Program

PROGRAM
IX Annual meeting of the Chilean Society of Neuroscience & VIII Ibero American Congress of Biophysics

Time	Tuesday, October 1st	Time	Wednesday, October 2nd	Time	Thursday, October 3rd	Time	Friday, October 4th
9:00-11:00	REGISTRATION	9:00-11:00	Symposium 3: Diversity of Chemical Senses. Chairs: Juan Bacigalupo, Julio Alcayaga Symposium 4: Exocytosis, neurotransmission and synapse structure. Chairs: Ana M Cardenas, Brigitte van Zundert	9:00-11:00	Special Young Scientist Symposium Chair: Juan Bacigalupo	9:00-11:00	SOBLA-Symposium: Lightning the structure and function of ion channels Chairs: Teresa Giraldez, Carlos González
11:15-11:30	Welcome	11:00-11:30	Coffee break	11:00-11:30	Coffee break	11:00-11:30	Coffee break
11:30-12:30	PLENARY LECTURE **Emanuel C. Mora**	11:30-12:30	PLENARY LECTURE **Gail Mandel**	11:30-12:30	PLENARY LECTURE **Paul Brehm**	11:30-12:30	SOBLA/LAFeBS PLENARY LECTURE **Marcelo Morales**
12:30-14.30	LUNCH	12:30-14:30	LUNCH ASAMBLEA DE SOCIOS	12:30-14:30	LUNCH	12:30-13:30	SOBLA AWARD LECTURE **Ramón Latorre**
14:30-16:30	Symposium 1: Membranes Chairs: Nuno Correia, Luis Bagatolli Symposium 2: New insights on calcium signaling. Chairs: Ariel Escobar, Eduardo Rios	14:30-16:30	Symposium 5: Calcium Channels Chairs. Enrique Jaimovich, Diego Varela Symposium 6: Protein Structure Chair: Eduardo Perozo	14:30-16:30	Symposium 7: Role and regulation of channels and hemichannels formed by connexins or pannexins in the nervous system. Chairs: Juan C. Sáez; Agustín Martínez Symposium 8: Synaptic Plasticity Chairs: Pablo Muñoz, Cecilia Hidalgo		
16:30-17:00	Coffee break	16:30-17:00	Coffee break		FREE AFTERNOON		
17:00-18:30	Posters I	17:00-18:30	Posters II				
18:30-19:30	PLENARY LECTURE **Enrico Nasi**	18:30-19:30	PLENARY LECTURE **Fernando Torrealba**				
19:30-21:00	Welcome Cocktail						

Tuesday, October 1st

9:00-11:00 REGISTRATION

11:15-11:30 WELCOME

11:30-12:30 PLENARY LECTURE **Emanuel C. Mora**
Host: Juan Bacigalupo
Auditorium

12:30-14:30 LUNCH

14:30-16:30 SYMPOSIUM 1: **Membranes**
Chairs: Nuno Correia, Luis Bagatolli
Dance Room
14.30-15:00 Nuno Santos
15:00-15:30 Manuel Prieto
15:30-16:00 Octavio Franco

14:30-16:30 SYMPOSIUM 2: **New insights on calcium signaling**
Chairs: Ariel Escobar, Eduardo Ríos
Auditorium
14.30-15:00 Rosana Bassani
15:00-15:30 Ariel Escobar
15:30-16:00 José Perez-Zoghbi
16:00-16:30 Eduardo Ríos

16:30-17:00 COFFEE BREAK

17:00-18:30 POSTERS I

18:30-19:30 PLENARY LECTURE **Enrico Nasi**
Host: Ana María Cárdenas
Auditorium

19:30-21:00 WELCOME COCKTAIL

Wednesday, October 2nd

9:00-11:00 SYMPOSIUM 3: **Diversity of Chemical Senses**
Chairs: Juan Bacigalupo, Julio Alcayaga
Auditorium
9:00-9:30 Julio Alcayaga
9:30-10:00 Sue Kinnamon
10:00-10:30 Kate Whitlock
10:30-11:00 Juan Bacigalupo

9:00-11:05 SYMPOSIUM 4: **Exocytosis, neurotransmission and synapse structure**
Chairs: Ana M Cardenas, Brigitte van Zundert
Dance Room
9:00-9:25 Ana M. Cárdenas
9:25-9:50 Guillermo Alvarez de Toledo
9:50-10:15 Fernando Marengo
10:15-10:40 Brigitte van Zundert
10:40-11:05 Ling-Gang Wu

11:00-11:30 COFFEE BREAK

11:30-12:30 PLENARY LECTURE **Gail Mandel**
Host: María Estela Andrés
Auditorium

12:30-14.30 LUNCH

14:30-16:30 SYMPOSIUM 5: **Calcium Channels**
Chairs. Enrique Jaimovich, Diego Varela
Auditorium
14.30-14:55 Diego Varela
14:55-15:20 Mariana Casas
15:20-15:45 Gustavo Brum
15:45-16:10 Enrique Jaimovich
16:10:16:35 Alan Neely

14:30-16:30 SYMPOSIUM 6: **Protein Structure**
Chair: Eduardo Perozo
Dance Room
14.30-15:00 Nelson Barrera
15:00-15:30 Mario Bianchet
15:30-16:00 Eduardo Perozo
16:00-16:30 Volker Doetsch

16:30-17:00 COFFEE BREAK

17:00-18:30 POSTERS II

18:30-19:30 PLENARY LECTURE **Fernando Torrealba**
Host: Brigitte van Zundert
Auditorium

Thursday, October 3rd

9:00-11:00 **SPECIAL YOUNG SCIENTIST SYMPOSIUM**
Chair: Juan Bacigalupo
Auditorium
9:00-9:30 Tatiana Adasme
9:30-10:00 Fernando Bustos
10:00-10:30 Jorge Vera
10:30-11:00 Christ Devia

11:00-11:30 COFFEE BREAK

11:30-12:30 PLENARY LECTURE **Paul Brehm**
Host: David Naranjo
Auditorium

12:30-14.30 LUNCH

14:30-16:30 SYMPOSIUM 7: **Role and regulation of channels and hemichannels formed by connexins or annexins in the nervous system**
Chairs: Juan C. Sáez, Agustín Martínez
Dance Room
14.30-15:00 Agustín Martínez
15:00-15:30 Christian Giaume
15:30-16:00 Juan Orellana
16:00-16:30 Raúl Russo

14:30-16:30 SYMPOSIUM 8: **Synaptic Plasticity**
Chairs: Pablo Muñoz, Cecilia Hidalgo
Auditorium
14.30-15:00 Paola Haeger
15:00-15:30 Pablo Muñoz
15:30-16:00 Pablo Castillo
16:00-16:30 Cecilia Hidalgo

FREE AFTERNOON

Friday, October 4rd

9:00-11:00 SOBLA-Symposium: **Lightning the structure and function of ion channels**
Chairs: Teresa Giraldez, Carlos González
9:00-9:20 Francisco Bezanilla
9:20-9:40 Peter Larsson
9:40-10:00 Andreas Reiner
10:00-10:20 Sebastian Brauchi.
10:20-10:40 Carlos Gonzalez Leon
10:40-11:00 Pablo Miranda

11:00-11:30 COFFEE BREAK

11:30-12:30 SOBLA/LAFeBS PLENARY LECTURE **Marcelo Morales**
Host: Alan Neely

12:30-13.30 SOBLA AWARD LECTURE **Ramón Latorre**
Host: Miguel Holgrem

Conferences

BAT ECHOLOCATION VS. MOTH HEARING: EVOLUTION OF TACTICS AND COUNTERTACTICS

Mora, E[1].,[1]Research Group in Bioacoustics and Neuroethology, Faculty of Biology, Havana University.

The arrival of echolocating bats in the Paleocene posed such a strong predatory pressure on moths, that it is widely accepted that ultrasonic hearing in these insects was originally in place as a defense mechanism against bat predation. When moths countered to the bat´s echolocation with ears tuned to ultrasonic frequencies and evasive flying maneuvers, some bats responded by moving the frequency of their calls away from the peak sensitivity of moth ears. Certain bats in the Caribbean however, specialized in hunting moths by using distinctive echolocation strategies that allow them to overcome moth hearing in the mid-frequency range, where moths hear best. *Molossus molossus* alternate call frequency to fool tone-deaf moth hearing. By performing as if flying away for the moth's ear, this bat masks its acoustic tracking behavior. Multiharmonic call design used by mormoopids is another strategy that allowed these other species to detect and classify insects while minimizing their acoustic conspicuousness. But the evolution of moth hearing also allowed the prey to counteract against the high frequencies used by bats. Combining laser-Doppler vibrometry, distortion-product otoacoustic emissions and electrophysiology techniques, we have discovered that the moth´s ear undergoes a mechanism of dynamic tuning, therefore able to match its frequency of best audition with that of the echolocation of sympatric bats. The bat-moth story is a "magic well" that continues to provide scientistswith refreshing insights. Within the ongoing bat-moth acoustic arms race, echolocation diversified into sophisticated strategies, but in response to echolocation, the moth ears alreadycounteracted.

FROM MICROVILLAR VISUAL RECEPTORS TO CIRCADIAN DETECTORS: MELANOPSIN TRANSDUCTION IN AMPHIOXUS

Nasi, E[1].,[1]Instituto de Genetica, Ciencias, Universidad Nacional de Colombia.

Melanopsin, the photopigment of mammalian 'circadian' receptors (ipRGGs), is homologous to invertebrate rhodopsins, but its signaling mechanisms and evolutionary history are poorly understood. Melanopsin is also found in the neural tube of amphioxus, the most basal chordate. It expresses in two types of photosensitive microvillar neurons, co-localizing with a G_q. Light triggers an inward photocurrent carried by Na and Ca, abolished by PLC-inhibitors, and accompanied by internal release of calcium. Because $[Ca^{2+}]$ increase precedes the photocurrent, and responsiveness is suppressed by BAPTA, a causal role of Ca in light-excitation is suggested; the minute temporal lag makes additional downstream steps unlikely. Ca photorelease from DM-nitrophen rapidly triggers a large inward current with similar kinetics, conduction, and pharmacology as the light-evoked current. Calcium may thus be the internal messenger of the photoresponse - or a component thereof - in these cells, likely precursors of ipRGCs. To examine evolutionary changes that may have optimized melanopsin phototransduction for circadian functions we dissected the determinants of their photosensitivity. Examination of single-photon responses reveals a minute transduction gain, like in their vertebrate counterparts. Comparing light-activated single-channel currents, quantum responses, and saturating photocurrents, we concluded that few ion channels are recruited per absorbed photon, the limiting factor being the mobilization of messenger molecules. However, the level of melanopsin expression – gauged from photo-isomerization currents – is orders of magnitude higher than in ipRGCs. This accounts for the intermediate sensitivity and the retention of the microvillar architecture in amphioxus, which was subsequently lost in vertebrates.

REPRESSORS AND GLIA: LIFE SUPPORT FOR NEURONS

Mandel, G[1].,[1]Vollum Institute, Investigator / Senior Scientist, Howard Hughes Medical Institute / Oregon Health and Science University.

Rett Syndrome is a severe autism spectrum disease affecting 1 in 10,000 live births of girls. It is due to sporadic mutations in a DNA binding protein, termed Methyl CpG Binding Protein 2, or MeCP2. Initially thought to be purely a neuronal disease, we find evidence for aberrant neuronal glial interactions that contribute to the neuropathology. I will summarize the evidence and provide new information on potential underlying mechanisms and future treatment.

FUNCTIONS OF THE INSULAR CORTEX IN RATS

Torrealba, F[1].,[1]Fisiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile.

The insular cortex (IC), largely neglected in Neuroscience textbooks, has been described as a control zone for autonomic, specially gastrointestinal function, as a control region for global brain states; as an association cortex, a multisensory area, a sensory cortex devoted to taste and as an interoceptive cortex, in different mammalian species. Connection studies seem to support any of the above descriptions, in part because there are several areas within the IC with different neural connections and functional significance. We stand by the idea that IC somehow maps most of the activity of several dozens of sensory receptors that collectively form the peripheral part of the interoceptive system, which in turn is the interface between body and brain. We postulate that the perceptions that arise from the IC are a large variety of bodily needs and emotions, both classes of perceptions being closely related to motivation. Examples that have been explored by us using behavioral, early-gene expression and electrophysiological approaches are: hunger, thirst, pain, drug craving, gastrointestinal malaise and fear (conditioned and innate fear). Financed by Proyecto Anillo ACT-66, Milenio P10-063-F and Fondecyt 1130042.

DIFFERENT SOURCES OF INTRACELLULAR CALCIUM MEDIATE SYNCHRONOUS AND ASYNCHRONOUS SYNAPTIC TRANSMISSION

Brehm, P[1].,[1]Vollum Institute, Senior Scientist, Oregon Health and Science University.

Asynchronous transmission plays a prominent role at certain synapses but lacks the mechanistic insights of its synchronous counterpart. The current view posits that triggering of asynchronous release during repetitive stimulation involves expansion of the calcium domains underlying synchronous transmission. Now, live imaging and paired patch clamp recording at the zebrafish neuromuscular synapse reveal distinct calcium sources for each mode. Synchronous release is tied to calcium entry into synaptic boutons via P/Q type calcium channels whereas asynchronous release utilizes an intracellular calcium wave that is initiated at off-synaptic locations in the axon and axonal branch points. This secondary calcium source fully accounts for the features distinguishing asynchronous from synchronous release, which includes persistence, delay and sensitivity to slow calcium buffers. The fact that the neuromuscular junction and CNS neurons share these features raises the possibility that secondary calcium sources are a common feature of synapses with prominent asynchronous release.

