Golgi apparatus where the carbohydrate chains are added prior to their appearance at the cell surface. The *Shaker* K<sup>+</sup> channel protein is glycosylated on two asparagine residues, N259 and N263, in the first extracellular loop. Papazian and their group showed that mutating these residues to glutamine abolishes glycosylation but does not prevent the cell surface expression of functional channels in *Xenopus laevis* oocytes. Furthermore, the voltage dependence of unglycosylated channels is similar to that of wild-type *Shaker* channels, indicating that glycosylation has no consequences for the assembly of functional channels nor their transport to the cell surface (Santacruz-Tolosa et al (1994) *Biochemistry* 33, 5607–5613). Macroscopic currents of *Shaker IR* channels showed that the unglycosylated channels displayed functional differences compared with wild-type, including cell surface expression and activation kinetics. Glycosylation promotes the incorporation to the plasma membrane and speeds activation. Funded by ACT46

**P23. SR-MARCO-MEDIATED ACTIVATION OF ASTROCYTES AND MICROGLIA: THEIR ROLE IN NEUROINFLAMMATION. Activación de astrocitos y microglías mediada por SR-MARCO: su rol en neuroinflamación.** Bárbara Godoy and Rommy von Bernhardt. Neuroscience Laboratory, Neurology Department, Faculty of Medicine, Pontificia Universidad Católica de Chile. Alzheimer's disease is associated to neuroinflammation and cytotoxicity. These responses depend mainly on the activated microglial cells and astrocytes associated to the  $\beta$ -amyloid (A $\beta$ ) aggregates. Up to date it is unknown which receptors mediate the participation of A $\beta$  on the induction of inflammatory or cytotoxic responses. One of the candidates is the scavenger receptor (SR) MARCO, a member of SR-A family. SR-MARCO is able to bind A $\beta$  and is expressed in rat astrocytes and microglia. It is the only member of SR-A family expressed by astrocytes. In this work we studied the effect of SR-A ligands and A $\beta$  on the activation of rat primary glial cultures. Inflammatory response was evaluated by quantifying nitric oxide (NO) production by the Griess assay, and the induction of interleukin-1 $\beta$  (IL-1 $\beta$ ) expression, by western blot. We observed that microglial cultures exposed to SR-A ligands increased NO production, effect that was potentiated by the presence of A $\beta$ . In contrast, neither SR-A ligands nor A $\beta$  increased production of NO by astrocyte cultures. Moreover, microglial production of NO induced by SR-A ligands was reduced by the presence of astrocytes. However, stimulation of astrocytes with SR-A ligands increased the expression of IL-1 $\beta$ , an effect that was potentiated by co-stimulation with A $\beta$ . Pretreatment of microglia cultures exposure to SR-A ligands with the IL-1 receptor antagonist IL-1ra did not reduce NO production. All together these results suggest that IL-1 $\beta$  was not responsible of the modulation of NO production. We propose that microglial activation mediated by

receptors of the SR-A family (SR-A and SR-MARCO) could promote cytotoxicity through increased NO production, an effect that was further increased in the presence of A $\beta$ . Instead, SR-MARCO-mediated response in astrocytes could be neuroprotective by inhibiting microglial NO production. Supported by grant FONDECYT 1040831.

**P24. PROTEIN KINASE CK2 REGULATES THE INHIBITORY EFFECT OF THE PDZ2 DOMAIN OF PSD-95 ON NMDAR ACTIVITY.**

La proteína CK2 regula el efecto inhibitorio del dominio PDZ2 de PSD-95 sobre la actividad del NMDAR. Dennisse González<sup>1</sup>, Estíbaliz Ampuero<sup>1</sup>, Mauricio Sandoval<sup>1,3</sup>, Juan José Marengo<sup>2</sup>, Ursula Wyneken<sup>1</sup>. [neuroc@uandes.cl](mailto:neuroc@uandes.cl). <sup>1</sup>Laboratorio Neurociencias, Universidad de los Andes; <sup>2</sup>ICBM, Universidad de Chile; <sup>3</sup>Facultad de Ciencias, Universidad de Chile. In the postsynaptic density (PSD) of excitatory synapses, NMDA receptors (NMDARs) interact with the PDZ 1 and PDZ 2 domains of the scaffolding protein PSD-95. Although it is known that PSD-95 participates in the trafficking and anchoring of glutamate receptors to synapses, less is known about its direct effects on NMDAR activity. To measure NMDAR currents, we have incorporated isolated PSDs into giant liposomes to do patch clamp recordings on them. The addition of a GST fusion protein containing the PDZ1 domain of PSD-95 to the intracellular face of the PSD led to a two-fold increase in channel activity, whereas PDZ2 inhibited the NMDAR by 50%. We had previously shown that PSD-95 is a major protein kinase CK2 substrate in PSDs. Here, we show that PDZ2 and PDZ1 are phosphorylated by CK2, and this abolished their modulatory effects on NMDAR currents. Site-directed mutagenesis of threonine 235, one of the most probable CK2 substrates within the PDZ2 domain that lies in the NR2 C-terminal binding pocket, abolished its inhibitory effect on NMDARs but not the interaction between both proteins. We have therefore identified a single amino-acid within the PDZ2 domain, a probable CK2 substrate, that mediates its inhibitory effect on the NMDAR. This might represent a new mechanism involved in synaptic plasticity. Financiamiento: Farmacias Cruz Verde, BMBF(UW), Universidad de los Andes.

**P25. ADDITION OF CHARGES TO THE SHAKER POTASSIUM CHANNEL VOLTAGE SENSOR.**

Adición de cargas al sensor de voltaje del canal de potasio tipo Shaker. Vivian M. González<sup>1,2</sup>, Katica Boric<sup>1,2</sup>, Tania Estévez<sup>1</sup> and David Naranjo<sup>1</sup>. <sup>1</sup>Centro de Neurociencias de Valparaíso. Universidad de Valparaíso. <sup>2</sup>Programa de Doctorado en Neurociencia, Fac. de Ciencias, Universidad de Valparaíso. Voltage-gated potassium channels contain a voltage sensor domain in each of its four subunits that confers its exquisite sensibility to membrane potential. The fourth  $\alpha$ -helical transmembrane segment (S4) of each subunit has seven highly conserved basic amino acids, periodically spaced by two hydrophobic residues. At least the four outermost arginine residues in S4 completely move through the membrane electrical field upon depolarization. This charge movement (12-13  $e_0$  per channel) promotes a conformational change leading the opening of the channel conduction pathway. We carried out punctual substitutions of V363 (located between the two outermost arginines R362 and R365) for either an arginine or an aspartate. Surprisingly, both additional charge mutations decreased the charge movement coupled to the opening (6-8 $e_0$  per

channel) measured by the limiting slope method. To test whether the reduced effective valence introduced by the additional charge was caused by a disruption of the electric field sensed by the S4, we assessed the accessibility of methanethiosulfonate derivatives for the double-mutants: R362C /V363R or R362C /V363D. We did not detect differences in the rate of modification within a ten-fold change in the overall open probability; suggesting a lack of state dependent in the modification rate in the double-mutants. We speculate that in the mutant channels with additional charges, Arg 362 could reside outside the electric field. Funded by ACT-46. Vivian.M. Gonzalez and Katica Boric are CONICYT Fellows.

## **P26 DIFFERENTIAL REGULATION OF THE FUSION PORE STABILITY AND EXPANSION BY THE DISTINCT $\text{Ca}^{2+}$ CHANNEL SUBTYPES.**

Regulación diferencial de la estabilidad y expansión del poro de fusión por los distintos subtipos de canales de  $\text{Ca}^{2+}$ . Arlek M González-Jamett, Alvaro O. Ardiles, Jaime Maripillan, María José Guerra, David Naranjo, Alan Neely y Ana M. Cárdenas. Centro de Neurociencia de Valparaíso, Universidad de Valparaíso, Chile. Various studies have focused in the relative contribution of different voltage-activated  $\text{Ca}^{2+}$  channels (VACC) to total transmitter release. However, how  $\text{Ca}^{2+}$  entry through a given VACC subtype defines the pattern of individual exocytotic events remains unknown. To address this question, we have used amperometry and intracellular  $\text{Ca}^{2+}$  measurements in bovine chromaffin cells, in the presence of different  $\text{Ca}^{2+}$  channels inhibitors. L, N and P/Q channels were individually or jointly blocked with flunarizine,  $\omega$ -conotoxin GVIA,  $\omega$ -agatoxin IVA or  $\omega$ -conotoxin MVIIC. L, N and P/Q channel types contributed similarly to cytosolic  $\text{Ca}^{2+}$  signals induced by 70 mM  $\text{K}^+$ . When these three channel types were simultaneously blocked with flunarizine plus  $\omega$ -conotoxin MVIIC, the depolarization-induced cytosolic  $\text{Ca}^{2+}$  signals was reduced by a 95%, indicating that R channels have a minimal contribution in bovine chromaffin cells. Interestingly, when two channels were simultaneously blocked, the remaining channel population exhibited different contributions to the frequency of exocytotic events. Thus, when  $\text{Ca}^{2+}$  entry occurred uniquely through L, N, or P/Q channels, the frequency of exocytotic events was  $0.04 \pm 0.02$ ,  $0.13 \pm 0.03$  and  $0.32 \pm 0.05 \text{ s}^{-1}$ , respectively. Additionally, we found that the fusion pore stability (the time period between pore formation and its expansion) and its kinetics of expansion were differentially regulated by L, N and P/Q channels. The  $\text{Ca}^{2+}$  entry through P/Q channels significantly accelerated the ascending phase of the amperometric spikes, while  $\text{Ca}^{2+}$  entry through N channels had the contrary effect on that amperometric parameter. Further,  $\text{Ca}^{2+}$  entry through P/Q channels significantly decreased the percentage of events with foot signals and shortened the foot duration. On the other hand, the effects of the individual blockade of L-type channels with flunarizine suggest that  $\text{Ca}^{2+}$  entry through L channels slows the expansion of the fusion pore and increases its stability. Taken together our results indicate that  $\text{Ca}^{2+}$  entry through a given VACC subtype not only determine the occurrence of an exocytotic event, but it also defines the fusion pore dynamics. This work was supported by Fondecyt 1020812 and Anillo de Ciencia y Tecnología (ACT-46)