CHLORIDE CHANNELS IN HEALTH AND SICKNESS: PAST, PRESENT AND FUTURE

Marcelo Morales

Federal University of Rio de Janeiro – Biophysics Institute.

It is well known that mutations in Cystic Fibrosis transmembrane conductance regulator (CFTR) lead to a defect of chloride secretion by epithelial cells causing the Cystic Fibrosis disease (CF). Our group has been studying the expression of CFTR in lungs and also in the kidneys. In lung (the most affected organ in CF) the transporter defect reduces the ability of epithelial cells in the airways to transport chloride in response to cAMP leading repetitive lung infection and consequent lung fibrosis.

We found that different mutations in CFTR (transiently expressed in Xenopus oocytes and in human airway epithelial cells) have different electrophysiological pattern (delta 508, A445E and G551D). Also we could find the new splicing variant of CFTR expressed mainly in renal medulla. This new variant of CFTR contain only the first transmembrane domain (TMD), the first nucleotide binding domain (NBD) and the regulatory domain and it was called TNR-CFTR. TNR-CFTR has cAMP-dependent PKA activation stimulated single Chloride channel characteristic and regulation of PKA activation of outwardly rectifying Chloride channel that are very similar to those of wild-type CFTR. TNR-CFTR is produced by an unusual mRNA processing mechanism involving two small nuclear RNAs: U11 and U12.

Our group is also involved in the study of an efficient vector to delivery genes in lungs for future gene therapy in CF by delivering CFTR in sick lungs. We could find (in mice) that adeno-associate virus type 5 (rAAV5) but not type 2 (rAAV2) is more appropriate vector. There was no evidence of inflammatory response or apoptosis in any group using rAAV5. rAAV5 did not induce an inflammatory process, mechanical or morphometric changes in the lungs. AAV5 may be an appropriate vector for lung gene therapy.

We also study the possibility to reduce the lung fibrosis in lung disease using bone marrow derived mononuclear cell therapy (BMDMCT). We have different animal models for lung disease. In lung silicosis fibrosis mice model we could find that BMDMCT is able to reduce lung granuloma's activity and the consequent fibrosis. This result show the possibility to treat and avoid lung fibrosis and it could be useful for treatment of other lung diseases such as CF. Recently we tested the BMDMCT in 5 patients with lung silicosis and the procedure showed to be safe.

MECHANISMS OF THE B1 SUBUNIT MODULATION OF THE CA^{2+}- AND VOLTAGE-ACTIVATED (BK) CHANNEL.

Ramón Latorre
Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso.

BK channels are involved in a large variety of physiological processes and regulatory β subunits are one of the mechanisms responsible of creating BK channel diversity fundamental to their adequate function of many tissues. Regardless the proven importance of this channel little is known about its detailed structure. Here using gating and macroscopic ionic current measurements we determined the extent to which β subunits modulate the BK voltage sensor and found that channel opening proceed in two steps. Moreover, the use of chimeric constructs between β1, β2 and β3 subunits allowed us to identify the molecular determinants in the β subunits responsible for the functional coupling with the pore-forming α subunit. On the other hand, a glimpse to the external architecture of BK channels was obtained using Luminescence Resonance Energy Transfer (LRET). We used a genetically encoded Lanthanide Binding Tag (LBT) that binds Tb^{3+} as LRET donor and a scorpion toxin, iberiotoxin (IbTx), labeled with a fluorophore, Bodipy as the acceptor for in vivo spectroscopic studies of intramolecular distances of the BK channel. By introducing LBTs in the external aspect of the β1 subunit we determined intermolecular distances between the LBTs and the Bodipy The methodology presented gives us a view to the BK channel external surface architecture with and without the β-subunit and reveals that the presence of the β1 subunit alters the α subunit structure.

Symposia

Symposium 1: **Membranes**
Chairs: Nuno Correia, Luis Bagatolli

AFM-based force spectroscopy probing of dengue virus capsid protein binding to lipid droplets and lipoproteins. Towards a new drug target.

Carvalho, F[2].,Martins, I[2].,Faustino, A[2].,Carneiro, F[1].,Assunção-Miranda, I[1].,Mohana-Borges, R[1].,Castanho, M[2].,Almeida, F[1].,Da Poian, A[1].,**Santos, N[2]**.,[1]Instituto de Bioquímica, Universidade Federal do Rio de Janeiro.2Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa.

Dengue virus (DENV) affects millions of people, causing more than 20 thousand deaths annually. No effective treatment is currently available. We studied the interaction between DENV capsid (C) protein and lipid droplets (LD), recently shown to be essential for viral replication. Atomic force microscopy (AFM)-based force spectroscopy measurements were performed with DENV C-functionalized AFM tips, tapping at the surface of the sample until the binding between tip and LD, and measuring afterwards the force necessary for the unbinding at the single-molecule level. AFM studies were complemented with NMR, zeta-potential and cell biology studies. DENV C-LD interaction is dependent on the high intracellular concentrations of potassium. Inhibition of Na+/K+-ATPase in DENV-infected cells resulted in a 50-fold inhibition of virus production. Limited proteolysis of LD surface impaired the interaction. Force measurements in the presence of specific antibodies indicate perilipin 3 (TIP47) as the major DENV C ligand on LD. A peptide designed based on a conserved segment of DENV C intrinsically disordered N-terminal domain (pep14-23) was also shown to successfully inhibit DENV C-LD interaction, and the related specific binding of DENV C to very low-density lipoproteins (VLDL). These advances pave the way to drug development approaches, in which inhibitory efficiency may be improved through lead optimization. A similar strategy may be used for other flaviviruses.

Carvalho et al. (2012) J. Virol., **86**, 2096-2108.
Martins et al. (2012) Biochem. J., **444**, 405-415.
Patent WO/2012/159187, PCT/BR2012/000162.
Faustino et al. (2013) Nanomedicine, in press.

Size and dynamics of PI(4,5)P2 clusters, induced by calcium concentrations in the physiological range, in model systems of membranes: Application of advanced fluorescence and microscopy methodologies.

M. J. Sarmento, A. Coutinho, F. Fernandes, M. Prieto

Centro de Química-Física Molecular and IN-Institute for Nanoscience and Nanotechnology, Instituto Superior Técnico, Universidade de Lisboa, Portugal (e-mail: manuel.prieto@ist.utl.pt)

Prieto, M[1].,[1]Centro de Química-Física Molecular, Instituto Superior Técnico, Universidade de Lisboa.

The lipid phosphatidylinositol 4,5-biphosphate is a critical minor component of the plasma membrane, since it is associated to e.g., membrane trafficking, endocytosis and exocytosis, as well as to the remodeling of the actin cytoskeleton. Although most of these processes are related to its localized lateral enrichment in the membrane, the mechanisms involved in this lipid clustering are however not fully elucidated.

We carried out a fluorescence study of a derivatized PI(4,5)P2 in several types of model membrane systems, and determined that its lateral distribution is very sensitive to the calcium concentrations in the low micromolar concentration, i.e., in the physiological range. This was determined from steady-state and time-resolved fluorescence techniques, using fluorescence self-quenching and energy migration (energy homotransfer) interactions. This effect was observed in both liquid-disordered (cholesterol poor), or liquid-ordered (cholesterol rich/raft model membranes). A model was developed to rationalize the fluorescence depolarization, and its fitting to the data allowed to conclude that the average cluster size is about 15 molecules of PI(4,5)P2. Evidence for clustering was also directly obtained from fluorescence correlation

spectroscopy (FCS), which showed a slower diffusion in the membrane due to the formation of the aggregates. In this way, PI(4,5)P2 could operate a as reliable sensor for calcium concentrations at the plasma membrane.

Acknowledgments: FCT Portugal for financial support (PTDC/QUI-BIQ/112067/2009; PTDC/QUI; PTDC/BBB-BQB/2661/2012; SFRH/BPD/64320/2009; SFRH/BD/80575/2011)

The challenges of bacterial infection control by using membrane-disrupting peptides.

Franco, O[1].,[1]Pós-Graduação em Ciencias Genomicas e Bioquímicas, , Universidade Catolica de Brasilia. (Sponsored by CNPq, CAPES, FAPDF And UCB)

In the last few years, the number of bacteria with enhanced resistance to conventional antibiotics has dramatically increased, becoming a major challenge for immune-depressed patients in particular. Among compounds that have been strategically researched to control these pathogens, antimicrobial peptides have become the potential drugs since they could act on pathogen membranes disruption. Moreover, such peptides could also act on cognate hosts improving immunological system function. In this view, antimicrobial peptides from plants and marine organisms have been reported here as modulators of immune response to bacterial infection, with independent activity of mechanisms that lead to an antibiotic effect. Several *in vitro* bioassays were performed in order to evaluate cytotoxic, antibacterial, antifungal, anti-tumor and anti-viral activities. The possible mechanisms of action of such peptides were studies in models of phospholipid membranes by Electrical Impedance Spectroscopy (EIS) and electron microscopy. Furthermore, the immunomodulatory activities of peptides against RAW 264.7 monocytes were here investigated. Furthermore, *in vivo* genotoxicity assays and neutrophil migration in response to both peptides were also performed. Finally the efficacy of treatment using peptides in a surgical wound *in vivo* S. aureus infection model and severe polymicrobial sepsis were also evaluated by using rats as animal models. Due to their multiple activities these promiscuous peptides are potential compounds for the treatment of serious bacterial infections such as skin infection and sepsis, showing enormous and remarkable biotechnological promise.

Symposium 2: **New insights on calcium signaling**
Chairs: Ariel Escobar, Eduardo Rios

Sarcoplasmic reticulum and excitation-contraction coupling in the neonatal myocardium: a star on the reserve bench

Bassani, R[1].,Oshiyama, N[1].,Bassani, J[1].,[1]Centro de Engenharia Biomédica, , Universidade Estadual de Campinas.

The sarcoplasmic reticulum (SR), which provides most of the contraction-activating Ca^{2+} in the adult myocardium, is structurally and functionally immature in the neonatal myocardium. In comparison with adult cells, ventricular myocytes from neonatal rats develop smaller and slower Ca^{2+} transients, in addition to showing lower SR Ca^{2+} content and release at a twitch. However, beta-adrenergic stimulation produces a similar increase in Ca^{2+} transient amplitude at both ages. Although the relative increase in L-type Ca^{2+} current (the main release trigger) and SR Ca^{2+}-ATPase (SERCA) activity is similar, the enhancement in SR Ca^{2+} load and release is greater in neonates. Inhibition of SR function with thapsigargin suppresses most (>90%) of the increase in the total Ca^{2+} flux after beta-adrenoceptor stimulation in both adults and neonates. It thus may be concluded that, although with limited contribution to excitation-contraction coupling under basal conditions, the SR function seems to be crucial for recruitment of the inotropic reserve in the immature mammalian myocardium, probably involving SERCA stimulation. Support: FAPESP (2008/54795-6 and 2011/19805-3) and FAEPEX/UNICAMP (78013)

Relationship between intracellular Ca^{2+} release and electrical excitability in intact beating mouse hearts

Escobar, A[1].,[1]Bioengineering, Engineering, University of California.

During the action potential in heart ventricle, contraction is triggered by calcium released from the sarcoplasmic reticulum intracellular stores (SR). This massive and short-lived increase in intracellular calcium occurs during systole as a result of the summation of many local events of calcium release. In this presentation, the focus will be in explaining how the dynamic modulation of RyR2 by Ca^{2+} binding proteins in SR can reshape the repolarization phase of the ventricular action potential by activating a plasma membrane transporter, the Na-Ca exchanger. The studies were carried out by combining measurements of RyR2-mediated Ca^{2+} signaling and electrical signaling of cardiomyocytes in the beating heart and provide hints on how the coupling between intracellular Ca^{2+} and membrane potential can promote arrhythmogenesis.

Regulation of airway smooth muscle contraction by calcium signaling and calcium sensitivity

Perez-Zoghbi, J[1].,[1]Cell Physiology and Molecular Biophysics, Texas Tech University.

The excessive contraction of the smooth muscle cells that surround the small intrapulmonary airways in response to bronchoconstrictos is characteristic of asthma and other obstructive lung diseases. However, the mechanisms that airway smooth muscle contraction in health and disease are not fully understood. We used 'living' lung slices in combination with imaging, biochemical, and pharmacological techniques to investigate the regulation of airway contraction by Ca^{2+}-dependent and Ca^{2+}-independent (Ca^{2+} sensitivity) mechanisms. We found that bronchoconstrictors such as acetylcholine induce Ca^{2+} oscillations in smooth muscle cells that are mediated by Ca^{2+} release from the sarcoplasmic reticulum through the IP3 receptor. Importantly, the frequency of these Ca^{2+} oscillations positively correlates with airway contraction. In addition, these agonists increase the sensitivity of the contractile apparatus to Ca^{2+} by inhibiting the activity of the myosin light chain phosphatase to increase myosin light chain phosphorylation. In contrast, bronchodilators such as b2-adrenergic agonists, nitric oxide, or hydrogen sulfide induce relaxation by inhibiting the Ca^{2+} oscillations or by causing Ca^{2+} desensitization. The mechanism for which bronchoconstrictors and bronchodilators induce changes in Ca^{2+} signaling and Ca^{2+} sensitivity will be discussed. The understanding of the mechanisms that regulate smooth muscle contraction in heath and disease is fundamental to the search of new retakes to manage asthma and other pulmonary diseases.

Termination of calcium signals: Deactivation, Attrition, CDI, Luminal Control

Ríos, E[1].,Blatter, L[1].,Fill, M[1].,[1]Molecular Biophysics and Physiology Rush University.

In striated muscles, skeletal and cardiac, cytosolic calcium transients must end rapidly, so that mechanical relaxation can be fast. The first mechanism of termination of calcium release is deactivation, reversal of the processes that activate Ca release. We will show evidence for three others. Two of them, Calcium-Dependent Inactivation —CDI— and “Pernicious Attrition” (also known as “Induction Failure”) act on the cytosolic side to close release channels. A third, Luminal Control, is irresistibly intriguing, but incompletely integrated into the current insight.

Symposium 3: Diversity of Chemical Senses

Chairs: Juan Bacigalupo, Julio Alcayaga

Chronic hypoxia modifies transduction in the carotid body receptor cells and/or synaptic responses in the chemo-afferent neurons.

Alcayaga, J[1].,Iturriaga, R[2].,Varas, R[2].,Ortiz, F[2].,[1]Biologia, Ciencias, Universidad de Chile.[2]Ciencias Fisiologicas, Ciencias Biologicas, Pontificia Universidad Católica de Chile.

The carotid body (CB), the main arterial chemoreceptor, comprises type-I (receptor) cells that are innervated by sensory neurons located in sensory ganglia of the glossopharyngeal (petrosal ganglion; PG) and vagus nerves. Acute hypoxic challenges reduce background and voltage-dependent K^+ currents, leading to type-I cells depolarization, increasing its intracellular [Ca^{2+}], inducing exocytotic release of one or more neurotransmitters that in turn increase the discharge in the afferent nerves. Chronic hypoxia induces an increase in basal chemosensory afferent activity and responses to acute hypoxia, both by peripheral and central mechanisms. Isolated type-I cells from rats subjected to chronic intermittent hypoxic (CIH) present background K^+ currents that are inhibited by hypoxia as in control animals, but the inhibition was larger and faster in cells from animals subjected to CIH. Similarly, the magnitude of the depolarization was larger in the cells subjected to CIH than in control cells. In cats subjected to CIH the responses induced in the PG by the CB putative transmitters were unmodified with respect to control animals. Thus, CIH appears to modulate the afferent activity by increasing the inhibition of K^+ background currents in type-I cells, without modification of the communication process between the type-I cells and fferent nerve terminals. On the other hand, responses of PG neurons to CB putative transmitters were largely increased in duration in animals subjected to chronic normobaric hypoxia with respect to control animals. Thus, chronic hypoxia increases chemosensory responses by modification of either type-I responses or synaptic communication depending on the chronic hypoxic challenge.

Transduction and afferent signaling in taste buds

Kinnamon, S[1].,Finger, T[2].,[1]Otolaryngology, , University of Colorado School of Medicine.[2]Cell and Developmental Biology, , University of Colorado School of Medicine.

Taste buds are the transducing elements of gustatory sensation. Each taste bud comprises 50-100 receptor cells, which extend from the basal lamina to the surface of the tongue where they encounter sapid molecules in the oral cavity. Three distinct taste cell types have been identified: Type II or “receptor cells” contain the GPCRs and downstream effectors for bitter, sweet, and umami stimuli; Type III cells contain apically located ion channels for sour and possibly salty transduction, and Type I cells provide a support function, similar to glial cells in the nervous system. We recently showed that ATP is the transmitter that links taste transduction to activation of gustatory afferent neurons. ATP is released from taste cells via non-vesicular mechanisms to activate ionotropic purinergic receptors on gustatory afferent fibers. While ATP release has been demonstrated only from Type II cells, ATP is required for transmission of all taste qualities to the nervous system. Mice lacking P2X2 and P2X3 (P2X DKO mice) lack responses to all taste qualities and this effect is phenocopied by AF-353, a selective P2X3 antagonist. Recent experiments have focused on the role of NTPDase2, the ectoATPase located on the membranes of Type I taste cells and which degrades the ATP released from other taste cells. NTPDase2 knockout mice have severely reduced responses to all taste stimuli, presumably due to desensitization of the ionotropic P2X receptors on the nerve fibers.

Olfaction: a scents of plasticity

KE Whitlock

CINV, Dept. de Neurociencia, Universidad de Valparaiso, Valparaiso, Chile

The olfactory sensory system is a fantastically plastic neuronal interface between the external environment and the central nervous system. The olfactory sensory neurons (OSNs) regenerate throughout life in all vertebrates and the genes coding the olfactory receptors are the largest gene families in the genome (1000 genes mouse/~160 gene in zebrafish). During early development the odor environment can affect gene expression in the differentiating olfactory epithelium and these changes in gene expression are correlated with the formation of long-term olfactory memories or olfactory imprinting (Harden et al., 2006; Whitlock, 2006). We have proposed that the expression of olfactory receptors, i.e. selectivity, in the OSNs set the external filters though which animals sense the world and these "filters" are biased toward early life experiences. Thus odors present during early development will bias receptor expression in their favor through epigenetic effects on receptor expression. Previously we have shown that fish will imprint on the odorant phenyl ethyl alcohol (PEA) and this behavior is correlated with changes in gene expression including the transcription factor *otx2* (Harden et al 2006). More recently we have demonstrated that PEA can affect the levels of olfactory receptor expression during early development (Calfun, Dominguez, Perez-Acle and Whitlock in prep). Through bioinformatic analyses we have found potential regulatory mechanisms that explain the reduction of olfactory receptor in PEA exposed animals.

Chemotransduction in pheromone sensory neurons of the main olfactory epithelium

Bacigalupo, J[1].,López, F[1].,Delgado, R[1].,López, R[2].,Restrepo, D[2].,[1]Biología, Ciencias, Universidad de Chile.[2]Cell and Developmental Biology, Rocky Mountain Taste and Smell Center, University of Colorado Anschutz Medical Campus.

Pheromones are not only detected by the vomeronasal organ (VNO), but also by the main olfactory epithelium (MOE). In transduction odorant binding to a receptor coupled to G-protein activates adenylyl cyclase (ACIII) generating cAMP, which directly gates cyclic-nucleotide-gated channels (CNG), allowing the influx of Ca^{2+} which in turn opens Ca^{2+} -activated Cl- channels, principally ANO2. Both channels generate a depolarizing response. Previous evidence (Lin et. al. PNAS 104:2471, 2007) suggested that a small subpopulation of olfactory sensory neurons (OSNs) expressing the Ca^{2+} -activated monovalent cation-selective channel TRPM5 were implicated in pheromone transduction by an unknown mechanism. Using TRPM5-GFP mice we determined by loose patch recording that TRPM5-GFP^{+} OSNs (express TRPM5) respond to putative pheromones but not to odorants, while those not expressing TRPM5 (TRPM5-GFP^{-}) respond to both. In both cases there was a rise in intraciliary [$Ca^{2+}{}_{i}$] of extracellular origin. Inhibitors of CNG suppressed $TRPM5^{+}$ and $TRPM5^{-}$ OSNs responses, while the TRPM5 inhibitor TPPO abolished TRPM5-GFP^{+} OSNs pheromone responses but had no effect on TRPM5-GFP^{-} OSNs. Single-TRPM5 channels were recorded from TRPM5-GFP^{+} OSNs cilia but not from TRPM5-GFP^{-} OSNs. Immunocytochemistry showed that TRPM5-GFP^{-} OSNs express ANO2 while TRPM5-GFP^{+} OSNs do not. The results show that in$TRPM5^{+}$ OSNs pheromones lead opening of CNG, which mediates an increment in [$Ca^{2+}{}_{i}$] in the cilia and Ca^{2+} gates TRPM5, giving origin to a depolarizing transduction current. FONDECYT 1100682 (JB), CONICYT fellowship (FL), DC04657 (DR), DC006070 (DR), TW007920 (DR, DR).

Symposium 4: Exocytosis, neurotransmission and synapse structure

Chairs: Ana M Cardenas, Brigitte van Zundert

Regulation of exocytosis by actin dynamics and dynamin-2 in neuroendocrine cells

Cárdenas, A[1].,González-Jamett, A[1].,[1]Centro Interdisciplinario de Neurociencia de Valparaíso, , Universidad De Valparaíso.

During exocytosis, a narrow channel called “fusion pore” connects the vesicular and extracellular compartments, allowing the slow release of transmitter molecules. Later, the fusion pore expands leading to massive release of the vesicular content, or alternatively it may close, leading to the partial release of transmitters. The GTPase dynamin appears to be involved in both fusion pore expansion and closure via a still unknown mechanism. We propose that this mechanism depends on dynamin ability to direct actin dynamics. Consistent with this idea, inhibition of dynamin GTPase activity restrained cortical actin assembly, a process that was calcium-dependent, and also disturbed cortical actin organization. Expression of a siRNA against dynamin-2, the predominant isoform in chromaffin cells, also altered cortical actin organization. Disruption of either actin polymerization or dynamin-2 function delayed fusion pore expansion and enlarged catecholamine quantal release. Both actions were not additive indicating that dynamin-2 and F-actin control last steps of exocytosis by a common mechanism.

Synaptotagmin-7 controls the size of the reserve pool of synaptic vesicles in hippocampal neurons

Alvarez, G[1].,Duran, E[1].,Montes , M[1].,Jemal, I[1].,[1]Physiology and Biophysics, Medicine, Seville.

Proper brain function resides in the fine tuning of synapses by means of potentiation and depression. An efficient synaptic transmission depends on the availability of synaptic vesicles, the number of vesicles fused with the presynaptic terminal and recycled during activity. In resting conditions only the ready releasable pool of vesicles come into action for neuronal communication, meanwhile reserve vesicles are recruited for synaptic transmission under high frequency stimulation. We have studied the role of Synaptotagmin-7 (Syt-7) in the control of synaptic vesicle pools in cultured hippocampal neurons. Syt-7 does not participate as Ca^{2+} sensor for transmitter release as its isoform Syt-1, since Syt-7 KO animals have a normal release probability and normal ready releasable pool. By contrast, Syt-7 KO animals reveal an increase in the size of the reserve pool of vesicles with a proportional decrease in the resting vesicles, although the total pool of vesicle increases. These data suggest that Syt-7 plays a significant role in vesicle translocation from the reserve to the resting pool, making them unavailable for normal synaptic transmission. Also, the protein may facilitate reuse of vesicles near the plasma membrane. This effect could explain basal synaptic activity but promote depression on high levels of synaptic stimulation.

Highly Coupled exocytosis in chromaffin cells

Marengo, F[1].,[1]Departamento Fisiología, Biología Molecular y Celular. IFIByNE - CONICET., Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

The immediately releasable pool (IRP) is a group of ready releasable vesicles rapidly released by short depolarizations. Using a pharmacological approach and KO animals for P/Q Ca^{2+} channels, we demonstrated in mouse chromaffin cells that the exocytosis of this pool is specifically coupled to P/Q Ca^{2+} current. The Ca^{2+} is 8 times more effective to induce exocytosis in response to short stimuli than the current flowing through L-type channels. We also investigated the mechanism that may generate the coupling between IRP and P/Q-type channels. The expression of synprint (an aminoacidic sequence located in the cytosolic domain of the P/Q channel α_{1A} subunit) reduced markedly IRP and diminished the efficiency of Ca^{2+} current to induce exocytosis. Finally, to evaluate the possible participation of IRP during repetitive stimulation, we studied the recovery of this pool after total depletion. The refilling rate resulted to low (7 s) to expect a significant participation of IRP at physiological stimulation frequencies. However, we found that a small fraction of IRP, which is released by an action potential like stimuli, recovers 8 times faster. These results show that IRP vesicles are specifically coupled to P/Q-type Ca^{2+} channels through a specific interaction at the molecular level, and they may participate of exocytosis during trains of stimuli applied at basal physiological frequencies.

Dendritic arbor development in mature hippocampal neurons is restricted by occlusion of synaptic located NR2B-NMDA receptors

Van Zundert, B[1].,[1]Center for Biomedical Research, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andrés Bello. (Sponsored by Fondecyt 1101012, Anillo ACT1114)

In the rodent CNS, and in neuronal cultures, dynamic large-scale structural changes in dendrites occur during the first postnatal week, but then become limited and cell-type specific. Considerable evidence points to a role for NMDARs in regulating neuronal architecture during the early developmental period. Based on the strongly correlation between structural alterations and the developmental switch from the NR2B subunit to NR2A, we argue that NR1NR2B receptors promote structural reorganization of connections, whereas NR1NR2A receptors facilitate stability. In the present work, with the use of specific constructs that alter the synaptic composition, we were able to reactivate dendritogenesis in mature cultured neurons. Our results indicate that obstruction of synaptic clustering of NR2B-NMDARs with maturation restricts reactivation of dendritic branching.

The trigger of endocytosis at nerve terminals

Wu, L[1].,[1]National Institute of Neurological Disorders and Stroke National Institutes of Health.

Endocytosis recycles exocytosed vesicles, prevents exhaustion of vesicles, and thus maintains the ability of exocytosis and synaptic transmission, an process essential for nearly all of our brain activities. How is endocytosis initiated? In this presentation, I will discuss recent progresses in our lab towards addressing this question at a central nerve terminal. We found that both calcium influx and exocytosis are needed to initiate endocytosis. Calcium influx activates calmodulin and calcineurin to trigger endocytosis, whereas exocytosis, particularly the SNARE proteins are involved in initiating endocytosis.