## **P27. VISUAL EXPLORATION OF FACES WITH DIFFERENT EMOTIONAL EXPRESSIONS**

(Exploración visual de caras con diferentes expresiones emocionales) A. Helo, J.P. Ossandón, J. Álvarez & P.E. Maldonado. CENI y Programa de Fisiología y Biofísica (ICBM), Facultad de Medicina, Universidad de Chile. Seeing faces is one of the most common visual experiences. This visual behavior has been strongly linked to social behavior like the ability to identify and recognize other people and infer their emotional and mental states. As vision is an active phenomena in which we are continuously exploring visual field through ocular movements it seems important to

have a description of this gaze patterns for the understanding of face processing. While previous studies about face exploration generally have not considered emotional expressions, they are important in that they may reveal top-down influences in visual gaze control. Here we described ocular movement and explorations patterns in visual observations of face with five emotional expressions (neutral, sad, happy, fear and angry). Overall, fixation durations were longer than previously reported times of fixations over other types of natural scenes (median face fixations=232 95% CI: 230-236; median natural fixations=214 95% CI: 212-216). When analyzed by different facial expression there were also significant differences in fixation durations between expressions type (Kruskal-Wallis,  $p=0.04$ ). These longer fixations are mainly due to eyes and mouth locations. Patterns of movements and region of interest analysis shows a strong bias to fixate in eyes (45% of all fixations and 54% of early fixation) more than in other areas and in a highly stereotypical gaze patterns. These results are consistent with evidence that show that there are brain areas specialized in the processing of visual information about the eyes and other face components, probably used as a cue of other people's intentions or expectancies. Supported by ICM P04-068-F and Fundación Guillermo Puelma

**P28. EFFECT OF INTERLEUKINE-1 $\beta$  ON SPINAL WIND-UP ACTIVITY IN NORMAL AND MONOARTHRITIC RATS WITH AND WITHOUT PROPENTOFYLLINE PRETREATMENT** (Efecto de interleukina 1 $\beta$  en el *wind-up* espinal de ratas normales y monoartríticas con y sin pretratamiento con propentofilina). Laurido C, Hernández A, Arriagada O, Pelissier T\*, Constandil L. Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile; \*ICBM, Facultad de Medicina, Universidad de Chile. When a direct traumatic injury is applied to the spinal cord, it responds with a robust glial reaction characterized by decreased ramification, hypertrophy, proliferation, and the up-regulation of immunoregulatory molecules such as cytokines. The contribution of glia-neuron interactions in the spinal mechanisms underlying hyperalgesia remains to be clarified. To investigate the functional contribution of glial cells in the spinal cord nociceptive transmission, the effect of intrathecally administered IL-1 $\beta$  was studied in both normal and adjuvant-induced, arthritic adult male Sprague Dawley rats with or without pharmacological glial inhibition. Four weeks after induction of monoarthritis, rats were treated with the glial cell inhibitor propentofylline, 10  $\mu$ g i.t. daily during ten days. The rats were then submitted to a C-fiber-mediated reflex paradigm evoked by single and repetitive (*wind-up*) electric stimulation. Both the propentofylline treatment and the monoarthritic condition modified the stimulating current required for threshold activation of C reflex responses. Results showed that IL-1 $\beta$  increased spinal cord wind-up activity in rats without propentofylline pretreatment, but resulted in decreased wind-up activity in propentofylline treated animals. Thus, glial inactivation reverted into inhibition the excitatory effect of IL-1 $\beta$  on spinal cord wind-up, irrespective the normal or monoarthritic condition of rats. We can conclude that the excitatory effect of nanomolar doses of IL-1 $\beta$  on spinal wind-up in healthy rats is produced by an unidentified glial mediator, while the inhibitory effects of IL-1 $\beta$  on wind-up activity in animals with inactivated glia resulted from a direct effect of the cytokine on dorsal horn neurons. Supported by grants 1050099 and 1070115 from Fondecyt.

**P29. PHARMACOLOGICAL STUDIES OF MINIATURE END-PLATE CURRENTS FROM DROSOPHILA LARVAE.** (Estudios Farmacológicos de las Corrientes Miniatura del Terminal Sináptico de la Larva de *Drosophila*) †F. López, \*M. Sanhueza and \*R. Delgado. \*Departamento de Biología, Facultad de Ciencias and Instituto Milenio de Dinámica Celular y Biotecnología, Universidad de Chile and †Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. According to the quantal hypothesis proposed by Katz and colleagues, miniature synaptic signals are produced by the spontaneous exocytic discharge of the total content from a synaptic vesicle. However, available data from various preparations, including mammalian central synapses, show that frequency histograms of the size of spontaneous events often deviate from a normal distribution, showing unexpected additional components. In both vertebrates and invertebrates, two separate vesicle pools are observed in nerve terminals, the readily-releasable pool (RRP), consisting of vesicles docked at the active sites of plasma membrane and a cytoplasmic reserve pool (RP). Vesicle size can be modified by specific experimental manipulations in chromaffin cells and neurons. Interestingly, in *Drosophila* NMJ synapsis it was recently shown that the size of glutamatergic vesicles and the quantal size are increased by experience, due to an enhanced replenishment of the reserve pool vesicles. Also in this model, synaptic vesicles coming from the RP reach the active zone after intense electrical stimulation or by enhancement of cAMP-dependent signaling pathway. While the differential regulation of transmitter release and vesicle recycling by  $Ca^{2+}$  or other second messengers may be crucial for synaptic transmission and plasticity, the underlying mechanisms remain unknown. To investigate these regulatory mechanisms, we carried out a pharmacological study of miniature end-plate currents (mEPCs) of third instar *Drosophila* larvae, conducting two electrode voltage-clamp recordings. We observed that a permeable cAMP analog increased the frequency of mEPCs, without significant amplitude changes. This effect was antagonized by a protein kinase A inhibitor. We also found intriguing differential effects of the exposure to diverse  $Ca^{2+}$  channel blockers.  $La^{3+}$  produced a persistent increase in the frequency of mEPCs, while flunarizine caused a transitory increase that lasted only a few minutes.  $Cd^{2+}$  produced a reduction in the spontaneous frequency of release. The use of these tools may contribute to disclose the regulation of the different processes involved in neurotransmitter release and the participation of distinct vesicle pools in synaptic function. FONDECYT 1040772 (RD), Anillos Ciencia y Tecnología ACT-45, P. Bicentenario CONICYT; MIDEPLAN ICM-P05-001-F y JS Guggenheim Fellowship (JB).

**P30. BIPHASIC ATP-INDUCED MODULATION OF LONG-TERM POTENTIATION IN CA1 OF RAT HIPPOCAMPAL SLICES.** (Modulación bifásica inducida por ATP de la potenciación a largo plazo en rebanadas de hipocampo de rata). R. A. Lorca<sup>1</sup>, S. Moreira-Ramos<sup>2</sup>, J. P. Huidobro-Toro<sup>1</sup> and B. Morales<sup>2</sup>. <sup>1</sup>Laboratorio de Nucleótidos, Centro de Regulación Celular y Patología, Instituto MIFAB, Departamento de Fisiología, P. Universidad Católica de Chile. <sup>2</sup>Laboratorio de Neurociencias, Departamento de Biología, Universidad de Santiago de Chile. The cellular mechanisms and purinergic receptor subtypes involved in ATP modulation of long-term potentiation (LTP) in the CA1 area of rat hippocampus remain unclear. Our aim is to identify subtypes of purinergic P2X and/or P2Y receptors in LTP induction. Hippocampal slices (400  $\mu$ m thick) were obtained from 2-3 week-old Sprague-Dawley rats. LTP was recorded in CA1 by electrical stimulation of high frequency (TBS) applied from Schaffer collaterals, using field excitatory postsynaptic potentials (fEPSP)

technique. 1  $\mu$ M ATP, incubated 10-min before and 10-min after TBS, potentiated the induction of LTP from  $137.2 \pm 8.8$  to  $178.9 \pm 17.3$  ( $n=13$ ,  $p<0.05$ ), whereas 5 and 10  $\mu$ M ATP inhibited LTP induction to  $107.5 \pm 14.5$  ( $n=12$ ,  $p<0.01$ ) and to  $101.5 \pm 10.4$  ( $n=8$ ,  $p<0.01$ ) respectively, demonstrating the biphasic nature of this ATP response. Neither, 100 nM 2-MeSADP, a P2Y<sub>1,12,13</sub> agonist, nor 1  $\mu$ M adenosine had an effect on LTP induction, suggesting that neither P2Y<sub>1,12,13</sub> nor the adenosine receptors modulate the induction of LTP, although these agonists slightly inhibited basal fEPSPs. To approach the possible P2X receptor subtype involved, and considering that trace metals differentially modify P2X<sub>4</sub> receptor activity (Acuña-Castillo et al, 2000), we next assessed whether 1-10  $\mu$ M copper or 1-10  $\mu$ M zinc modulate LTP. Trace metals were applied in the same manner as ATP; whereas copper inhibited in a concentration-dependent manner, zinc likewise facilitated LTP induction. 5 and 10  $\mu$ M copper significantly diminished LTP from  $175 \pm 18.1$  to  $131.3 \pm 9.7$  ( $n=11$ ,  $p<0.05$ ) and from  $177.4 \pm 15.9$  to  $123.5 \pm 8$  ( $n=12$ ,  $p<0.01$ ), respectively; 10  $\mu$ M zinc increased LTP from  $161.2 \pm 5.1$  to  $210.4 \pm 16.5$  ( $n=10$ ,  $p<0.01$ ). These findings suggest the possible involvement of P2X<sub>4</sub> receptors in LTP. However, the exact relation between trace metal modulation and the role of purinergic receptors in LTP remains to be further established. Funded by FONDAP 13980001, MIFAB, DICYT-USACH and CONICYT Fellowship AT 23070142.