Symposium 5: Calcium Channels

Chairs. Enrique Jaimovich, Diego Varela

$Ca_V\beta_2$ variants and calcium homeostasis in newborn rat cardiomyocytes

Moreno, C[1].,Hermosilla , T[1].,Sarmiento, D[2].,Simon, F[2].,**Varela, D[1]**.,[1]CEMC & ICBM, Medicina, Universidad De Chile.[2]MIII & Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas and Facultad de Medicina, Universidad Andrés Bello. (Sponsored by FONDECYT 1120240 (DV) And 1121078 (FS))

In the heart, L-type currents are carried by a multi-subunit membrane complex that includes CaV1.2 as the pore forming subunit that co-assembles with the auxiliary $Ca_V\alpha_2\delta_1$ and, preferentially, with the $Ca_V\beta_2$ subunits. To date, five distinct $Ca_V\beta_2$ transcriptional start site (TSS)-variants ($Ca_V\beta_{2a\text{-}e}$) differing only in the composition and length of the N-terminal domain have been described, each of them conferring distinct biophysical properties to the L-type current. However, the impact of $Ca_V\beta_2$ TSS-variants over Ca^{2+}-handling in cardiomyocytes has not been explored. Here we show that only four of these variants are present in neonatal rat cardiomyocytes. Recombinant adenoviruses were used to over express each variant in cultured newborn rat cardiomyocytes to explore the impact on endogenous L-type channel and Ca^{2+} homeostasis. As expected, all $Ca_V\beta_2$ TSS-variants increased the whole-cell L-type current density and the maximal gating charge moved with depolarization, producing distinctive changes on channels activation and inactivation kinetics. Calcium transients, induced by extracellular pulses, where found to be strongly dependent on the TSS-variant over expressed. The amplitude of calcium transients was found to be higher in $Ca_V\beta_{2a}$ transduced cardiomyocytes and smaller in $Ca_V\beta_{2d}$. Interestingly, percentage contribution of Ca^{2+}-influx and Ca^{2+}-release over total calcium transients, as well as the sarcoplasmatic calcium content, was found to be TSS variants-dependent. Overall, we demonstrated that changes in $Ca_V\beta_2$ TSS-variants expression produce deep modification in calcium homeostasis in cardiomyocytes.

Cav1.1 controls ATP release in adult muscle fibers

Jorquera , G[2].,Jacquemond, V[1].,Contreras-Ferrat, A[2].,Buvinic, S[3].,Jaimovich, E[2].,**Casas, M[2]**.,[1]Centre de Genétique et de Physiologie Moléculaire et Cellulaire, CNRS UMR 5534, Université de Lyon .[2]Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad De Chile.[3]Ciencias Básicas y Comunitarias, Facultad de Odontología, Universidad De Chile. (Sponsored by Anillo ACT1111, FONDECYT 1110467)

In adult muscle fibers, Cav1.1 acts as voltage sensor for both excitation-contraction coupling and the activation of a signaling cascade that regulates gene expression. We have shown that ATP is released trough pannexin-1 channels after electrical stimulation at 20 Hz, having a key role in the induction of transcriptional changes related to fast-to-slow muscle fiber phenotype transition. Myotubes lacking the Cav1.1a1 subunit displayed almost no ATP release after electrical stimulation. The same was observed in adult fibers treated with the Cav1.1 antagonist nifedipine (25µM), showing that Cav1.1 has a central role controlling ATP release.

These results suggest that Cav1.1 controls ATP release trough Pannexin-1 channels, activating it after low stimulation frequencies and blocking ATP release during resting. The loss of this control perturbs the normal transcriptional response to electrical activity of adult muscle fibers.

The voltage sensor of excitation-contraction coupling in mouse skeletal muscle: voltage and Ca^{2+} dependence.

Brum, G[1].,[1]Biofísica, Facultad de Medicina, Universidad de la República.

In frog skeletal muscle the voltage sensor of excitation-contraction coupling produces two well differentiated charge movement distributions corresponding to the so called charge 1 and charge 2. Changes in membrane potential and in external cation concentration promote interconversion between these two charge species. We studied charge movement in single fibers isolated from the FDB muscle of the mouse and found that it has similar properties to its counterpart in frog muscle. We will discuss our findings in the framework of a simple model of the voltage sensor.

The multiple cellular functions of voltage gated L type calcium channels

Jaimovich, E[1].,[1]Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad De Chile.

Voltage gated calcium channels have been linked to a number of physiological processes acting either as carriers of a calcium current or as voltage sensors for signaling processes involved in diverse cellular functions. Structural aspects of the L type calcium channel molecules will be presented by Alan Neely and how structural changes affect calcium channel function in the heart muscle cell will be discussed in the presentation of Diego Varela. Gustavo Brum will address recent questions on a different role of Cav1.1 in skeletal muscle where these channels act as voltage sensors for the process of excitation contraction coupling. Mariana Casas will dig into an additional role for these channels, acting also as voltage sensors but now in the process of excitation-transcription coupling in skeletal muscle fibers, where they also participate in a mechanism that discriminates the stimulation frequency.

Regulation of backward versus forward traffic of calcium channels by auxiliary subunits

Neely, A[1,2].,[1]Neurociencia, Ciencias, Universidad De Valparaíso.[2]D. de Neurociencia, Facultad de Ciencis, Universidad de Valparaíso.

Calcium channels of the High Voltage-activated subfamily are multiprotein complexes in which a central pore-forming $\alpha_1$subunit that by itself support voltage-dependent calcium influx it associates to several auxiliary subunits that regulate function. Among them the so-called β subunit is the most potent regulators has been propose to act at several stage of the channel biogenesis from the release of $\alpha_1$subunit from the endoplasmic reticulum to removal of channels by endocytosis, including several aspect of channel function. Weighting the relative importance of these regulatory steps has been hampered by seemingly contradictory results obtained with different expression system. I will review the evidence that emphasize the role of the β subunit on backward traffic of calcium channels in heterologous expression system as well as in primary cultured cells.

Symposium 6: Protein Structure

Chair: Eduardo Perozo

Mass spectrometry of membrane proteins: from proteomics to intact complexes

Barrera, N[1].,[1]Physiology, Biological Sciences, Pontificia Universidad Católica De Chile.

Structural biology on membrane proteins has been an evolving field over the years. For a number of years Mass Spectrometry (MS) has been applied to characterize the sequence of subunits forming membrane protein complexes, however only recently the stoichiometry of intact membrane protein complexes has been obtained via a new methodology based on MS.In this symposium recent insights from the MS application on intact membrane protein complexes will be discussed, in particular molecular systems regulated by lipid binding. Funded by Millennium Science Initiative P10-035F, Fondecyt 1120169 and Wellcome Trust grants.

Redox-control of axon guidance by mical-1

Bianchet, M[1].,Al-Qassim, S[2].,Simon, O[2].,Urquiza, M[2].,Amzel, M[2].,[1]Neurology, Biophysics & Biophysical Chemistry, Assistant Professor, Johns Hopkins Medical School.[2]Biophysics & Biophysical Chemistry, , Johns Hopkins Medical School.

After nerve injure, the regeneration of axons along previous paths requires overcoming repulsive signals present in the scar. At molecular level, these repulsive signals are manifested as soluble or membrane-attached molecules that are recognized by guidance receptors at the plasma membrane of the axon growth-cone. For example, the detection of a soluble Sema3A by PlexinA/Neuropilin receptors generates changes in PlexinA's cytoplasmic portion that recruits MICAL-1 among few other proteins to the plasma membrane. MICAL-1 is multidomain enzyme that mediates the cytoskeleton reorganization required to steer away the axon from tissue or extracellular media displaying repulsive cues. MICAL's activity has been shown as an essential part of the guidance-cue transduction to the cytoskeleton reorganization associated with the local collapse of the axon growth-cone and as consequence of it identified as a possible target for nerve regeneration pharmacotherapies. The actin-modifying redox activity of MICAL-1 resides in its N-terminal monooxygenase domain that is followed by three cytoskeleton-interacting domains. The C-terminal domain of MICAL-1 interacts with PlexinA; have been shown that such interaction relieves the autoinhibited state of the enzyme. Previously, we have determined the structure and characterized the high NADPH-oxidase activity of the isolated monooxygenase domain. Since with a "divide and conquer approach", we have been studying the structural relations between MICAL-1 domains and the contribution of the non-enzymatic domains to the enzyme activities using x-ray diffraction and small angle x-ray scattering, as well as binding thermodynamic and kinetic characterizations. The findings of our studies will be discussed.

Structural Basis of Lipid-Driven Conformational Transitions in the KvAP Voltage Sensing Domain

Li, Q[1].,Sompornpisut, P[1]., **Perozo, E[2]** .,[1]Biochemistry and Molecular Biology, University of Chicago.[2]Deprt of Biochemistry and Molecular Biology , University of Chicago. (Sponsored by Eduardo Perozo)

Voltage-gated ion channels are responsible for the electrical and chemical signaling that underlies cellular communication and signal transduction. They respond to transmembrane electric fields through reorientations of the positively charged S4 helix within the voltage-sensing domain (VSD). Despite a wealth of structural data, the details of this conformational change remain controversial. Recent electrophysiological evidence showed that conformational equilibrium between the resting (Down) and activated (Up) conformations of KvAP's isolated VSD can be biased through reconstitution in lipids with or without phosphate groups. We investigated the structural transition between these functional states using site-directed spin labeling and EPR spectroscopic methods. Solvent accessibility and inter-helical distance determinations suggest that KvAP gates through a novel mechanism involving a ~3 Å upward tilt and simultaneous ~2 Å axial shift of S4. This motion leads to large accessibly changes in the intracelluar water-filled crevice and supports a novel model of gating that combines structural rearrangements and electric field remodeling.

Combination of cell-free protein expression and liquid state NMR spectroscopy for the structure determination of membrane proteins

Doetsch, V[1].,Reckel, S[1].,Sobhanifar, S[1].,Laguerre, A[1].,Proverbio, D[1].,Loehr, F[1].,Bernhard, F[1].,[1]Biophysical Chemistry, , Goethe University.

Cell-free expression systems are ideal for the production of proteins that cannot be overexpressed in E. coli cells. In particular for NMR spectroscopy, cell-free expression provides major advantages since it allows for amino acid type selective labeling without metabolic scrambling. We have developed several labeling protocols that allow us to obtain the backbone assignment of membrane proteins in an efficient way. The TMS-labeling (Transmembrane segment enhanced labeling) is based on the fact that 60% of the amino acids of the transmembrane helices consist of only six different amino acid types. Double labeling membrane proteins with these six amino acids produces large consecutive stretches of labeled amino acids that can be analyzed with standard triple resonance experiments with a significantly reduced peak overlap. In addition, we have developed a combinatorial labeling scheme that allows us to assign specific amino acids. Using these methods we have obtained the backbone assignment for the C-terminal fragment of presenilin, the catalytic component of the g-secretase which is responsible for cleaving the amyloid precursor protein. This peptide forms the plaques that are found in the brains of Alzheimer patients. We have determined its structure as well as the structure of the retinal binding protein Proteorhodopsin.

Special Young Scientist Symposium
Chair: Juan Bacigalupo

BDNF-induced synaptic plasticity and RyR increase require ca^{2+}-induced-ca^{2+} release and ros

Adasme, T[1].,Bruna, B[1].,Lobos, P[1].,Paula-Lima, A[2].,Hidalgo, C[3].,[1]CEMC,BNI, Departamento de Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile. [2]Departamento de Ciencias Básicas y Comunitarias, Facultad de Odontología, Universidad de Chile. [3]CEMC,BNI,ICBM, Departamento Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile. (Sponsored by Cecilia Hidalgo Tapia)

Ca^{2+}-induced- Ca^{2+} release (CICR) mediated by the ryanodine receptor (RyR) amplifies activity-dependent Ca^{2+} entry signals, providing a major fraction of the Ca^{2+} increase displayed by hippocampal neurons in response to specific stimuli. Ca^{2+} signals and reactive oxygen species (ROS) are involved in synaptic plasticity. Along with Ca^{2+} signals, BDNF upon binding to its TrkB receptors initiates complex signaling pathways essential for hippocampal synaptic plasticity and spatial memory. The participation of CICR and ROS downstream of BDNF signaling, which has been poorly studied, is the focus of our research. To this aim, we determined RyR expression and activity in hippocampal neurons incubated for 6 h with BDNF, and explored also if BDNF-induced spine remodeling requires RyR-mediated Ca^{2+} release. RyR activity was suppressed by pre-incubating cultures with inhibitory ryanodine. Additionally, cultures were transfected with a specific RyR2 antisense oligonucleotide or shRNA. To evaluate ROS participation in BDNF signaling we added the antioxidant N-acetylcysteine (NAC) during BDNF incubation. We found that BDNF-treated neurons displayed larger RyR-mediated Ca^{2+} signals, increased RyR2/RyR3 mRNA and protein contents, and significantly increased spine length and density. Overall inhibition of RyR activity with ryanodine suppressed the RyR2/RyR3 protein expression enhancement and the spine remodeling induced by BDNF. Moreover, selective inhibition of RyR2 expression or NAC suppressed both the enhanced RyR2 mRNA/protein levels and the spine remodeling induced by BDNF. These combined results suggest that RyR2-mediated Ca^{2+} signals and ROS are key components of the complex neuronal plasticity processes induced by BDNF. FONDECYT 3120093, 11110322, 1100052 and BNIP-09-015F.

Role of PSD95 in neuronal branching: epigenetic regulation of its expression during neuronal development

Bustos, F[1].,Montecino, M[1,2].,Van Zundert, B[1].,[1]Center for Biomedical Research, , Universidad Andrés Bello. [2]FONDAP Center for Genome Regulation, , Universidad Andrés Bello.

PSD95 is the most abundant scaffolding protein in the post-synaptic density that regulates spine maturation, dendritic stabilization, and LTP induction. Increased expression of PSD95 is correlated with limited plasticity in the adult brain. Knockdown and overexpression experiments report that PSD95 plays a key role regulating arborization. To determine whether epigenetic mechanisms regulate PSD95 expression, we first identified a pattern of histone tail modifications that strongly correlate with the increasing PSD95 expression during hippocampal development; thus, we found enrichment of activating marks (e.g. H3K4me3). In order to alter PSD95 expression and recover plasticity in mature neurons, we generated an artificial transcription factor (ATF) that binds the PSD95 gene promoter and change the pattern of histone tail modifications present at the promoter. We used an ATF alone (NOED; no-effector domain), or fused to either activation (VP64) or repression (SKD, G9a, Suvdel) effector domains. Infection of hippocampal neurons with ATF-VP64 leads to an upregulation of PSD95 expression and increased histone acetylation (H3Ac). Conversely, SKD, G9a and Suvdel repressed PSD95 expression, decreased H3Ac, and enhanced H3K9me. To test a physiological effect, we quantified the number of dendritic branches of hippocampal neurons. We found that similar as overexpression of PS95, ATF-VP64 decreases branching. By contrast, similar as shRNA-PSD95: ATF-SKD, ATF-G9a or ATF-Suvdel increases arborization. Together, our results demonstrate that we were able to generate an ATF that is capable of silencing PSD95 gene expression by epigenetic means, hereby reactivating dendritic arborization in mature neurons.

Recreated high conductance state modulates subtreshold θ resonance of mammalian neurons through changes in input resistance.

Vera, J[1].,Pereira, U[2].,Deichler, A[1].,Faunes , M[1].,Restrepo, D[3].,Bacigalupo, J[1].,Sanhueza, M[1].,[1]Biología, Ciencias, Universidad De Chile.[2]Fisica, Ciencias, Universidad De Chile.[3]Biología Celular y del Desarrollo, Centro Médico Anschutz, Universidad de Colorado.

θ-range subthreshold resonance (TSR) is the property of some neurons of generating a maximal voltage response when stimulated with an oscillatory current of a frequency between 3-12 Hz (the resonance frequency, f_{res}). TSR relies on HCN and/or KCNQ channels and is amplified by a persistent Na^+ conductance. If TSR is coupled to spike threshold, neurons display selective firing at θ and this may be transmitted to downstream cells, stabilizing network oscillations. In conventional slice experiments, neuronal input resistance (R_{in}) is 100-200% higher than in the intact brain, as synaptic bombardment from the active network is strongly reduced. The so-called *high conductance state* (HCS) in the brain has dramatic effects on signal processing and unexplored impacts on TSR.

Objective: To investigate the degree of coupling between TSR and firing, and the impact of HCS on TSR in rat hippocampus and olfactory amygdala.

Methodology: Whole-cell recordings from brain slices, recreation of HCS in individual cells by the dynamic-clamp technique, computational modeling, neuronal tracing and behavior.

Results:
i) R_{in} reduction during HCS increases f_{res} and decreases resonance strength.
ii) Olfactory amygdala neurons couple TSR with spike generation, in contrast to hippocampal neurons that do not.
iii) Selective firing in the amygdala is preserved in HCS.

Conclusion:
Network activity modulates TSR through changes in R_{in}. Considering the strong θ activity of the olfactory circuits *in vivo* and the fact that a high R_{in} allows resonance preservation in amygdala neurons, we propose a functional role for TSR as a frequency selector mechanism during olfactory coding.

Phase synchronization patterns on EEG signals during sustained visual perception.

Devia, C[1].,Maldonado, P[1],[2].,[1]Physiol. and Biophysics, ICBM; Biomedical Neuroscience Institute, Faculty of Medicine, Universidad De Chile. [2]Santiago, Chile, CENEM. (Sponsored by ICM P09-015F, ICM P10-001-F, Conicyt Doctoral Fellowship (C.D.) And The Guillermo Puelma Foundation.)

Several mechanisms have been proposed to understand the basic principles underlying brain functional connectivity. One of the best supported mechanisms relates to neural coordination. A common measure for neural coordination in human electrophysiology is phase synchronization. It has been amply demonstrated that phase synchronization is modulated immediately after brief stimuli presentations (~500ms). However, on natural vision, visual percepts can be sustained for long periods of times. It is still unknown whether spatio-temporal patterns of phase synchrony can account for this sustained perception. In this work, we examined patterns of phase synchronization in a classical bistable visual task, the Necker cube. In this paradigm, one lasting physical stimulus has two possible interpretations, and the subject reported each time his perception change from one to the other interpretation, by a button press (BP). Twelve human subjects were exposed to the Necker cube stimulus and to several other control conditions while electroencephalographic signals and eye movement behavior were simultaneously recorded. This stimulus presented the advantage that the subjects frequently reported sustained perception periods over 1 sec. Sustained perception during visualization of the Necker cube exhibited three statistical significant synchrony patterns. These patterns were mainly due to perceptual activity as we compared them through a permutation test against activity occurring because of the BP alone. Our results show that these synchronization patterns directly relate to perceptual activity and that discrete episodes of neuronal synchronization can be associated with sustained perception occurring during natural vision.

Symposium 7: Role and regulation of channels and hemichannels formed by connexins or pannexins in the nervous system.

Chairs: Juan C. Sáez; Agustín Martínez

"How to learn to play the Tsuzumi without becoming deaf in the try?"

Martínez, A[1].,Garcia , I[1].,Ceriani, R[1].,Maripillán, J[1].,Pinto, B[1].,[1]Centro Interdisciplinario de Neurociencia de Valparaíso, Ciencias, Universidad de Valparaíso.

Gap junction channels (GJCs) and hemichannels (HCs) are major pathways for intercellular signaling, they are made of proteins named connexins (Cxs). Mutations in the Cx26 gene can give rise to non-syndromic or syndromic deafness, if they affect exclusively the audition or if they also produce skin disease, respectively. How do these different clinical phenotypes arise is under speculation. To gain insight about this issue, we studied several non-syndromic and syndromic mutations targeting the amino-terminal and first transmembrane domains of Cx26. We found that all Cx26 mutants associated with non-syndromic or syndromic deafness present impairments in the formation and/or function of GJCs. In addition, some syndromic mutants were dominant negative of the GJC function of wild type Cx43 (a connexin that normally does not hetero-oligomerize with wild type Cx26). Since Cx43 and Cx26 are co-expressed in the cochlea and skin we explored a possible interaction as consequence of the N-terminus mutations. We found that all Cx26 syndromic mutants, but not non-syndromic mutants, co-oligomerize with Cx43, suggesting that these syndromic mutations affected compatibility between connexins. Surprisingly, in cells co-expressing any of the syndromic mutants with Cx43 we observed increments in HC activity. Moreover, we found that these aberrant heteromeric HC have reduced regulation / gating by extracellular calcium ions, which correlates with susceptibility to cell death. Since Cx43 is co-expressed with Cx26 in the skin the mechanism by which Cx26 mutants induce deafness may differ from the mechanism that induces skin disease. (Financed by Millennium Institute-CINV, FONDECYT-1130855 and Anillo grant ACT-1104).

Activity-dependence of astroglial connexin channels in the olfactory bulb

Giaume, C[1].,[1]Center of Interdisciplinary Research in Neuroscience, Collège de France, Collège de France, Paris.

Because they express a high rate of connexins (Cx43, Cx30) astrocytes have the capacity to communicate through gap junction (GJ) channels and hemichannels (HCs). This adds another level of complexity when considering the contribution of neuroglial interactions to brain functions. We studied how neurons and astrocytes reciprocally modulate their network properties in olfactory glomeruli where neuronal connectivity is highly ordered. We observed: (1) a compartmentalized organization of GJ-mediated astroglial networks that overlaps neuronal functional units; (2) that these networks are plastic and modulated by neuronal activity. Interestingly, this modulation specifically targets Cx30-mediated GJ communication within glomeruli and depends on extracellular potassium generated by neuronal activity. Then, we addressed the contribution of astroglial Cxs to neuronal network activity. We observed that mitral cells (MCs) exhibit spontaneous and periodic activity (<1Hz) similar to the 'slow' wave oscillations recorded during slow wave sleep. UP and DOWN state fluctuations of MC membrane potential depend on glutamatergic interactions that take place at the level of the glomeruli. Importantly, these slow oscillations are modulated by the expression of Cxs. Indeed, in mice devoided of astroglial Cxs, the amplitude of slow oscillations is impaired. Also TTX treatments affect the UP state amplitude and firing rate of MCs. Those alterations are mimicked by a blockage of Cx43 HCs in the Cx30 KO mice. These results indicate that Cx43 HC function is promoted by neuronal activity and modulates neuronal network activity.Altogether these results indicate that astroglial Cxs play a role in bidirectional interactions between astroglial and neuronal networks.

Role of single membrane channels formed by connexins or pannexins in glial cells

Orellana, J[1].,[1]Departamento de Neurología, Escuela de Medicina, Pontificia Universidad Católica De Chile.

During the last two decades, it became increasingly evident that glial cells accomplish a more important role in the brain function than previously thought. Glial cells express pannexins and connexins, which are member subunits of two protein families that form membrane channels termed hemichannels. These channels communicate intra- and extra-cellular compartments and allow the release of autocrine/paracrine signaling molecules (e.g., ATP, glutamate, NAD^+ and PGE_2) to the extracellular milieu, as well as the uptake of small molecules (e.g., glucose). An increasing body of evidence has situated glial hemichannels as potential regulators of the beginning and maintenance of homeostatic imbalances observed in diverse brain diseases. Here, we review and discuss the current evidence about the possible role of glial hemichannels on neurodegenerative diseases. A sub-threshold pathological threatening condition leads to microglial activation, which keep active defense and restores the normal function of the central nervous system (CNS). However, if the stimulus is deleterious, microglial cells and the endothelium become overactivated, both releasing bioactive molecules (e.g., glutamate, cytokines, prostaglandins and ATP) that increase the activity of glial hemichannels, reducing the astroglial neuroprotective functions, and further reducing neuronal viability. Because ATP and glutamate are released via glial hemichannels in neurodegenerative conditions, it is expected that they contribute to neurotoxicity. More importantly, toxic molecules released via glial hemichannels could increase the Ca^{2+} entry in neurons also via neuronal hemichannels, leading to neuronal death. Thereby, blockade of hemichannels expressed by glial cells and/or neurons during neuroinflammation might prevent neurodegeneration.

Connexin 43 defines spatial domains of progenitors in a stem cell niche of the spinal cord

Russo, R[1].,[1]Neurofisiología Celular y Molecular, , Instituto de Investigaciones Biológicas Clemente Estable.

The cells lining the central canal (CC) of the spinal cord derive from the ventral part of the neural tube, and in some vertebrates are responsible for the functional recovery after spinal cord injury. In the turtle, the region surrounding the CC contains proliferating cells that seem to generate both glia and neurons. Understanding the biology of spinal progenitors with the potential to generate new neurons “in situ” could give important clues for cell replacement therapies. Thus, we aimed to identify and characterize precursor cells in the spinal cord of *Trachemys dorbignyi*. To evaluate the population of proliferating cells, 5-bromo-2’-deoxyuridine (BrdU) was injected every 4 h (50 µg/g, i.p.) during 24 h. We found BrdU+ nuclei around the CC with a higher density in the lateral quadrants, where whole cell patch clamp recordings showed extensive dye coupling of cells on the lateral aspects of the CC. Carbenoxolone (100 µM) increased the input resistance suggesting strong gap junction coupling among precursors. The expression of brain lipid binding protein -a marker of a subtype of radial glia- and Pax6 matched the location of clusters of dye coupled cells, suggesting they belong to a domain of neurogenic precursors. These domains were delimited by a high density of Cx43 located on the endfeet of CC contacting cells. Our findings indicate that spinal precursors share basic properties with those in the embryo and neurogenic niches of the adult brain and support a key role of communication via Cx43 for spinal cord neurogenesis and plasticity.

Symposium 8: Synaptic Plasticity
Chairs: Pablo Muñoz, Cecilia Hidalgo

Role of CaMKII in NMDA receptor-mediated long-term plasticity at hippocampal mossy fiber-CA3 synapses

HAEGER, P[1],[2].,Younts, T[2].,Lujan, R[3].,Castillo, P[2].,[1]Departamento Ciencias Biomédicas, Facultad de Medicina, Universidad Católica Del Norte, Coquimbo-Chile.[2]Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx-USA.[3]Instituto de Investigación en Discapacidades Neurológicas (IDINE), Departamento de Ciencias Médicas , Facultad de Medicina, Universidad Castilla-La Mancha, Campus Biosanitario, Albacete, Spain. (Sponsored by NIH/NIMH (R01-MH081935), Pew Latin American Fellows Program In The Biomedical Sciences, Becas Chile.)