### **P31. ORGANOPHOSPHORATE CHLORPYRIFOS PESTICIDE EFFECTS ON THE MECHANISMS OF SPATIAL LEARNING AND MEMORY IN RODENTS**

Efectos del pesticida organofosforado Clorpirifos sobre los mecanismos de memoria y aprendizaje en roedores. <sup>1</sup> Maceiras, S. <sup>1</sup> Subiabre, P. <sup>2</sup> Berrios-Bravo, C. <sup>1</sup> Valenzuela-Harrington, M. <sup>1</sup> Facultad de ciencias naturales y exactas, Universidad de Playa Ancha de Ciencias de la Educación, <sup>2</sup> Facultad de ciencias químicas y farmacéuticas, Universidad de Chile. The capacity of orientation of rodents is an evolutive matter of importance in these animals. It could study in an experimental way carrying out laboratory tests using labyrinths. Their success depends on the animal's neurological process performed at a spatial level. The aim of the present work was recognize pesticide substance effect; in our case organophosphorate chlorpyrifos (0,0- diethyl 0-3,5,6-trichloro-2- pyridyl phosphorothioate) over spatial learning and memory in a group of male Sprague – Dawley rats. This group was put to test, administrating chlorpyrifos (100 mg/Kg per day) for several days. The treatments compared a control group (treated with a vehicle) and the other treated with the pesticide diluted in vehicle. The rats were put to spatial learning test in T labyrinths and also anxiety open field tests. A preliminary analysis of collected data indicated that the spatial learning and anxiety levels in rodents might be affected.

### **P32 EFFECT OF INTRATHECAL D-SERINE IN SPINAL NOCICEPTIVE TRANSMISSION OF NORMAL AND MONOARTHRITIC RATS**

(Efecto de D-serina intratecal en la transmisión nociceptiva espinal en ratas normales y monoartríticas). Mariqueo T<sup>1</sup>, Laurido C<sup>1</sup>, Vergara A<sup>1</sup>, Tornabene L<sup>1</sup>, Pelissier T<sup>2</sup>, Flores F<sup>1</sup>, Hernández A<sup>1</sup>, Constandil L<sup>1</sup>; <sup>1</sup>Laboratorio de Neurobiología, Universidad de Santiago de Chile; <sup>2</sup>ICBM, Facultad de Medicina, Universidad de Chile. D-serine is a ligand for the glycine modulatory binding site of NMDA receptors, a receptor subtype that plays a key role in pain transmission in the spinal cord. At low concentrations, D-serine has been shown to produce facilitation of the tail-flick reflex and to inhibit the

antinociceptive effect of NMDA antagonists. We studied the effect of three doses of intrathecal D-serine (10, 100 and 300 µg) on spinal nociceptive transmission of normal and monoarthritic (intra-articular Freund adjuvant-induced arthritis) adult male Sprague-Dawley rats, by submitting the animals to a C-fiber-mediated reflex paradigm evoked by single and repetitive (wind-up) electric stimulation. Results showed that 10 µg of intrathecal D-serine did not modify the nociceptive transmission of either normal or monoarthritic rats, while 100 and 300 µg showed antinociceptive effect in monoarthritic and normal rats, respectively. The results showed that high doses of intrathecal D-serine decreased pain sensitivity in rats, monoarthritic animals being more sensitive to spinal D-serine than normal ones. This effect of D-serine could be explained by the displacement by D-serine of endogenous glycine bound to glycine B sites, together with the higher calcium current induced by D-serine compared to glycine. Funded by grants 1050099 and 1070115 from Fondecyt.

**P33. SALIENCY ROLE IN THE HUMAN ATTENTION ALONG THE INSPECTION OF STATIC IMAGES.** (Rol de las Saliencias en la atención humana durante la inspección de imágenes estáticas) Muñoz Silva F.<sup>1,2,3</sup>, Ossandon J. P.<sup>2</sup>

Loncomilla P.<sup>3</sup> Ruiz del Solar J.<sup>3</sup> Maldonado P.<sup>2</sup> · 1 Centro de Neurociencia de Valparaíso, Universidad de Valparaíso; 2 Centro de Neurociencias Integradas y Programa de Fisiología y Biofísica, Universidad de Chile; 3 Departamento de Ingeniería Eléctrica, FCFM, Universidad de Chile. The human vision is selective to subsets of the visual stimuli. This results in visual fixations in specific areas in natural scenes. The central question about that is: How are fixation locations chosen in a static image? Computational models, such as the saliency model of Itti y Koch (1998) describe the focal attention as processing intrinsic characteristics of the image, as contrast, intensity, color or direction. This model intent to emulate the primary visual pathway in primate and the computation of the intrinsic characteristics allow building a map of potential locations for visual fixation. The accuracy of this saliency model does conflict with new experimental data and now several works dispute this approach. In the present study, we investigated the performance between the saliency model and human experimental data. For this propose we use seven classes of pictures, which consisted in landscapes, human faces, outdoor scene, cosmos, white noise, color noise and fractals. Each image was display in a computer monitor in a random fashion four second while we recorded the subject's eye movements with and EyeLink II eye tracker. Our results indicate that consistent divergence between the experimental data and computational models for natural images (landscape, human face and outdoor scene), but not for fractals or white noise, converging the performance with the theoretical saliency models. Theses results implicate that attention or other high level cognitive processes strongly modulate the sequence of eye movements, and thus fixation locations. Supported by Iniciativa Científica Milenio ICM P04-068-F

**P34. EFFECT OF HYPOXIA ON GLIAL CELL ACTIVATION AND SCAVENGER RECEPTORS EXPRESSION INDUCED BY INFLAMMATORY STIMULI.** Efecto de la hipoxia en la activación glial y la expresión de Receptores Scavenger inducida por estímulos inflamatorios. Loreto Olavarría & Rommy von Bernhardt.

Laboratorio de Neurociencia, Departamento de Neurología, Facultad de Medicina, Pontificia Universidad Católica de Chile. The unbalance between inflammatory activation and neuroprotective-modulatory activity of microglia and

astrocytes can lead to neurotoxicity. Such a mechanism could be involved in the neurodegenerative process of Alzheimer disease (AD). The unbalance could be promoted by physiopathological conditions like hypoxia, which is related to alterations of brain perfusion during aging. Hypoxia/reperfusion is responsible for the production of radical species which can activate glial cells increasing their reactivity to molecules such as Amyloid Beta ( $A\beta$ ) and proinflammatory factors. Also, hypoxia and inflammation could modify the expression of some receptors involved in  $A\beta$  internalization like Scavenger Receptors (SR). We studied the effect of a hypoxic condition (2%  $O_2$ , 5%  $CO_2$ ; for 0.5, 2 and 4 h) in the activation of primary astrocytes cultures and the microglial line cell EOC-20, by evaluating Nitric Oxide (NO) production at 24 and 96 h after the hypoxic stimulus. After 4 h of hypoxia + LPS or LI (1 $\mu$ g/ml LPS + 10 ng/ml IFN- $\gamma$ ), NO production by astrocytes increased 12-fold at 24 h and 14-fold at 96 h compared to normoxic control. A 2.3-fold increase compared to normoxic proinflammatory conditions. In EOC-20 cells, NO production induced by hypoxia + LPS or LI increased 120-fold respect normoxic control at 24 h and 140-fold at 96 h. A 2.2-fold increase compared to normoxic proinflammatory condition.  $A\beta$  did not induce NO production for neither cell type. We studied by western blot the effect of hypoxia on the expression of SR-MARCO and SR-BI by astrocytes. We observed that the expression of both SRs decreased under hypoxic conditions compared to normoxia + proinflammatory molecules. Our results suggest that hypoxia increased glial reactivity potentiating induction of NO production by proinflammatory molecules and decreased expression of both SRs. Those effects could contribute to the impairment of  $A\beta$  uptake, promoting the formation of aggregates and perpetuating the inflammatory glial activation characteristic of AD.

Supported by FONDECYT 1040831.

**P35. DOPAMINERGIC MODULATION OF RECURRENT NETWORK ACTIVITY THROUGH CONTROL OF THE H-CURRENT** (Modulación dopaminérgica de la actividad recurrente a través del control de la corriente  $I_h$ ). Carolina Oliva<sup>1, 2</sup> and David McCormick<sup>2</sup>. <sup>1</sup>Centro de Neurociencias de Valparaíso, Universidad de Valparaíso and <sup>2</sup>Department of Neurobiology, School of Medicine, Yale University. We are currently examining the mechanisms that may underlie rapid changes in network activity and neuronal excitability and the interaction of these with the slow changes controlled by neuromodulators. One form of rapid change is achieved through the activation of “reverberating” networks of cortical neurons. A wide variety of factors may influence the generation of recurrent network activity, including the activation of intrinsic conductances or changes in synaptic strength between these cells. The prefrontal cortex (PFC) is critical for higher order cognitive functions, guiding our behavior, thoughts and emotions using representational knowledge; that is, short-term mnemonic task or working memory. Dopaminergic modulation of prefrontal cortex plays an important role in cognitive functions and has been suggested to be critically involved in numerous normal and abnormal cognitive processes including schizophrenia. However, despite intense research, there is still a lack of clear understanding of the basic principles of actions of DA in the PFC. One difficulty to solve this is that DA is a neuromodulator and is clearly not an excitatory or inhibitory neurotransmitter. DA acting through the same receptor can produce bidirectional modulation via different intracellular cascades and can exert bidirectional effects on a physiological measure at different concentrations. One target for the effect of neuromodulators in the brain is the hyperpolarization-activated h-current ( $I_h$ ). Neuronal

$I_h$  channels subserve fundamental physiological functions such as contribution to membrane potential, integration of synaptic input to neurons and the rhythmicity of various brain regions. However, the effects of DA on the h-current may be synapse and neuron specific, leading to depolarization in some cases or reducing excitability in others. Here, I use brain slices of ferret PFC to study how dopamine may modulate recurrent network activity and intrinsic mechanisms in layer 5 pyramidal cells of the PFC. Specifically, I study how dopamine can modulate the biophysical properties of hyperpolarization activation h-current. In this work, I show how dopamine increases the excitability of this cell in a dose-dependent manner, changing the activation time course of this current.