NMDA receptors (NMDARs) are ligand-gated ion channels that play crucial roles in synaptic plasticity. NMDARs are heterotetramers typically composed of two GluN1 and two GluN2 (GluN2A or GluN2B) subunits. CaMKII, a major protein constituent of excitatory synapses is central to the regulation of glutamatergic synapses and to the induction of classical AMPAR LTP. CaMKII may act as a central organizer of excitatory synapses, which works as an activity-dependent scaffold to regulate synaptic NMDARs. Recently, our group identified a selective form of long-term potentiation of NMDAR-mediated transmission at the mossy fiber (mf) to CA3 pyramidal cell (mf-CA3) synapse in the hippocampus (NMDAR-mfLTP). In this study, we examined the role of CaMKII in NMDAR-mfLTP using rodent brain slice electrophysiology and molecular manipulations to interfere CaMKII signaling and CaMKII-GluN2B interaction. We found that the selective CaMKII inhibitor KN-93 blocked NMDAR-mfLTP, whereas the inactive analog KN-92 had no effect. We also loaded CA3 pyramidal neurons with a CaMKII inhibitory peptide (CaMKII-Ntide), which is derived from an endogenous CaMKII inhibitory protein and operates by a distinct mechanism from that of KN-93. Interestingly, CaMKII-Ntide strongly inhibited NMDAR-mfLTP. Finally, we took advantage of a recently developed GluN2B knock-in mouse where the CaMKII-GluN2B interaction is strongly impaired by mutating Leu1298 to Ala and Arg1300 to Gln160. NMDA-mfLTP was severely impaired in GluN2B-KI mice compared with littermate controls. Together, these findings indicate a novel role of CaMKII-GluN2B interaction as a key step of NMDAR plasticity.

Synaptic plasticity and aging

Muñoz, P[1].,[1]Neurociencias, Ciencias, Universidad de Valparaíso, Chile..

Aging is a stochastic process associated with a decline in cognitive function that can, in part, be explained by changes in synaptic plasticity (SP). Progressive oxidative accumulation, as well as, alterations on transcriptional regulation take place during aging. To understand the potential contribution of aging to transcriptional machinery that sustains SP and memory, we used behavioral, electrophysiological and molecular approaches. To that end, we evaluated young (3 months) or aged (18 months) rats in an object recognition task. After finishing the behavioral task, we explored changes on SP either hippocampal or perhirrinal cortical slices from trained animals, due to both temporal lobe structures are considered anatomical substrates for recognition memory. On the other hand, we also assessed the expression levels of ryanodine receptor (RYR), critical protein for SP, as well as the levels of oxidation of DNA, in animals of different ages. We found that both groups have significant differences in hippocampal- dependent memory, but not in cortical- dependent memory. Additionally, we found that the levels of RyR are higher in aged rats compare with young rats, which correlates with both dysfunction in SP and an increase in the oxidation of DNA. Altogether, our data suggest that the hippocampus has a greater susceptibility to the aging process than perhirrinal cortex and an increase in the oxidation of DNA can be responsible for the alterations in the expression of genes related to plasticity and memory.

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Bidirectional NMDA receptor plasticity in the hippocampus

Hunt, D[1].,**Castillo, P[1]**.,[1]Neuroscience, Albert Einstein College of Medicine. (Sponsored by NIH R01 MH081935)

NMDA receptors (NMDARs) are glutamate-gated ion channels that are crucial for neuronal communication, neurodevelopment and synaptic plasticity. While classically known as coincidence detectors for the induction of long–term synaptic plasticity, NMDARs contribute significantly to fast excitatory synaptic transmission. In the hippocampus, NMDARs have been implicated in CA3–dependent spatial memory functions that likely rely on dynamic cellular ensemble encoding of space. The unique functional properties of both NMDARs and mossy fiber (MF) projections to CA3 pyramidal cells place MF–NMDARs in a prime position to influence CA3 ensemble dynamics. In this study, we have investigated whether *in vivo*–like patterns of DGC and CA3 pyramidal cell activity could elicit bidirectional NMDAR plasticity at MF–CA3 synapses. To this end, we have adapted the spatially selective burst–firing patterns of place cells to the paradigm of spike timing–dependent plasticity (STDP). Our results demonstrate that the temporal relationship between pre and postsynaptic bursting activity can dictate the polarity (LTP or LTD) and magnitude of NMDAR–mediated transmission. We further report that bidirectional NMDAR plasticity can modulate MF–driven burst–firing output, spike temporal fidelity, and mediate heterosynaptic metaplasticity between MF synapses and neighboring associational/commissural (AC) synapses established between CA3 pyramidal cells. These forms of plasticity could contribute to the formation, storage, and recall of cell assembly patterns in the hippocampal CA3 subfield.

Hippocampal synaptic plasticity requires ryanodine receptor-mediated calcium release

Hidalgo, C[1].,Adasme, T[2].,Barrientos, G[3].,Haeger, P[4].,More, J[2].,Paula-Lima, A[2,5].,Valdes, I[2].,[1]BNI and CEMC, Physiology & Biophysics Program, ICBM, Faculty of Medicine, Universidad de Chile.[2]BNI and CEMC, Faculty of Medicine, Universidad de Chile.[3]Physiology and Biophysics Program, ICBM, Faculty of Medicine, Universidad de Chile.[4]Department of Biomedical Sciences, Faculty of Medicine, Universidad Católica Del Norte.[5]Department of Basic and Communitarian Sciences, Faculty of Dentistry, Universidad De Chile. (Sponsored by Biomedical Neuroscience Institute (BNI))

Hippocampal neurons (rat) express ryanodine receptor (RyR) channels that possess highly reactive cysteine residues and act as intracellular redox sensors. Our previous results indicate that controlled RyR oxidation stimulates RyR-mediated Ca^{2+} release from the endoplasmic reticulum in hippocampal primary neurons leading to the activation of Ca^{2+}-dependent signaling cascades required for synaptic plasticity, and makes possible RyR-mediated Ca^{2+}-induced Ca^{2+} release in electrically stimulated hippocampal neurons. Synaptic plasticity entails changes in synaptic efficacy and dendritic spine morphology. Results will be presented showing that RyR-mediated Ca^{2+} signals, which are prevented by the antioxidant N-acetylcysteine, are essential for the spine elongation and growth produced by brain-derived neurotrophic factor (BDNF) in primary hippocampal neurons. Moreover, inhibition of RyR channel activity prevents the induction of LTP by theta burst stimulation (CA3 to CA1) of hippocampal slices, which express the three mammalian RyR isoforms, but does not affect paired-pulse responses suggesting a postsynaptic mechanism. LTP induction produced after 1 h a significant increase in RyR1 and RyR2 mRNA levels and RyR2 protein content, but did not modify RyR3 mRNA and protein levels. We interpret these results as an indication that RyR-mediated Ca^{2+} release plays a central role in hippocampal synaptic plasticity. Supported by FONDECYT 1100052, FONDECYT 3120093 and MILLENNIUM BNI P-09-015F.

SOBLA-Symposium: Lightning the structure and function of ion channels
Chairs: Teresa Giraldez, Carlos González

Conformational changes tracked by spectroscopy

Bezanilla, F[1].,[1]Biochemistry and Molecular Biology, , University of Chicago.

Voltage dependence of ion channels, transporters and some voltage-dependent enzymes is conferred by a voltage sensor (VS). The VS is normally a set of charges or dipoles embedded in the membrane protein that move in response to a change in the membrane potential. The movement of the charged voltage sensor produces gating (or sensing) currents which can be measured as transient currents in the external circuit under voltage clamp. Gating currents provide information of conformational states of the protein with a high level of temporal resolution and they have been the basis of detailed kinetic models that describe the gating of channels, transporters and pumps. Although these electrical measurements are precise, they give no information on what parts of the structure are moving. Fluorescence spectroscopy provides spatial resolution with high sensitivity, to the single molecule level, and the measurements can be done while the protein is functional. Fluorescence spectroscopy is done after fluorescent probes are introduced in specific sites of the protein allowing the detection of time dependent quenching, spectral shifts, electrochromicity and energy transfer with simultaneous electrical measurement. In this presentation I will give examples of how fluorescence spectroscopy has provided detailed information on voltage sensing. Studies on Na^+ and K^+ channels and the voltage sensitive phosphatase Ci-VSP have provided a detailed picture of how a voltage sensor operates to reach different conformational states while revealing the molecular basis that makes Na^+ channels faster than K^+ channels, which is crucial for the generation of the nerve impulse.
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Subunit interactions during cooperative opening of voltage-gated proton channels

Peter Larsson
D. of Physiology and Biophysics
Miller School of Medicine
University of Miami, USA

Unlike other voltage-gated cation channels, voltage-gated proton (Hv1) channels have only four transmembrane domains and lack a centrally located pore formed by the assembly of subunits. Instead, the proton permeation pathway in the Hv1 channel is within the voltage-sensing domain formed by the four transmembrane domains of each subunit. The gating mechanism of this pathway is still unclear. Mutagenic and fluorescence studies suggest that the fourth transmembrane segment, S4, functions as the voltage sensor and that there is an outward movement of S4 during channel activation. Using thermodynamic mutant cycle analysis, we found that the conserved positively charged residues in S4 are stabilized by counter charges in the other transmembrane segments both in the closed and open state. Based on this information, we constructed a model of both the closed and open state of Hv1. These structural models suggest that electrostatic interactions between transmembrane segments in the closed state pull hydrophobic residues together to form a hydrophobic plug in the center of the subunit. Outward S4 movement during channel activation induces conformational changes that remove this hydrophobic plug in the voltage-sensing domain, thereby allowing the formation of a hydrogen-bond chain across the channel that allows proton permeation. This suggests that the salt-bridge networks and the hydrophobic plug function as the gate in Hv1 channels and that outward movement of S4 leads to the opening of this gate.

Fast photoswitching of tethered ligands to study ionotropic glutamate receptor activation and desensitization

Reiner, A[1].,Isacoff, E[1].,[1]Molecular & Cell Biology, University of California Berkeley.

Ionotropic glutamate receptors (iGluRs), which are gated by the neurotransmitter glutamate, are an important family of ion channels that mediates excitatory synaptic transmission and plasticity in the brain. Binding of glutamate to the four receptor subunits both activates and desensitizes these receptors, generating a transient current. However, little is known about how the ligand occupancy at each subunit contributes to gating, as detailed studies are complicated by the probabilistic nature inherent to binding of diffusible ligands. To circumvent this problem, we use tethered ligands, which are covalently attached at the ligand binding site and which encompass a photoswitchable azobenzene linker that allows to induce ligand binding and unbinding with light of different wavelengths. Ultrafast photoswitching to control specific subunits in two kainate-type iGluRs, GluK2 homotetramers and GluK2/GluK5 heterotetramers, provides a real-time measure of gating and reveals how different ligand occupancies lead to activation and desensitization.

Site-specific incorporation of fluorescent un-natural amino acids into proteins expressed in mammalian cells

Brauchi, S[1].,[1]Physiology, , Universidad Austral De Chile.

The use of un-natural amino acids (UAAs) into proteins had emerged as a promising strategy to manipulate, with unbeatable precision, protein structure and protein-protein interaction in vitro and in vivo. To date, fluorescent UAAs have been successfully incorporated in proteins expressed in bacteria and Xenopus oocytes. Here, we engineer a novel tRNA/RS pair, allowing for the incorporation of the fluorescent aminoacid tyr-coumarin in recombinant proteins expressed in HEK-293T cells. TRPV1 channels were labeled at different positions, and single molecules were individually resolved by TIRF microscopy. The advantages and limitations of this experimental approach will be discussed. FONDECYT 1110906.

Permeation mechanism in Hv channels

Baez, D[1].,Otarola, E.,Contreras , G[1].,Castillo, K[1].,Larsson, H[2].,Latorre, R[1].,**Gonzalez, C[3]**.,[1]Neurociencias, CINV, Universidad De Valparaíso.[2]Physiology & Biophysics, Miller School of Medicine, University of Miami.[3] Neurociencias, CINV, Universidad De Valparaíso. (Sponsored by Ramon Latorre)

Recently, a family of voltage-gated proton channels, called (Hv) was cloned from different organisms. Hv channel shares a common structure with the Voltage Sensor Domain (VSD) of voltage-gated K channels. Despite off the high homology between Hv channel and the VSD, they have distant quaternary structures. Hv channel lacks a pore domain, and is fully functional either as a monomer or dimer. The S4 was proposed as the possible permeation pathway, resembling the omega current present in some mutants of Shaker K channel. However the permeation pathway is still elusive. Fluorescence experiments suggests that the voltage activation in Hv is in two steps, where the second step is a cooperative conformational change, involving interactions between subunits that opens both permeation pathways in a concerted way in the dimer. However, the relationship between the H pore, and the voltage sensor activation is not fully understood for the monomer. Here we presented the first Hv gating currents recordings from the monomeric Hv form. Interestingly, the voltage sensor activation precedes the H conduction, suggesting that voltage sensor and H pore, are two different structures allosterically coupled. The Q-V present a zδ of 1.6e0, in agreement with the first step of voltage activation from fluorescence experiments. The data presented support the hypothesis that channel opening is associated to a second conformational state of the voltage sensor, associated to an extremely slow component in the gating current. Supported by Fondecyt grants ACT 1104 and Fondecyt 1120802 to CG and 1110430 to R.L. CINV is a milleniun institute.

Divalent cation - dependent motion of the bk channel gating-ring reported by state-dependent fret

Miranda, P[1].,Giraldez, T[2].,Holmgren, M[3].,[1]National Institutes of Neurological Disorders and Stroke, , National Institutes of Health.[2]Research Division, , University Hospital NS Candelaria, Tenerife, Spain.[3]National Institute of Neurologial Disorders and Stroke, , National Institutes of Health, Bethesda, MD, USA

Large conductance voltage- and calcium-dependent potassium channels (BK channels) are important controllers of cell excitability. The channel's open probability is regulated both by changes in transmembrane voltage and by intracellular calcium and magnesium concentration. The voltage sensor resides within the transmembrane region of the channel, while Ca^{2+} binding is sensed by a large C-terminal intracellular region, where eight Regulator of Conductance for K^+ (RCK) domains form a "gating ring". It has been demonstrated in intact BK channels that there are remarkable large rearrangements of the gating ring upon Ca^{2+} binding, much larger than the rearrangements predicted by existing X-ray structures of the isolated gating ring. Other divalent cations like magnesium and barium can activate the BK channels . In the present study we investigate the magnesium, barium and voltage-dependence of conformational changes in the intact human BK channel by patch-clamp recordings and simultaneous measurements of fluorescence energy transfer between CFP and YFP variants of the green fluorescent protein, inserted into the BK gating ring. Our results demonstrate that upon Mg^{2+} and Ba^{2+} binding there is structural rearrangement of the gating ring that are not obligatorily coupled to the opening of the pore and are not strictly cooperative. These movements are dependent of the ion involved, presenting a different voltage dependent behaviour for Mg^{2+} and Ba^{2+}. Our results point to a complex movement of the gating ring induced by divalent cations and this movement could be not directly related to the opening of the pore.

Posters

POSTERS I

1. Effects of chloride channel blockers on Ca^{2+} release in frog skeletal muscle.

Ferreira Gregorio, J[1].,Pequera, G[1].,**Brum, G[1]**.,[1]Biofísica, Facultad de Medicina, Universidad de la República. (Sponsored by CSIC Grant To GB)

Disulfonated stilbene derivatives chloride channel blockers have potentiating effects on the skeletal muscle ryanodine receptor (RyR) incorporated in bilayers (Kawasaki & Kasai, 1989; O'Neil et al., 2003) increasing the open time probability. Similar effect was reported on the cardiac RyR (Zahradníková & Zahradník, 1993) in addition to an increase in channel conductance (Hill & Sitsapesan, 2002). 9-anthracenecarboxylic acid (9AC) is another chloride channel blocker. We have explored the effects of SITS, DIDS and 9AC on Ca^{2+} release. In voltage clamped cut fibers all three agents increased the peak and the steady component of Ca^{2+} release when applied intracellularly from the cut ends. Both stilbene derivatives added to the external solution at high doses (2 mM) produced similar effects. We also studied the effects of these agents on Ca^{2+} sparks in permeabilized fibers. At low doses (1-8 μM) SITS or DIDS produced in some experiments a small increase in spark frequency, while in others suppressed spark occurrence and induced the appearance of long lasting events. At high doses (up to 1 mM) both drugs induced sparse prolonged events and an increase in basal fluorescence indicating that cytosolic [Ca^{2+}] augmented. Regular sparks disappeared. 9AC had similar effects, but at higher doses. In the range of 100 to 400 μM it induced an increase in spark frequency (n=10). At 200 μM the frequency increase was 25%+0.19. The effect of 9 AC was antagonized by high Mg^{2+} concentrations. The present results are in line with previous observations on single channels and indicate that Ca^{2+} release can be severely altered in the presence of these agents.

2. Is thermosensor DesK a bifunctional protein having kinase and channel activities?

Cybulski, L[1].,Inda, M[1].,De Mendoza, D[1]., [1]Microbiology, Fac. de Ciencias Bioquímicas y Farmacéuticas - Instituto de Biología Molecular y Celular de Rosario- CONICET, Universidad Nacional de Rosario.

DesK is involved in detecting temperature changes in *Bacillus subtillis*. At low temperatures it behaves as a kinase, phosphorylating the response regulator DesR, which in turn activates expression of a desaturase, thereby regulating membrane fluidity. We designed a chimerical protein in which both sensing and transmission of DesK(which has five transmembrane segments), were captured into one single chimerical transmembrane segment (MS-DesK). Employing genetics, biophysical and molecular biology tools, we found that both the N-terminus and the C-terminus of DesK and MS-DesK have motifs that render DesK sensitive to membrane thickness and interfacial hydration, which would in turn depend upon temperature changes. Both motifs must interact to form the trigger. Our proposed mechanism of thermal regulation holds true for both, the full length DesK and the Minimal sensor DesK.

Then the emerging questions are : *What is the role of the other transmembrane segments? Why were they conserved along evolution if they could have been replaced by only one TMS?*

Our hypothesis that DesK is a bifunctional protein with two separatable activities: **i)** a thermosensitive histidine kinase that responds to low temperatures activating desaturase expression and **ii)** an ion chanel. The idea that DesK could be also anion channel is reinforced by : **i)** the presence of several Ser and Thr residues in the TM region, **ii)** DesK has an cluster of charged resiudes at the interfacial region connecting the TMS with the catalytic domain conserved in several ion channels and **iii)** DesK activity is strongly influenced by salt concentrations.

3. Purinergic-dependent contraction and calcium oscillations in smooth muscle of small intrapulmonary veins.

Henriquez, M[1].,Torres, A[1].,Baigorri, R[1].,Perez-Zoghbi, J[2].,[1]Lab. Bronchovascular Dynamics & Lung Injury, Program of Physiology and Biophysics, Medicine, Universidad De Chile.[2]Cell Physiology & Molecular Biophysics, , Texas Tech University Health Sciences Center. (Sponsored by BECAS-CHILE And ICBM To MH And The American Heart Association And TTUHSC To JFPZ.)

Small Intrapulmonary Veins (SPV) could play an important role during the development of some forms of pulmonary edema and pulmonary hypertension. Furthermore, under many lung conditions, ATP is commonly released to extracellular space and may affect the control of vascular tone by stimulating purinergic receptors. However, the effects of ATP on smooth muscle cells (SMC) in SPV are unknown. Thus, we investigated the effects of extracellular ATP on the contraction and Ca^{2+} signaling in SMC in SPV. Living lung slices were prepared from SD rats and used to measure SPV contraction and Ca^{2+} signaling with phase-contrast and confocal microscopy, respectively. Also, indirect immunofluorescence was used to detect the P2Y receptor in SMC. The SPV (~100 µm)perfused with ATP contracted reducing their lumen area in ~60%. The response to ATP was concentration-dependent (EC_{50} = 36 ± 5 µM). ATP-induced vein contraction was accompanied with an increase of intracellular [Ca^{2+}] in the SMC characterized by Ca^{2+} oscillations. In absence of extracellular Ca^{2+}, both Ca^{2+} oscillations and contraction were initiated, meaning that Ca^{2+} release from intracellular stored is involved. The ATP-induced contraction was inhibited by Suramin and U73122, a purinergic receptor and PLC inhibitor respectively and the P2Y2 receptor was detected by immunofluorescence. Finally, 2-APB but not Ryanodine inhibited both Ca^{2+} signaling and SMC contraction. Our results suggest that SPV contract in response to extracellular ATP through the activation of P2Y metabotropic receptors in their SMC and the subsequent induction of Ca^{2+} oscillations mediated by Ca^{2+} release from intracellular stores through IP_3 receptors.

4. Complex regulation of connexin hemichannels and pannexin-1 channels by NO in endothelial cells

Lillo, M[1].,Gaete, P[1].,Puebla, M[1].,Figueroa , X[1].,[1]Fisiologia, Ciencias Biologicas, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 1100850, Biophysical Society)

Intercellular channels formed by connexin(Cx) proteins are involved in vasomotor tone. Connexins also form hemichannels,which are structurally related to the channels formed by pannexin.The expression and regulation of connexin-and-pannexin hemichannels in endothelial cells are unknown. We evaluated hemichannel activation by ethidium uptake and connexin or pannexin S-nitrosylation in response to ACh, an endothelium-dependent vasodilator.The expression of connexins-and-pannexins on the membrane of endothelial cells was evaluated by protein biotinylation. Cx37, Cx40, Cx43 and Panx-1 were detected in the membrane of endothelial cells and ACh increased ethidium uptake, which was abolished by the pannexin channel blockers probenecid and ^{10}Panx, but attenuated by the connexin hemichannel blocker La^{3+} and the connexin mimetic peptides $^{37-43}$Gap26 and ^{43}Gap26. ^{40}Gap27 did not affect the ACh-induced ethidium uptake. Inhibition of NO synthase with N^G-nitro-L-arginine, guanylate cyclase soluble with ODQ or PKG with KT5823 inhibited the ACh-evoked ethidium uptake. Moreover, the cGMP analog 8Br-cGMP induced a probenecid-sensitive increase in ethidium uptake. Cx37, Cx40 and Cx43, but not pannexin-1, were found to be S-nitrosylated in basal conditions, and ACh enhanced the S-nitrosylation of Cx40 and Cx43. Interestingly, dithiothreitol (DTT), a reducer of sulfhydryl groups, increased the activity of hemichannels, which was blocked by La^{3+} and $^{37-43}$Gap26, but not by probenecid or ^{43}Gap26. These results indicate that NO signaling induces opening of both connexin- and pannexin-based hemichannels. While ACh-evoked NO production activates Cx43-based hemichannels by S-nitrosylation, this posttranslational modification inhibits Cx37-formed hemichannels. Moreover, pannexin-formed channels are activated by cGMP-PKG signaling pathway

5. Quantal properties of voltage dependent Ca release in frog skeletal muscle studied in voltage inactivated and SR depleted fibers.

Pizarro, G[1].,Olivera, J[1].,[1]Biofísica, Medicina, Universidad de la República, Montevideo, Uruguay.

Using a double-pulse protocol the fast inactivation of Ca release flux in skeletal muscle follows an arithmetic rule, if the conditioning depolarization is less than or equal to the test depolarization then decay (peak minus steady level) in the conditioning release is approximately equal to suppression (unconditioned minus conditioned peak) of the test release. This is due to quantal activation by voltage, analogous to the quantal activation of IP3 receptor channels (Pizarro et al, J. Physiol, 501(2)289-303, 1997). Either subsets of channels with different sensitivities to voltage or the clusters of Ca-gated Ryanodine Receptor (RyR) beta in the parajunctional terminal cisternae (Felder and Franzini-Armstrong, PNAS,99(3)1695-1700, 2002) might constitute the quantal units. If Ca-dependent gating is involved, quantal release should be modified by strong release inhibition. We inhibited Ca release by voltage dependent inactivation to reduce global Ca release without modification of the single RyR channel current and by sarcoplasmic reticulum Ca depletion which reduced the single RyR current as well as global release flux. The effect of both procedures on the quantal properties of Ca release in frog skeletal muscle fibers under voltage clamp was studied. The quantal properties of release were preserved regardless of the inhibitory maneuver applied. These findings limit the role of the Ca-activated component of release in generating quantal release. This is consistent with the observation of quantal release in mammalian skeletal muscle where Ca-activated release is thought to be absent (Szentesi et al, J Physiol 528(3)447-456, 2001).Funded by grant CSIC I+D06 to GP.

6. Calcium signaling in muscle cells from patients with RyR mutations causing Malignant Hyperthermia.

Figueroa, L[2].,Kraeva, N[1].,Manno, C[2].,Edwards, J[2].,Riazi, S[1].,**Ríos, E[2]**.,[1]Malignant Hyperthermia Unit (MHIU), , University of Toronto.[2]Molecular Biophysics and Physiology, , Rush University.

Malignant Hyperthermia, MH, is a hereditary sub-clinical condition characterized by events of uncontrolled release of calcium, deadly if untreated. 60% of MH cases are due to mutations of the RyR1 calcium release channel. A puzzling property of these mutations is their phenotypic variability. We are comparing calcium signals in cells from “MHS” patients (positive for 2 standard tests) with those from normal donors (MHN). Subjects are genotyped for the proteins of interest. *Gracilis* muscle biopsies are received overnight from MHIU and studied acutely with voltage clamp and calcium imaging, or used to generate cultures. Myotubes were imaged for $[Ca^{2+}]_{cyto}$ (fluo-4) and V_m (di-8-ANEPPS). In response to brief field stimuli, normal donors had brief action potentials (t ~15 ms) and a monophasic Ca transient, which in rare occasions was followed by a slow wave. Cells from a MHS patient with the mutation G341R always had a 2-phase response —small initial transient followed by a large propagated wave. Two MHS patients with positive reaction to only one of the 2 tests had Ca transients with intermediate features. E Jaimovich and associates have shown that slow Ca release occurs through IP3 receptors. We are currently using pharmacology and local photorelease of IP3 to explore the possibility that IP3-mediated Ca signals are altered and cause some of the phenotypic variability in familiar MH. These approaches should allow a characterization of cellular signaling defects in multiple mutations, linked to MH and other muscle diseases. *Supported by the NIH (USA) and University of Toronto.*

7. Acute Stress Induces Memory Extinction During Reconsolidation in a Water Maze

Campos, G[1].,González, P[1].,Dagnino, A[1].,[1]Laboratory of Behavioral Neurobiology, Center for Neurobiology and Brain Plasticity, Faculty of Sciences, Universidad De Valparaíso. (Sponsored by FONDECYT 1100413 Grant (Alexies Dagnino))

Introduction: Emotional arousal induced by stress increases memory consolidation. Thus, lasting memories of our important experiences are stored in the brain. Moreover, stress effects on memory reconsolidation remain unclear.
Objective: The aim of this study was to determine whether acute stress affects memory reconsolidation.
Methods: Male *Sprague-Dawley* rats were trained in a Morris water maze to study reconsolidation of spatial memory. A day after training, animals were randomly assigned to the following experimental groups:
1) control without memory reactivation,
2) control with memory reactivation,
3) stress (immersion in cold water for 15 minutes) without memory reactivation,
4) stress with memory reactivation. Twenty-four hrs later, the spatial memory was evaluated in a probe trial. Memory strength was determinate by number of annulus crossings, defined as the number of times a rat crossed an ideal circle located at the original platform position.
Results: Stressed rats that were subjected to memory reactivation showed lower number of annulus crossings in the target quadrant than that of controls.
Conclusions: Conversely to the stress effects on memory consolidation, acute stress induces extinction of specific memories during reconsolidation, only the memories that were reactivated before acute stress. This study opens a new approach to understand the stress effects on long-term memories.