**P36 DO HUMANS SCAN STIMULI FOR SPECTRAL CHANGES IN DEPTH PERCEPTION?**

¿Exploran los humanos los estímulos en busca de diferencias espectrales para la percepción de profundidad? Jose P. Ossandon<sup>1</sup>, Peter Zaenen<sup>2</sup>, Pedro Rosas<sup>1,2,1</sup>CENI y Programa de Fisiología y Biofísica. Facultad de Medicina. Universidad de Chile. <sup>2</sup> Katholieke Universiteit Leuven. Given that the receptive fields of simple cells in V1 are well modelled by Gabor filters spanning different frequency and orientation tuning it has been suggested that spatial vision is accomplished by performing a spectral decomposition of stimuli. We are interested in assessing this hypothesis in a rather high-level task such as depth perception. Here we report results for the gaze of human subjects performing slant-discrimination using temporal two-alternative-forced-choice. If spatial-frequency decomposition is critical for this type of task, we expected to observe that fixations would follow the change of spectra relevant for slant discrimination. To assess such question we constructed an ideal observer of slant discrimination based on tracking the Average Peak Frequency as suggested by Sakai & Finkel (1997). Such a model predicted the best locations on our stimuli depending on the slant level and the texture type used in the experiment. By comparing those predictions with the empirical data collected we wanted to assess whether the scanning strategies of human observers are consistent with an observer tracking spectral changes in the stimuli.

**P37. ELECTRICAL RESONANCE IN THE  $\theta$  FREQUENCY RANGE IN OLFACTORY AMYGDALA. (Resonancia eléctrica en el rango de frecuencia  $\theta$  en la amígdala olfatoria)**

M. Pezzoli, J. Bacigalupo, and M. Sanhueza. Depto. de Biología, Fac. de Ciencias e Inst. Milenio ICDB, U. de Chile. The amygdala complex is involved in processing emotionally relevant sensory information. Sensory input from the olfactory bulb (OB) enters this complex through the cortical nuclei of the amygdala. In rats, both OB mitral cells and layer II pyramidal neurons of the anterior cortical nucleus of the amygdala (ACo), present membrane potential intrinsic oscillations in the  $\theta$  range (2 – 12 Hz). Oscillatory activity in this frequency range has been proposed to be relevant for explorative behaviors as well as for memory formation. Accordingly, similar  $\theta$ -oscillations have been observed in the basolateral amygdala (BLA), a structure that receives ACo connections and is involved in emotional learning for different sensory modalities. It has been proposed that neurons endowed with intrinsic oscillatory electrical properties could be more reliably activated when stimulated presynaptically at their *natural* frequencies. In this work we studied the impedance (Z) amplitude profile (ZAP) of layer II ACo neurons to trace any “electrical frequency preference” or

resonance behavior of these neurons around the  $\theta$  range. We conducted whole-cell patch-clamp recordings in current-clamp mode in coronal brain slices of juvenile Sprague Dawley rats. A sinusoidal current of linearly decreasing frequency (15 – 0 Hz) was intracellularly injected and the voltage oscillatory output wave was recorded. Using fast Fourier transform (FFT) analysis we obtained the impedance magnitude profile by the equation:  $|Z| = |\text{FFT output (mV)}| / |\text{FFT input (pA)}|$ . We observed that a fraction of ACo neurons displayed a resonant profile (i.e. a peak in the impedance) in the range of 2 – 5 Hz. The neuronal resonance proved to be an intrinsic electrical property not dependent on neural network connections, as it persisted in the presence of glutamatergic and GABAergic synaptic inhibitors. The resonant profile was more prominent at hyperpolarized membrane potential values (i.e. more negative than -75 mV) and at depolarized values (i.e. more positive than -55mV) than around resting membrane potential (i.e. -59 mV). This suggests that different voltage-dependent conductances may participate in resonance generation in these two different potential ranges. To our knowledge this is the first work reporting neural electrical resonance in this brain area. Anillos Ciencia y Tecnología ACT-45, P. Bicentenario CONICYT; MIDEPLAN ICM-P05-001-F y JS Guggenheim Fellowship (JB)

**P38 EARLY ACTIVATION OF THE TUBEROMAMMILLARY NUCLEUS IS A COMMON FACTOR IN APPETITIVE BEHAVIORS IN RATS.** La activación temprana del núcleo tuberomamilar es un factor común en conductas apetitivas en ratas. M. Quispe, M. Contreras, M.E. Riveros, J.L. Valdés and F. Torrealba. Departamento de Ciencias Fisiológicas, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile. Histamine neurons of the tuberomammillary nucleus show an earlier activation during the appetitive phase of feeding, compared to the other arousal system nuclei. To test if in different appetitive behaviors also these histaminergic neurons become active first, we studied changes in Fos-ir in arousal nuclei during sexual, drinking and drug-seeking behavior. Male rats were exposed to sexually receptive or to non-receptive female rats, allowing sensory but not sexual contact. Receptive females elicited increased sniffing time which positively correlated with Fos-ir in the dorsal raphe, laterodorsal tegmental nucleus, orexin hypothalamic neurons and tuberomammillary nucleus. Non receptive females induced less sniffing and no increased Fos-ir. Other male rats were deprived of water for 48 h and presented with an empty water bottle to induce appetitive behavior. The presentation of an empty water bottle to thirsty rats induced increased approaches to the bottle while they tried to drink. While water deprivation *per se* increased Fos-ir in the dorsal raphe and the locus coeruleus, the presentation of the bottle increased Fos-ir in the tuberomammillary nucleus and induced a further Fos-ir increase in the locus coeruleus. Other male rats group was conditioned using the place preference paradigm, where one of two chambers was repeatedly paired with the administration of amphetamine (1.5 mg/Kg i.p.). Conditioned rats, but not rats injected with saline instead of amphetamine, showed a significant preference for amphetamine-paired room and increase in the number of Fos-ir in the tuberomammillary nucleus, orexin hypothalamic neurons and locus coeruleus. To evaluate the importance of the histaminergic neurons in the appetitive phase of these motivated behavior, we lesioned tuberomammillary nucleus using saporin conjugated to the hypocretin 2. The histaminergic neurons lesion blunted the appetitive phase in all motivated behaviors studied, without affecting general motor capacities. Taken together our results indicate that the histaminergic neurons become active at the onset of different motivated behaviors and they are key in the arousal that is essential in

motivation. Other arousal nuclei may participate depending on the particular behavior. Fondecyt 1020718, Fondecyt 1060476 and DIPUC.

**P39. TUBERALIN II: A NOVEL MEMBER OF THE PARS DISTALIS HORMONES?** (Tuberalin II: una nueva hormona de la pars distalis?). Carvajal AM, Guerra M, Vásquez P and Rodríguez EM. Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral de Chile. Valdivia, Chile. The pars tuberalis (PT) is a specific region of a adenohypophysis located in close spatial relationship with the median eminence of the hypothalamus. Although PT function has not yet been clarified there is evidence that it plays a role on the release of *pars distalis* (PD) hormones through the secretion of specific compounds called “tuberalins”. We have raised antibodies against two tuberalins (tuberalin I and tuberalin II) and have obtained evidence supporting their hormonal nature. The present investigation deals with the biochemical characterization of both tuberalins using bidimensional electrophoresis (2D) and immunohistochemistry (IHC). 2D *western blot* analysis using anti-tuberalin II revealed several immunoreactive spots with a mass of 20-25 kDa and electrofocussing pHs ranging between 5 and 9. Two of these spots correspond to LH and TSH, suggesting a molecular relationship between tuberalin II and PD-glycoprotein hormones. Using bioinformatics we identify conserved regions in LH, FSH and TSH, and selected a sequence common to these hormones, most likely corresponding to the binding site of the alpha subunit (common to the three hormones). A synthetic peptide was obtained and used to immunoabsorb anti-tuberalin II prior to immunostaining. Non-absorbed antituberalin II immunoreact with cells of the PD and the PT cells; the immunoabsorbed antibody does not longer immunoreact with the PD cells but continue to react with the PT cells. The results suggest that tuberalin II is a protein different form PD-glycoprotein hormones that is specifically expressed in PT cells, further supporting the possibility that tuberalin II is a the novel pituitary hormone. (Supported by Proyecto Bicentenario and DID-UACH grants to AC, MECESUP AUS0107 and DID-UACH grants to MG and Fondecyt 1030265 grant to ER)

**P40. PROINFLAMMATORY STIMULI INDUCE MICROGLIAL CELL-MEDIATED cAPP- AND  $A\beta$  NEUROTOXICITY IN HIPPOCAMPAL CULTURES.** Estímulos pro inflamatorios inducen la neurotoxicidad de  $A\beta$  y cAPP mediada por las células microgliales en cultivos hipocampales. Gigliola Ramírez, Sergio Rey and Rommy von Bernhardt. Laboratorio de Neurociencias, Departamento de Neurología, Facultad de Medicina, Pontificia Universidad Católica de Chile. Alzheimer’s Disease (AD) is a neurodegenerative disorder. One of its main histopathological hallmarks is the presence of senile plaques formed by aggregated  $\beta$ -amyloid peptide ( $A\beta$ ) surrounded by dystrophic neurites and activated glial cells. Glial cells, especially microglia, are the immune effector cells in the Central Nervous System (CNS). However, when glial cells are over-activated, they trigger crucial events in the development of neurodegenerative diseases, such as the sustained neuroinflammation observed in AD. We evaluated the response of hippocampal cells co-exposed to proinflammatory molecules, 1  $\mu$ g/mL LPS+10 ng/mL IFN- $\gamma$  (LI),  $A\beta$  ( $A\beta$ 1-42)- and APP244-C (APP244-C)-conjugated non-phagocytatable beads, and co-cultured with or without microglial cells. Co-cultures containing hippocampal and microglial cells

exposed to LI with or without APP244-C or A $\beta$ 1-42-conjugated beads decreased their reduction metabolism (MTT) by 50%, increased 13 to 14 fold the production of nitrites, evaluated through the Griess assay, and showed an increase of 5 to 6 fold on the expression of pro-Interleukin 1 $\beta$  (IL1 $\beta$ ) after 4 days of stimulus. In contrast, LI by itself was unable to significantly induce cell death, whereas LI with APP244-C or A $\beta$ 1-42-conjugated beads induced a significant increment of cell death. Those effects were not observed in the absence of microglial cells or when hippocampal cells were co-cultured with microglia for one day. Our results indicate that the neurotoxicity induced by A $\beta$ 1-42 or APP244-C -conjugated beads and pro-inflammatory molecules was a slow process developing over several days that depended on the presence of microglial cells. Pro-inflammatory molecules activated glial cells to produce nitric oxide (NO) and pro-IL1 $\beta$ . However, only in the presence of APP244-C or A $\beta$ 1-42 conjugated beads, inflammatory conditions generated a stressing condition, inducing cell death of both neurons and non-neuronal cells. The observed cytotoxicity could be the consequence of a vicious cycle in which elevated concentrations of active IL-1 $\beta$  and radical species support persistent activation of glial cells and cell damage. FONDECYT 1040831.

**P41. NMDA-TYPE GLUTAMATE RECEPTORS REGULATE THE EXPRESSION OF SYNAPTIC VESICLE-RELATED PROTEIN SYNAPSIN I IN SUBSTANTIA NIGRA IN A RAT MODEL OF PRESYMPTOMATIC PARKINSON'S DISEASE.** Receptores de glutamato tipo NMDA regulan la expresión de la proteína de vesículas sinápticas sinapsina I en sustancia nigra de ratas en un modelo de enfermedad de Parkinson presintomático. Eduardo Riquelme, Cristian Leon, Jorge Abarca and Gonzalo Bustos. Lab. of Biochemical Pharmacology, Dept. of Cell and Molecular Biology, Pontificia Universidad Católica de Chile, Santiago. Studies from our laboratory have demonstrated early increases in the expression of both mRNA and protein of brain derived neurotrophic factor (BDNF) and extracellular glutamate levels in substantia nigra (SN) in a rat model of presymptomatic Parkinson's disease (PD). These changes could modify, in turn, the expression of vesicle-related proteins in SN, in order to facilitate transmitter release from remaining undamaged cells, following partial dopamine cell population disappearance. We have now evaluated, by means of immunohistochemical (IHC) techniques, both the expression and phosphorylation levels of the synaptic vesicle-related protein synapsin I in SN of rats in a model of presymptomatic PD and its possible regulation by NMDA-type glutamate receptors. Ipsilateral intrastriatal 6-OH-DA injection was used to partially damage the nigro-striatal DA pathway. After one day of neurotoxin injection, an increase in the number of phosphorylated synapsin I-immunoreactive (IR) cells was found in the ipsilateral SN. However, at this time period, no changes were detected in synapsin I IR. In contrast, one week after of 6-OH-DA injection we observed a decrease in the expression of synapsin I IR in the SN. Pretreatment of the injured animals with MK-801, antagonist of NMDA-type glutamate receptors, resulted in a blockade of both, the increase in the number of phosphorylated synapsin I- IR cells and the decrease observed in the expression of synapsin I IR. These results allow us to suggest that glutamate through NMDA-type glutamate receptors may regulate the synaptic communication in SN at early stages of presymptomatic PD by modifying both the expression and phosphorylation of the synaptic vesicle-related protein synapsin I. (Supported by grant FONDECYT # 105-0981)

**P42. LACK OF FORMATION OF REISSNER'S FIBER LEADS TO HYDROCEPHALUS. La Carencia de fibra de Reissner conduce a hidrocefalia.**

S.Rodríguez<sup>1</sup>, K.Vio<sup>1</sup>, F.Bátiz<sup>1</sup>, A. Ortloff<sup>1</sup>, R. Muñoz<sup>1</sup>, L.M. De Graff<sup>2</sup>, J.P.Graves<sup>2</sup>, D.J.Stump<sup>2</sup>, P.J.Blackshear<sup>2</sup>, D.C. Zeldin<sup>2</sup>, E.M. Rodríguez<sup>1</sup>. <sup>1</sup>Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral de Chile. <sup>2</sup>National Institute of Environmental Health Sciences (NIEHS; NIH) USA. The subcommissural organ (SCO) secretes glycoproteins into the cerebrospinal fluid (CSF) flowing through the Sylvius aqueduct (SA); these proteins either assemble to form an ever growing fiber (Reissner's fiber (RF)) extending along the SA and central canal. Overholser et al. (1954) have demonstrated that offspring littered by rats maintained on a diet deficient in folic acid and/or Vitamin B<sub>12</sub> lack a SCO and develop hydrocephalus. This led them to propose that a dysfunction of the SCO during development leads to stenosis SA and hydrocephalus. We have investigated several animal models in which the SCO plays a role in the pathogenesis of hydrocephalus. We have now performed a comparative analysis of the SCO-RF complex of these animal models, with the aim to find a landmark common to all hydrocephalus animals that might help to unfold the mechanism of the SCO-dependant hydrocephalus. Results. Model 1: Immunological blockage of RF formation by maternal transfer of antibodies against RF-glycoproteins: undamaged and secretory active SCO, missing RF, stenosed SA and moderate hydrocephalus. Model 2: hyh mice with a point mutation of  $\alpha$ SNAP gene: undamaged and secretory active SCO, missing RF, obliterate SA and severe hydrocephalus. Model 3: HTx rat: subcommissural portion (two distal thirds) of SCO missing, supracommissural portion (rostral third) of SCO secretory active, RF absent, obliterated SA and severe hydrocephalus. Model 4: Transgenic mice deficient in transcription factor RFX34-v<sub>3</sub>: subcommissural portion SCO missing, supracommissural portion SCO secretory active, RF absent, patent SA and moderate to severe hydrocephalus. Conclusions: 1.- The SCO is formed by two zones, the subcommissural and the supracommissural portions, 2.- Differentiation of both zones would be controlled by different genes, 3.- Subcommissural portion of SCO is essential for RF formation. Mutant and transgenic animals lacking this portion, although still have a secretory active supracommissural portion, do not form a RF, 4.- The only common feature to all animal models is the absence of RF, 5.- RF-glycoproteins appear to be essential for a normal flow of CSF throughout SA, 6.- Absence of RF could cause SA obliteration or turbulent CSF flow through SA what, in turn, would lead to hydrocephalus. Supported by FONDECYT 1070241 (Chile), the Intramural Program of the NIH, NIEHS (USA).

**P43. IVERMECTIN AND ZINC POTENTIATE THE P2X4 RECEPTOR GATED CURRENTS ACTING AT DISTINCT AND SEPARATE ALLOSTERIC MODULATOR SITES.**

Ivermectina y zinc potencian la actividad del receptor P2X4 actuando en sitios alostéricos distintos. F. Rodríguez-Tirado, C. Coddou, F. Godoy and J. Pablo Huidobro-Toro. Centro de Regulación Celular y Patología J.V. Luco, Instituto Milenio de Biología Fundamental y Aplicada MIFAB, Departamento de Fisiología, Laboratorio de Nucleótidos, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile. Ivermectin (IVM) is an anti parasitic drug of ample use in veterinary medicine; it was recently described to potentiate selectively the ATP-gated currents in the P2X4 purinoceptor through an allosteric mechanism that involves sites in the membrane domain of the P2X4 receptor. On the other hand, zinc facilitates the ATP-gated currents acting on a site on the extracellular domain of the P2X4 receptor.

Therefore, we hypothesized that the joint application of both modulators should produce additive effects, each acting at a distinct site. To further describe the sites of these modulators, we assessed these modulators in the C132A mutant and in a P2X4/2 chimera. cDNA for the P2X4 wild-type (wt), the C132A mutant and a P2X4/2a chimera was injected intranuclearly to *X. laevis* oocytes. 48 h later, the currents evoked by 10s ATP applications were recorded with the two-electrode voltage-clamp technique. 3  $\mu$ M IVM was applied 3-min prior to, and 10  $\mu$ M  $Zn^{2+}$  3-min prior and during the 10s ATP addition. Single additions of IVM and  $Zn^{2+}$  potentiated reversibly 6.9 $\pm$ 1.2-fold and 15.1 $\pm$ 3.7-fold (n=5, n=4 respectively) the ATP-evoked currents on the P2X4 wt receptor. Their joint application elicited an additive effect amounting to a 30.8 $\pm$ 5.5-fold increase in the ATP-gated current (n=4). In contrast, in the C132A mutant, resistant to the zinc-modulation, IVM potentiated 4.9 $\pm$ 0.7-fold (n=10) the ATP-evoked current. The joint application of both modulators only elicited a 7.1 $\pm$ 1.7-fold increase in the ATP-gated current (n=9). Finally, in the the P2X4/2a chimera  $Zn^{2+}$  potentiated 2.5 $\pm$ 0.7-fold (n=3) the ATP-gated current while this receptor was resistant to the IVM action (1.8 $\pm$ 0.4-fold; n=3). The co-application of both modulators only amounted to a 2.3 $\pm$ 1-fold increase in the ATP-gated current (n=1). Therefore, IVM and zinc are both positive allosteric modulators of the P2X4 receptor; their joint application is additive since they interact apparently at separate and distinct extracellular allosteric sites of the receptor. Funded by FONDAP grant 13980001 and the the MIFAB Institute.

**P44. GENERATION OF A SYMPTOMATIC ANIMAL MODEL IN AN INITIAL STAGE OF PARKINSONISM TO EVALUATE CELL REPLACEMENT THERAPIES: ANATOMIC AND FUNCTIONAL CHARACTERIZATION USING RATS WITH PARTIAL LESION OF NIGROSTRIATAL PATHWAY** (Generación de un modelo animal sintomático en un estadio inicial de parkinsonismo para evaluar terapias de reemplazo celular: caracterización anatómica y funcional en ratas con lesión parcial de la vía nigroestriada).

Francisco Javier Rubio<sup>1</sup>, Rodrigo Somoza<sup>1</sup>, Jose Tomás San Martín<sup>1</sup> and Carlos Juri<sup>2</sup>. <sup>1</sup>Instituto de Ciencias, Facultad de Medicina, Clínica Alemana-Universidad del Desarrollo, Santiago, Chile; <sup>2</sup>Departamento de Neurología, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile. Cell transplantation into the damaged brain to replace neurons in neurodegenerative diseases is one of the regenerative approaches used in the treatment of Parkinson's disease (PD). The effect of the transplant depends on the type of cells used and the brain zone where they are implanted. However, it also depends on the region and the level of degeneration of the Substantia Nigra pars compacta (SNpc). In PD, motor symptoms begin when around 60% of the dopaminergic neurons present in the lateral SNpc are underwent cell death. On the other hand, in experimental models of parkinsonism in animals lesioned with 6-OHDA there is a degeneration of the nigrostriatal pathway due to the death of more than 90% of the dopaminergic neurons of the medial SNpc. The aim of this study was to reproduce a hemiparkinsonism rat model with neurodegeneration similar in grade and topography to that found it in an initial stage of PD. For this purpose, we characterized rats with unilateral 6-OHDA-lesions using two motor behavioral tests in order to identify the time in which there is a partial lesion of nigrostriatal pathway due to tyrosine hydroxylase-immunoreactive cell loss in the lateral SNpc. We administrated 21  $\mu$ g of 6-OHDA in 3 deposits placed in the lateral striatum of rats and then evaluated the spontaneous and drug-induced behavioural manifestations at 1, 2 and 3 weeks post-lesion. Our results show that the induced degeneration ranges between 40-60% and is

related with asymmetry in the use of the forelimbs, but also with the beginning of the effect induced by metamphetamine. Thus, these two motor tests allow us to define the appropriate timepoints for therapeutic cell transplant and evaluation of neuroregeneration as well as functional recovery in this hemiparkinsonian model. Concluding, at 2 weeks post-lesion we can develop a parkinsonian model in the rat with neuroanatomic and functional characteristics similar to those of an initial symptomatic PD. Therefore, this rat model seems to be more adequate than other classical models to draw conclusions as regards to the implications and effectiveness of a cell therapy in humans. Supported by UDD 80.11.037 and Fundación Andes C-14060/60

**P45. INCREASED AROUSAL AND ACTIVITY OF HISTAMINERGIC NEURONS IN RESPONSE TO INCREASED REWARD VALUE IN APPETITIVE BEHAVIOR.** Incremento del arousal y la actividad de neuronas histaminérgicas en respuesta al incremento del valor de recompensa en la conducta apetitiva. Cristián A. Sánchez<sup>1</sup>, Fernando Torrealba<sup>1, 1</sup> Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. The behavioral arousing and autonomic responses that characterize motivated behaviors depend on the activity of the ascending arousal system (AAS). The appetitive phase of feeding-related motivated behavior is primarily associated with the activation of the tuberomammillary nucleus and presents an increment in behavioral and vegetative arousal, evidenced by increases in locomotor activity and core temperature respectively. The lesion of the tuberomammillary nucleus or its afferent infralimbic cortical area abolished the thermogenic response observed during the appetitive phase. An increment in motivational state by offering salami during the appetitive phase should increase the reward value and increase the activity of the AAS as well as the behavioral and vegetative responses. To address this issue, rats were accustomed in one group to food enriched with salami and other group to habitual food for one week before being rendered motivated for food by fasting them for 1 day and then were enticed with food. We evaluated the level of arousal and Fos-ir in the AAS during food enticing. We found in both groups a strong arousal and an early increase in Fos-ir in the histaminergic neurons from the tuberomammillary nucleus after 30 min of enticing, followed by increased Fos-ir in other nuclei of the AAS when food enticing was prolonged for 1 hour. Although fasting time was the same in both groups, the group enticed with salami-enriched food had twice the increase in thermogenic response, and a significant increase in the locomotor activity, and a three-fold increase in Fos-ir in histaminergic neurons, and a significant increased Fos-ir in the AAS nuclei respect to the group enticing with habitual food. We conclude that increasing the reward value during the appetitive phase increments the activity level in the whole AAS, especially in the tuberomammillary nucleus as well as increased in thermogenic response. Financed by Fondecyt 1060476.

**P46. No existe.**

**P47. INHIBITION OF NMDA-R CURRENTS BY THE NEUROTROPHIN BDNF IN CORTICAL PSD FOLLOWING STATUS EPILEPTICUS.** (Inhibición de las corrientes R-NMDA por la neurotrofina BDNF en DPSs corticales después de Status

Epilepticus). Sandoval R<sup>1,2</sup>, Calderón R<sup>1</sup>, Sandoval S<sup>1</sup>, Ampuero, E<sup>1</sup>, Wyneken U<sup>1</sup>.<sup>1</sup>Lab. Neurociencias, Univ. de los Andes; <sup>2</sup>Fac. Ciencias, Univ. de Chile. Status epilepticus (SE) is a brain insult that induces the development of temporal lobe epilepsy (TLE). In TLE, the hippocampus becomes hyperexcitable and therefore, the epileptic focus, while other brain areas are more resistant to the development of hyperexcitability. BDNF is critically involved in TL epileptogenesis, because its receptor, TrkB, promotes depolarization through interactions with cationic ion channels, including the NMDA receptor (NMDA-R). It also stimulates axonal and dendritic growth. TrkB is a transmembrane tyr-kinase that activates the PI3K, Erk1/2 and PLC $\gamma$  pathways. Both TrkB and the NMDA-R are anchored to the postsynaptic density (PSD) of excitatory synapse. The aim of this study was to compare the effects of TrkB on NMDA-Rs in PSDs isolated from brain cortex and hippocampus following SE. For this, we microtransplanted PSDs obtained from both brain structures into *Xenopus* oocytes to record the NMDA-R currents by two-electrode voltage clamping. In control PSDs, potentiation of NMDA-Rs was abolished by PI3K and Erk 1/2 inhibitors. In SE-PSDs, Western blots showed that the TrkB content increased by 600%, however TrkB was phosphorylated (active) in hippocampal, but not in cortical PSDs. In cortical SE-PSDs, BDNF inhibited the NMDA-R currents in 88% of the experiments (n=42), whereas inhibition of NMDA-Rs was never observed in hippocampal PSDs. The inhibition was reverted by anti-TrkB antibodies, PI3K and Erk 1/2 inhibitors, but not by K252a, an inhibitor of the TrkB kinase activity. Co-immunoprecipitation of TrkB from PSDs isolated from cortex and hippocampus showed changes in the protein complex associated to TrkB that might imply changes in its signaling capacity. In addition, total TrkB content was decreased in cortical but not hippocampal homogenates. By immunohistochemistry and Golgi staining, we show that the decrease of TrkB in cortical pyramidal cells was associated with retraction of neuronal soma, dendrite and spine density. Our results suggest that TrkB induces compensatory responses in cortical synapses that may counteract seizure propagation and epileptogenesis, and that for yet unknown reasons do not operate in the hippocampus. The understanding of endogenous protective mechanisms following SE may have important implications for the development of new strategies to prevent epileptogenesis. Univ. de los Andes MED03-06; MECESUP UCH0012

**P48. BLOCKADE OF INTERNAL MOUTH OF THE CALCIUM ACTIVATED POTASSIUM CHANNELS BY TETRAPENTHYLAMMONIUM.** Bloqueo de la boca interna del canal de potasio activado por calcio por tetrapentilamonio. Katherine Stack<sup>1</sup> and David Naranjo<sup>1</sup>. <sup>1</sup>Centro de Neurociencias de Valparaiso. Universidad de Valparaiso. The ionic conduction pathway is very conserved among potassium channels. Each one of the four subunits contribute equally to form the potassium selective pore. At its more external half of the pore rest the selectivity filter that coordinates two K<sup>+</sup> ions, while in the internal half rest an hydrophobic cavity to which quaternary ammonium compounds bind. Calcium activated potassium channels exhibit conductances of 100-300 pS, one order of magnitude higher than that of the classical voltage gated potassium channels. As an approximation to the dimension of the internal entrance of the pore, we used the quaternary ammonium tetrapentylammonium (TPeA) to block unitary currents and measured the kinetic of association and dissociation to the channel. Single calcium activated potassium channel currents were measured with the patch clamp technique in the inside-out configuration. The TPeA blockade was analyzed measuring the duration of the open and shut events. As the QA

ammonium concentration was increased the open duration got shorter in a reciprocal manner. The association rate constant at 0 mV was  $90 \pm 1$  with a shallow voltage dependency of 0.078 as effective valence. This Association constant is ~8-fold higher than that of classical voltage gated potassium channels. On the other hand, TpeA adds a population of 2-10 ms to the shut duration distribution. Our data suggest the TPeA binds with a 1:1 stoichiometry and speculate that the high association rate of TPeA could be correlated with the higher conductance of these channels.

**P49. INTERDISCIPLINARY MODEL FOR INTERVENTIONS OF SUBCLINIC HEPATIC ENCEPHALOPATHY: A CASE STUDY.**

Intervención desde un modelo interdisciplinario en el diagnóstico de un caso de Encefalopatía Hepática Subclínica. Marcela Tenorio D.<sup>1,2</sup>, Paulo Barraza R.<sup>1</sup>, Ricardo Rosas<sup>1</sup> & María Cristina Pinto D<sup>2</sup>. 1. Programa Doctorado en Psicología – Pontificia Universidad Católica de Chile. 2. Consultores en Psicología – Facultad de Psicología – Pontificia Universidad Javeriana - Bogotá . Hepatic Encephalopathy (HE) is a neuropsychiatric syndrome associated with an incorrect functional response from the liver that, besides observable physical damage in traditional medical practice, is linked with a particular neuropsychological function which involves important alterations in cognition, behaviour and personality. It is physiopathologically related to a compromise of the cerebral metabolism that results in the reduction of the amount of oxygen and glucose traveling from the brain, affecting the hematic filtering process and producing toxins that affect chemical processes in the central nervous system. New research has introduced differential symptoms that facilitate our understanding of acute and chronic HE. The first condition is characterized by diversity in its presentation levels, using different neurological change indicators, consciousness state and cognitive functioning. However, there are other symptoms like clumsiness, lack of concentration and daily forgetfulness which may be permanent and they are associated to chronic hepatic failure. When these symptoms appear, the syndrome is named Chronic or Subclinical Hepatic Encephalitis (SHE) and is a life-threatening condition, with a silent course that demands a timely diagnostic before consequences are irreversible. This document shows the integration of results from neuropsychological evaluation and both clinical and paraclinical mandatory exams from a 48 year old female who was received in Consultores en Psicología (Pontificia Universidad Javeriana – Colombia) with a request for neuropsychological evaluation and therapeutical support on account of personal and familiar positive antecedents for Hepatitis B induced Cirrhosis. The set of tested variables indicate neurophysiological and neuropsychological alterations, the latter being typified by a compromise in attention and visomotor coordination, associated to anomalous toxic deposits in the cerebral base nuclei, probably copper, ammonia and manganese; and also an alteration in relative functionality in the parietal lobe (cortico and sub-cortico regions). In demonstrating the course of a SHE, this study gives favourable evidence supporting interdisciplinary designs to optimize diagnosis and intervention.

**P50. TGF- $\beta$  REGULATES THE EXPRESSION OF SCAVENGER RECEPTORS IN MICROGLIAL CELLS.** TGF- $\beta$  Regula la Expresión de Receptores Scavenger en Células Microgliales. Tichauer J and von Bernhardt R. Neuroscience Laboratory, Neurology Department, Faculty of Medicine, Pontificia Universidad Católica de Chile. Alzheimer disease (AD) is the most common cause of dementia in the elderly. It is characterized by the accumulation of  $\beta$ -amyloid ( $A\beta$ ), neurofibrillary tangles and dystrophic neurites surrounded by activated microglial cells and astrocytes in the neocortex and hippocampus.  $A\beta$  and pro-inflammatory cytokines can induce several activation processes in glial cells, such as phagocytosis, secretion of cytokines, release of short lived cytotoxic factors and the activation of different signal transduction pathways. We have shown that astrocytes can modulate microglial cytotoxic activation through secretion of TGF- $\beta$  and previous reports have also shown that TGF- $\beta$  can increase phagocytosis of  $A\beta$ . However the receptors and transduction pathways involved on  $A\beta$  uptake mediated by TGF- $\beta$  have not been adequately described. AIM: To determine the effect of TGF- $\beta$  and the participation of its effector pathway Smad3, on the expression of SR-A, SR-MARCO and SR-BI by microglia. RESULTS: TGF- $\beta$  increased 2.5-fold SR-A expression whereas decreased SR-BI expression by 87% at 48h of exposure. TGF- $\beta$  did not change SR-MARCO expression after up to 48h of treatment. Inhibition of Smad3 through selective inhibitor SIS3, resulted in a decrease of the inducing effect of TGF- $\beta$  on scavenger receptor expression. CONCLUSION: TGF- $\beta$  could prevent  $A\beta$  accumulation through its modulatory effect on scavenger receptor expression. TGF- $\beta$  effect appeared to be at least partially mediated by the Smad pathway, modulation that could be relevant for the pathogenesis of AD. FONDECYT 1040831.

**P51. ASSESMENT OF THE EFFICACY OF A COGNITIVE STIMULATION TREATMENT IN CHILDREN WITH ADHD BETWEEN 9 AND 14 YEARS OLD.** Evaluación de la Eficacia de un Tratamiento de Estimulación Cognitiva en niños con ADHD entre 9 y 14 años. Toledo A.<sup>1</sup>, Manzur, H.<sup>2</sup> <sup>1</sup>Instituto de Restauración Neuropsicológica – Universidad SEK. <sup>2</sup>Programa de Doctorado en Ciencias Biomédicas - Universidad de Chile. One of the most frequent causes of impairment and disability among school age children is the Attention Deficit and Hyperactivity Disorder (ADHD). The worldwide estimated prevalence reaches the 9% in various studies. Which indicates that ADHD is a very important problem of public health. Also the ADHD has been associated with increased risk of comorbidity like personality disturbances, substance abuse and increased incidence of car accidents among others. The multifactorial aetiology of the syndrome has led to a multidisciplinary approach of the study as well as the treatment strategies. Among the accepted treatments the use of psychostimulants, like methylphenidate and amphetamine, are widely prescribed. However, these kind of pharmacological-only treatments on the modification of other aspects of the ADHD, like the disorganization of behavior, the lack of strategies of problem solving, the absence of metacognition among others, have a reduced impact. Other types of treatments like behavioral methods and the training on problem solving abilities, that seem to be more integrative, are not well studied. In this study we evaluate the efficacy of a newly developed Cognitive Stimulation Treatment (CST) as a coadjuvant treatment of ADHD. The CST includes a cognitive function (attention, memory, language, executive functions) oriented software, as well as elements of Feuerstein mediation in the interphase between the patient and the software. The elements of the Feuerstein mediation, in hands

of a trained profesional, are well suited for the aquisition of cognitive abilities in ways to complement the farmacological treatment of children diagnosed with ADHD

**P52. MICROGLIAL CELLS AND BYSTANDER EFFECT IN GLIOMAS.** (Células microgliales y efecto Bystander in gliomas). Federico Rodríguez, Eveling Inostroza, Francisco Nualart. frodrigu@udec.cl. Research Center for the Study of the Nervous System: Cell Biology and Biomedical Applications. Department of Cell Biology, Concepcion University, Chile. Gliomas are the most common brain tumours and one the most lethal cancers. These are characterized for having high levels of microglial infiltration and for resisting the cytostatic anti tumoural therapies. Vitamin C, a potent intracellular antioxidant that concentrates in the central nervous system protects normal cells from oxidative insults. This vitamin is incorporated into the cells by the sodium-ascorbate co-transporter SVCT2. Additionally, we have demonstrated in peripheral tumors that the local production of superoxide by activated cells causes the oxidation of extracellular AA to DHA, which is transported by neighboring cells (tumor cells) through the glucose transporters (Bystander effect). We postulate that a similar mechanism is produced in glioma tumors that have microglial infiltration. Using kinetic studies we demonstrated that TC620 glioma cells are able to capture almost ten times more oxidized vitamin C than AA, effect that we have associated to a high expression of GLUT1 and to the intracellular localization of SVCT2. Additionally, we have observed that microglial cells could be excellent mediators of the “Bystander effect” since they do not significantly express GLUT1 or GLUT3. Furthermore, we have confirmed these findings by transport assays showing that microglia from primary rat cultures are unable to capture significant levels of DHA. On the other hand, we have confirmed the expresion of the fructose transporter GLUT5 in microglia using RT-PCR and immunocytochemical determinations. Finally, we also determined the expression of p47Phox, a pivotal protein for the activation of the NADPH-oxidase. Our conclusion indicates that microglia would be poor competitors for the DHA produced when being activated by tumoural factors or by the cytostatic therapies themselves. Grant Anillo ACT02, \* CONICYT fellowship (FR).

**P53. COMPARISON BETWEEN THE EFFECT OF CHRONIC GLUCOCORTICOID ADMINISTRATION AND CHRONIC STRESS: EFFECT OF SERTRALINE ANTIDEPRESSANT.** Comparación entre el efecto de la administración crónica de glucocorticoides y el estrés crónico: Efecto del antidepresivo sertralina. JL Ulloa<sup>1</sup>, C Berríos<sup>1</sup>, G Díaz-Veliz<sup>2</sup>, S Mora<sup>2</sup>, K. Gysling<sup>3</sup>, J Fiedler<sup>1</sup>, 1.Laboratorio de Neurobioquímica, Departamento de Bioquímica y Biología Molecular, Facultad de Cs. Químicas y Farmacéuticas, Universidad de Chile; 2. Programa de Farmacología Molecular y Clínica-Oriente, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile, 3. Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. Although the etiology of depression is still unknown, it has been observed that certain environmental factors, like stress perhaps involving the activation of the hypothalamus-pituitary-adrenal gland axis (HPA) and hence an increase in glucocorticoids plasma levels. The chronic stress condition produces hippocampal atrophy, neuronal damage and impairment in the learning processes.

We evaluated in adults rats, using two experimental groups (n=7), if the chronic administration of glucocorticoid (corticosterone 30 mg/Kg/day) and chronic stress by motion restraint (both during 14 days) induce similar behavioral changes. Also we evaluated if these effects were prevented by the chronic administration of the antidepressant sertraline (10 mg/kg/day). It was observed that animals under chronic stress or with chronic administration of corticosterone displayed an increase in the immobility spent time in the forced swim test ie. a learned helplessness condition. This effect was prevented only by chronic sertraline administration in chronic stressed-animals. Also using the active avoidance test we observed that conditioned avoidance responses were reduced by restraint, being prevented by sertraline. But corticosterone administration alters differentially conditioned avoidance responses. Based in the observations that animals submitted to corticosterone administration, that is a component of the stress response and submitted to chronic stress by motion restraint shows distinct response to chronic sertraline administration we can suggest that pharmacological interventions in mood-related illness are associated to changes more complex than restoration of the HPA axis. Data were analyzed by Prism Graph Pad software and presented as mean± S.E.M. values. The statistical tests used were one-way analysis of variance (ANOVA) followed by Newman–Keuls’s multiple comparisons test. A probability level of 0.05 or less was considered statistically significant. Support: ENL1107.

**P54. ACIDOSIS DECREASES BETA-AMYLOID-INDUCED NEURODEGENERATIVE CHANGES OF HIPPOCAMPAL CELLS *IN VITRO*.**

(La acidosis disminuye los cambios neurodegenerativos inducidos por beta-amiloide en células hipocámpales *in vitro*.) Uribe R., Herrera-Molina R. and von Bernhardt R. Department of Neurology, Faculty of Medicine, Pontificia Universidad Católica de Chile, Marcoleta 391, Santiago, CHILE. Alzheimer’s disease (AD) is the most frequent neurodegenerative disease. Its progression associates to chronic inflammatory processes and vascular pathology, determining a deficit in local perfusion capable of producing local acidosis at the site of lesions. Acidosis can induce changes in amyloidogenesis contributing to neuronal death. Nevertheless, it has been described that acidosis also has neuroprotective effects by suppressing NMDA receptors, which reduces glutamate excitotoxicity in hypoxia models. We analyzed how acidosis affects A $\beta$ -mediated neurotoxicity. We evaluated morphological changes on neurons, LDH and nitrite production, reduction metabolism and TGFbeta1 expression in hippocampal cell cultures exposed to A $\beta$  and kept at pH 7.4, 7.15 or 7.05. The neurotoxic effect of fibrillar A $\beta_{1-42}$  on hippocampal cells resulted in a marked retraction of neuronal processes, as well as in an increased neural death and LDH production. Nevertheless, when cultures were kept at pH 7.15-7.05, A $\beta$  neurotoxicity was significantly attenuated, showing a 68% reduction on LDH release compared to cultures exposed to A $\beta$  at pH 7.4. Similarly, the MTT reduction metabolism increased 3.5-fold, whereas nitrite production induced by A $\beta$  in hippocampal cells was also reduced by 68% when exposed to acid pH media compared to basal pH. Acidosis also decreased the expression of TGFbeta1. Whereas TGFbeta1 is a cytokine capable of modulating glial reactivity, it is increased in the cerebrospinal fluid of AD patients and associates to potentially neurotoxic events. In AD as well as in TGFbeta over-expression models, high levels of TGFbeta1 induces an increased production of APP and A $\beta$ . Our results show that acidic pH protects hippocampal cells from the damage produced by the A $\beta_{1-42}$  peptide, suggesting that

acidosis could have a neuroprotective effect in the AD brain. Grant support by FONDECYT 1040831 is acknowledged.

**P55 ELECTROPHYSIOLOGICAL RECORDINGS DURING ASSOCIATIVE LEARNING IN A RAT MODEL *IN VIVO***

Registros electrofisiológicos durante aprendizaje asociativo en un modelo de rata *in vivo*. Valenzuela-Harrington<sup>2</sup>, M., Delgado-García J.M.<sup>1</sup>, Gruart A.<sup>1</sup> <sup>1</sup> División de Neurociencias, Universidad Pablo de Olavide, Sevilla - 41013, Spain, <sup>2</sup> Facultad de Ciencias Naturales y Exactas, Universidad de Playa Ancha de Ciencias de la Educación, Valparaíso, Chile. Learning and memory processes are manifested in multiple ways by different brain systems. The hippocampus is a very important brain structure implicated in learning and memory. The neural transmission through the hippocampus exhibits a high degree of modulation. The aim of the present was to design an experimental model that allows the study of integrative and functional properties present in hippocampal networks during an associative learning process, as the classical conditioning of eyelid responses in awake and freely-moving rats.

Twenty rats were chronically implanted with recording electrodes in the dentate gyrus, and in the CA1 and CA3 hippocampal areas, and with stimulating electrodes in the perforant path. In addition, bipolar stimulating electrodes were implanted in the suprorbital branch of the trigeminal nerve and bipolar recording electrodes were aimed to the orbicularis oculi muscle. Conditioning sessions consisted of 60 presentations of a conditioned stimulus (CS, a 20-ms, 2400-Hz, 85-dB tone) followed, 500 ms later, by an unconditioned stimulus (US, a 0.5-ms, square, cathodic pulse set at < 1 mA). A total of four habituation, 10 conditioning, and five extinction sessions were carried out. This experimental preparation allowed recording synaptic field potentials at selected recording sites of hippocampal circuits evoked by the electrical stimulation of the perforant path during the acquisition and extinction of conditioned eyelid responses.

The preliminary results of collected data indicate that specific hippocampal sites transform their responses to incoming perforant path inputs across learning. Moreover, the experimental model allows the *in situ* study of neuronal properties underlying the action of selected pharmacological agents (Ro 25-6981). The effects of Ro 25-698, a selective NMDA (NR1/2B) receptor antagonist, prevented the acquisition of classically conditioned eyelid responses and of some electrophysiological tests, as, for example, long-term depression (LTD).

**P56. COPPER AFFECTS SODIUM CURRENTS: A QUANTITATIVE STUDY**

(Cuantificación de los cambios inducidos por cobre sobre las corrientes de sodio). Vera J., Delgado R., Wolff D. and Vergara C. Laboratorio de Fisiología Celular, Facultad de Ciencias, Universidad de Chile. Different experimental approaches have demonstrated that extracellular copper can alter neuronal excitability by interacting with different voltage gated ion channels. Nevertheless, the molecular mechanism by which copper affects these channels has not been described. Previous findings in our laboratory, using olfactory neurons as a neuronal model system, suggest that copper acts in a biphasic manner on transient sodium current. At low concentrations (50-100 nM) copper increase the peak of sodium current and accelerate its kinetic, whereas at higher concentration (5  $\mu$ M) it has the opposite effect, diminishing the peak current by 70% and making the kinetic slower. The aim of this work is to quantify the changes in sodium current kinetics produced by copper using the Hodgkin-Huxley model. This

model describes the time course of the current based on kinetic parameters that will allow us to know which of them are affected. To this purpose, we used dissociated olfactory neurons from *Caudiverbera caudiverbera* under whole cell voltage clamp configuration. We applied external copper and recorded the effect on sodium currents after complete block of potassium currents with internal caesium. Using standard protocols we obtained the recovery of inactivation time constant ( $\tau_h$ ) and the steady state inactivation curve ( $h_\infty$ ), characterized by its slope ( $z_h$ ) and the voltage at which  $h_\infty$  equals 0.5 ( $V_h$ ). Preliminary results show that 0.1-1  $\mu$ M copper shift voltage-sensitivity of  $h_\infty$  towards hyperpolarizing potentials ( $V_h = -41,8 \pm 1,0$  mV for control (n = 6);  $-48,4 \pm 3,2$  mV for 100 nM copper (n=3); and  $-50$  mV and  $-49,2$  mV for copper 1  $\mu$ M), without a significant change in the curve slope. However, at 10-100  $\mu$ M copper in addition to the curve displacement produces a slope decrease. Moreover, copper accelerates the time course of recovery in all tested concentrations (0.01-10  $\mu$ M) with maximal effect at 1  $\mu$ M. These results will help us in the future to design experiments that allow us to identify putative sodium channel regions where copper can bind and exert its effects. Funding: Fondecyt 1040681, Anillos Ciencia y Tecnologia ACT-45, P. Bicentenario Conicyt.

**P57. THE CALCIUM CHANNEL  $\beta$ -SUBUNIT REGULATES DYNAMIN-1 AVAILABILITY DURING KISS-AND-RUN EXOCYTOSIS.** La subunidad- $\beta$  de los canales de  $\text{Ca}^{2+}$  activados por voltaje regula la disponibilidad de dinamina-1 durante el kiss-and-run. María José Guerra, Patricia Hidalgo, Arlek González Jamett, Alan Neely, Ana M. Cárdenas. Centro de Neurociencia de Valparaíso, Universidad de Valparaíso. Multiples cellular processes including synaptic transmission and endocrine secretion depend on the  $\text{Ca}^{2+}$  entry through voltage-activated  $\text{Ca}^{2+}$  channels (VACCs). VACCs are multi-protein complexes containing a pore-forming subunit ( $\alpha_1$ ) and various auxiliary subunits including the  $\beta$ -subunit ( $\text{Ca}_v\beta$ ). The latter regulates several aspects of VACC function such as gating, intracellular trafficking, and assembly.  $\text{Ca}_v\beta$  belongs to the membrane-associated guanylate kinase class of scaffolding proteins (MAGUK) that comprises a series of protein-interaction motifs. Two such domains are present in  $\text{Ca}_v\beta$ : a Src homology 3 (SH3) and a guanylate kinase domain. We have recently reported that the SH3 domain of the  $\text{Ca}_v\beta_{2a}$  ( $\beta_{2a}$ -SH3) interacts with dynamin-1 and promotes the endocytosis of VGCCs, in a dynamin dependent manner (*Gonzalez-Gutierrez et al 2007, J. Biol. Chem. 282:2156*). Dynamin-1 is reportedly necessary for kiss-and-run exocytosis in endocrine cells. In this exocytosis mode, secretory vesicles partially release their content, and are then retrieved intact through a mechanism dependent on dynamin-1. This protein seems to oligomerize in the vesicle “neck” and cause vesicle fission and when absent, vesicles collapse onto the plasma membrane and release all their content. Therefore,  $\text{Ca}_v\beta$  may interact with dynamin-1 and define the mode of exocytosis in endocrine cells. To address this hypothesis, we measured individual exocytotic events with amperometry in bovine chromaffin cells injected with  $\text{Ca}_v\beta_{2a}$  or  $\beta_{2a}$ -SH3. First, pull down assay show that either  $\text{Ca}_v\beta_{2a}$  or its SH3 domain interact with dynamin-1 from chromaffin cells. Intracellular injection of  $\text{Ca}_v\beta_{2a}$  increased the quantal size and duration of individual exocytotic events during KCl-induced depolarizations. Similar effects were observed when an antibody against dynamin-1 was injected. Conversely, intracellular injection of  $\beta_{2a}$ -SH3 dramatically decreased both the quantal size and duration of exocytotic events as if this protein induced a shift in the exocytotic mode from full-collapse to kiss-and-run. Co-injecting an anti-dynamin-1 antibody or a

peptide encompassing the recognition motif for SH3 domain-containing proteins in the dynamin-1, reverted  $\beta$ 2a-SH3 induced kiss-and-run. Taken together, our results suggest that VACC  $\beta$ -subunit has a role in regulating the availability of dynamin-1 for kiss-and-run exocytosis. This work was supported by Fondecyt 1020812 and Anillo de Ciencia y Tecnología (ACT-46)

**P58. EXPRESIÓN DE CONEXINA-36 Y SU PAPEL EN LA TRANSMISIÓN SINÁPTICA EN RETINA DE *OCTODON DEGUS*.** (Expression of Connexin-36 and its role in the synaptic transmission in the retina of *Octodon degus*). Angelina C. Palacios, Agustín D. Martínez, Adrián G. Palacios. Centro de Neurociencia Celular y Molecular de Valparaíso. Facultad de Ciencias, Universidad de Valparaíso. The expression, distribution and functional role of gap junction protein Cx36 were study in the retina of rat and *Octodon degus*, nocturnal and diurnal-crepuscular animals, respectively. Previous studies have shown a role of Cx36 gap junction in the visual signaling pathways of nocturnal animals. In addition, visual signaling is affected in the retina of mouse with targeting deletion of Cx36 gene (Cx36<sup>-/-</sup>). This study was controversial because Cx36 may be important for retina proper development. Here we carried a electroretinogram (ERG) physiological approach to study the in-vivo role of gap junction channels in retina visual signalling. First of all, we confirm the expression and distribution of Cx36 in *O degus* retina by western blot and immunofluorescence. Indicating, that Cx36 gap junction channels are present in *O degus* retina like previous findings in mouse and rats. To address the possible role of Cx36 in retinal visual transduction we took the advantage of a recently discovery; mefloquine, an anti malaria drug, specifically block Cx36 gap junction channels. We study the effect of intravitally injected Mefloquine on different parameters of the electroretinograph (ERG) of rat and *O degus* during scotopic and photopic illumination. During scotopic condition, mefloquine (100  $\mu$ M) induced a significant reduction in the amplitude of the b-wave, 43 and 31.9 % in rat and *O degus*, respectively. In addition, a highly significant reduction in the sensitivity for the b-wave was observed under this condition. Similar results were obtained after treatment of the retina with 18- $\beta$ -glycyrrithenic acid, a broad spectrum gap junctional blocker. Under photopic condition, mefloquine and 18- $\beta$ -glycyrrithenic, did not affect significantly the ERG parameters in both kind of animals. Our results suggest that Cx36 gap junction channels arte essential for the scotopic pathway of the visual transmittion in both, nocturnal and diurnal-crepuscular animals. GRANT ACT45, ACT46 PBCT-CONICYT.