8. Tanycytes GLUT2 inhibition in vivo affects feeding behavior.

Llanos, P.; Barahona, M.J.; Martínez, F.; Villagra, M.; Salgado, M.; Nualart, F.; Millán, G. y García, M.A. Depto. de Biol. Celular, Universidad de Concepción, mgarcia@udec.cl
Llanos, P[1].,[1]Biología Celular, Ciencias Biológicas, Universidad De Concepción.

Introduction:
We previously described the expression of GLUT2 in the hypothalamus, particularly in tanycytes, which are in close connection with ventromedial hypothalamic neurons known as the satiety center. We propose that inhibition of GLUT2 expression in tanycytes affects the regulation of food intake.

Methodology:
An adenovirus encoding a shRNA to inhibit GLUT2 expression (Ad-shGLUT2) was generated, which also contains the *EGFP* reporter gene. Its inhibitory capacity was confirmed in primary tanycyte cultures. Subsequently, GLUT2 expression was inhibited in rats through injection of Ad-shGLUT2 into the third ventricle using stereotactic surgery. GLUT2 expression was assessed by qRT-PCR and Western blot analyses. Feeding behavior was measured in two fast-refed cycles (24h-24h) by assessing the grams of food eaten and the period of satiety during the refed period.

Results:
At the first cycle, knockdown animals ate 75% more than the control animals, and during the second cycle, this difference increased to 100%. In parallel studies using real-time monitoring to establish the time of satiety at the beginning of each refed period, GLUT2 knockdown animals had 70% and 65% increased satiety establishment time during the first and second cycles, respectively.

Conclusion:
These results demonstrate that hypothalamic GLUT2 expression in tanycytes plays an important role in the feeding behavior of rats, affecting satiety response and thus increasing food intake.

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9. Prenatal Stress Modifies Long-Term Memory and Neuronal Activity in the Prefrontal Cortex

Negrón, I.,Neira, D.,Espinosa, N.,Fuentealba, P.,Aboitiz, F.,[1]Departamento de Psiquiatría, Escuela de Medicina, Pontificia Universidad Católica De Chile.2Centro Interdisciplinario de Neurociencia Pontificia Universidad Católica De Chile.

Prenatal stress is a risk factor for the development of psychiatric disorders in adulthood, which are characterized by increased behavioral perseverance and alterations of the functional connectivity between the prefrontal cortex (PFC) to other structures. The goal of this study was to address the impact of prenatal stress on the functional connectivity between the PFC and hippocampus (HPC), and its relationship with memory consolidation, a cognitive function dependent on the interplay between these structures. To this aim, pregnant mice were subject to restraint stress during the last week of pregnancy, and we assessed short- and long-term spatial memory under an aversive context in the adult offspring. One day after memory evaluation, we examined spontaneous neuronal activity in the PFC and HPC using *in vivo* extracellular recordings.

Prenatal stress did not affect short-term memory, and after short-term memory testing we did not find changes between groups in neuronal activity, neither in the PFC or HPC. In contrast, prenatal stress produced a persistence of long-term aversive memories that in control animals was extinguished. After long-term memory testing, prenatal stress decreased firing rate in the PFC respect to control animals. This finding was associated to a reduced proportion of PFC neurons cross-correlated with sharp-wave ripples in the HPC, and with an increased phase-locking of PFC single units to HPC delta band (1-4 Hz). Our data suggests that prenatal stress affects the functional connectivity related to memory consolidation.

10. Effects of auditory distractors in α9-nicotinic acetylcholine receptor knock-out mice trained in a visual discrimination task.

Terreros, G[1].,Madrid-Lopez, N[1].,Delano, P[1,2].,[1]Programa de Fisiologia y Biofisica, Medicina , Universidad De Chile.[2]Servicio de Otorrinolaringología, Medicina , Hospital Clinico Universidad de Chile. (Sponsored by Gonzalo Terreros Hernandez)

The auditory efferent system comprises descending pathways from the auditory cortex to the cochlea, allowing modulation of sensory processing even at the most peripheral level. This control is exerted by medial olivocochlear (MOC) neurons that make synapses with outer hair cells (OHC) through a particular inhibitory cholinergic channel formed by α9/α10 subunits. The functional role of the MOC is still under assessment, but a possible role in modulating attention to visual stimuli in the presence of auditory distractors has been proposed. Here, we trained wild type (WT) (n=6) and α9-nicotinic acetylcholine receptor knock-out mice (KO) (n=6) in a two-choice visual discrimination task. The behavioral performances were evaluated in the presence of two auditory distractors: (i) non-aversive tones and clicks and (ii) aversive white noise. We found a significant decrease in total correct responses and a significant increase in the number of omitted responses in KO mice compared to WT mice (n=6, t-test, $p<0.05$). Interestingly, this effect was greater in the basal and post training sessions without affecting performance, as expected, in the presence of auditory distractors. These results suggest that during selective attention to visual stimuli, auditory distractors could be suppressed by compensatory mechanisms in α9-nicotinic acetylcholine receptor K.O mice.

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11. Simultaneous recording of left and right hippocampus during slow-wave sleep

Villalobos, C[1].,Maldonado, P[1].,Valdes, J[1].,[1]Fisiologia, Medicina, Universidad de Chile. (Sponsored by CENEM & BNI)

Multi-neuron recording from live animals has become one of the most used techniques to study the neurophysiological basis of cognitive processes. Memory consolidation is thought to be mediated not by a single, isolated brain structure but by multiple, interconnected brain areas. The hippocampus has been accepted as the main structure leading to acquisition and consolidation of newly memory traces. The hippocampus comprises two homologus structures located on each brain hemispheres. Despite the overwhelming amount of studies supporting the role of hippocampus in memory little is known whether it works as a whole interconnected structure or not. Studies in humans and mouse models have documented right-hemisphere dominance suggesting that hippocampal spatial memory functions may be lateralized. The current work presents a modified recording device capable of obtaining high-density recordings from both hippocampi simultaneously allowing us to investigate coherence/synchrony of extracellular signals between right and left hippocampus. We successfully recorded LFPs and hippocampal spikes from both hippocampi, as revealed by histological examination. Analysis of simultaneously recorded EEG during slow-wave sleep (SWS) showed a despair degree of synchrony between LFPs. During SWS, lower frequency brain waves showed a higher degree of correlation than higher frequency brain waves (SWR, sharp-wave ripples). Within the SWR frequency spectrum (100-250Hz), we also observed variances in the coherence of events between both left and right hippocampus. We focused on sharp-wave ripples events since they are known to be mostly present during SWS and to be relevant for the memory consolidation process carried out by the hippocampus.

12. Odorant modulation of gene expression in the olfactory epithelium mediated in zebrafish (Danio rerio)

Calfun, C[1].,Dominguez, C[2].,Perez-Acle, T[2].,Whitlock, K[1].,[1]Centro Interdisciplinario de Neurociencias de Valparaíso, , Universidad De Valparaíso.[2]Computational Biology Laboratory, , Fundación Ciencia y Vida.

Zebrafish form olfactory memories of odors experienced during early development (imprinting) including the artificial odorant phenylethyl alcohol (PEA). Correlated with this olfactory memory it was shown that the transcription factor *otx2* is upregulated in the olfactory epithelium (OE) of PEA-imprinted fish, demonstrating a role for peripheral nervous system (PNS) in olfactory memory. In order to further analyze odorant modulation of the gene expression in the OE, we focused on the olfactory receptor (OR) as potential odorant regulated genes through regulation by *otx2*. We identified ORs containing OTX2 binding sites in their control regions and analyzed their expression by qPCR. Exposure to PEA resulted in the down regulation of five of the eight ORs analyzed. In order to found regulation sequences for ORs, we perform a bioinformatic analysis looking for binding site of TFs. Our preliminary data show multiple binding sites for transcription factors, including Otx2, in regions flanking ORs sequences. Interestingly, according the literature, some of these TFs can to form repressive complexes with Otx2 to control gene expression, and are located upstream the odorant-modulated ORs. Currently we are performed a high-throughput analysis in order to study the odorant effect over the zebrafish genome, including the ORs repertoire. This study supports the model where the environment can modulate gene expression during early development and suggests a role for the ORs in the memory generation.

13. Assembly of dhpr tetrad arrays requires the participation of the carboxy-terminal domain of DHPR-β1a subunit

Perez, C[1].,Thomas, M[1].,Franzini-Armstrong, C[2].,[1]Department of Anesthesia, , BWH, Harvard Medical School.[2]Department of Cell and Developmental Biology, , University of Pennsylvania. (Sponsored by Supported By NIH Grants 5K01AR054818 (to CFP) And AR055104 (to CFA) And The William F. Milton Fund/Harvard University (to CFP).)

Lack of expression of DHPR-β1a subunit in skeletal muscle cells severely compromises the DHPRs-RyR1 stereospecific association that results in the assembly of ordered DHPR tetrads arrays, leading to the disruption of depolarization-induced Ca^{2+} release signal. In our previous studies we found that aa residues 488-512 of the carboxyl-terminal (C-term) domain of DHPR β1a subunit plays a critical role in the restoration of this Ca^{2+} signal. Here we further explore the role of C-term tail of β1a in restoring the DHPR-RyR1 association responsible for Ca^{2+} signaling in muscle cells and how this correlate with their ability to restore DHPR tetrad array. Wt- and C-term truncated β1a clones were expressed in mouse β1-null myotubes and tested for their ability to 1) restore depolarization-induce Ca^{2+} release and 2) DHPR tetrad arrangement. Expression of wt-β1a showed to fully restore Ca^{2+} signaling, as well as normal DHPR tetrads arrays. Construct β14, lacking 14 aa of the C-terminal tail of β1a, still supports normal Ca^{2+} signaling and also shows normal arrangement of tetrads. However, β14 clone containing mutated residues that prevent Ca^{2+} release in muscle cells failed to restore tetrad arrays. These results suggest that 1) C-term tail of β1a plays an essential role in permitting and/or maintaining the precise positioning of four DHPRs relative to the four RyR subunits; 2) DHPR positioning in tetrads is essential to Ca^{2+} signaling and 3) the structure of the C-term tail is relevant for the role of β1a in the DHPR-RyR structural and functional relationship.

14. CoREST interacts with the SUMOE3-ligase PIASγ and is modified by SUMO2

Sáez, J[1].,Barrios, A[1]., Arredondo , C[1].,Andrés, M[1].,[1]Cellular and Molecular Biology, Biological Sciences, Pontificia Universidad Católica De Chile.

The CoREST protein is a transcription regulator which belongs to a transcriptional repressor complex that also includes the histone demethylase, LSD1 and the deacetylases HDAC1/2. CoREST was first identified as an interacting of the transcription factor REST/NRSF. Together REST/NRSF and CoREST repress the expression of neuronal genes in non-neuronal cells.Posttranslational modification by SUMO peptides is a key regulatory mechanism of transcription factors. There are 3 SUMO peptides, SUMO1, SUMO2 and SUMO3 that can be conjugated to an acceptor lysine (K) in a 3 step enzymatic reaction. The first step is the SUMO activation by SAE1/SAE2 enzymes; second is SUMO conjugation by a unique Ubc9 enzyme and third the ligation of SUMO peptides, function exerted by several SUMOE3-ligases enzymes, among them the PIAS family of proteins. SUMOylation is reversed by the SUMO peptidases or SENP proteins. We detected the interaction between CoREST and the SUMO-E3-ligase, PIASγ,through yeast two hybrid assays. Then, we evaluated the interaction in mammalian cell lines overexpressing recombinant Myc-CoREST and HA-PIASγ. We observed the interaction between CoREST and PIASγby co-immunoprecipitation assays of whole HEK293 cell extracts. In order to learn if PIASγ SUMOylate CoREST we overexpressed SUMO1, SUMO2 or SUMO3 along with PIASγ in cell lines, and performed western blot assays. A slower migrating band of about 100-130 kDa was observed when CoREST is overexpressed along with SUMO2. This band was stronger and better defined in the presence of PIASγ, suggesting that PIASγ is involved in the SUMOylation of CoREST by SUMO2.

15. Non-apoptotic cell death mechanisms during hereditary retinal degeneration

Paquet-Durand, F[1].,Trifunovic, D[1].,Sahaboglu, A[1].,Kranz, K[2].,Farinelli, P[1,6].,Michalakis, S[3].,Cottet, S[4].,Euler, T[5].,Ekstrom, P[6].,Ueffing, M[1].,Arango-Gonzalez, B[1].,[1]Institute for Ophthalmic Research, Universitätsklinikum Tuebingen, University of Tuebingen.[2]Neurobiology, , University of Oldenburg.[3]Center for Drug Research, , Ludwig-Maximilians University Munich.[4]IRO, Sion, , Institute for Research in Ophthalmology.[5]Centre for Integrative Neuroscience, , University of Tuebingen.[6]Ophthalmology, Clinical Sciences, Lund, University of Lund. (Sponsored by Kerstan Foundation; EU (DRUGSFORD: Health-F2-2012-304963; PANOPTES: NMP4-SL-2010-246180), DFG (PA1751/4-1), BMBF (HOPE2-FKZ01GM1108A).)

Cell death in neurodegenerative diseases is often thought to be governed by apoptosis. However, an increasing body of evidence suggests the activity of alternative cell death mechanisms. Here, we show that in hereditary forms of retinal neurodegeneration, apoptosis play only a minor role and that, instead, non-apoptotic degenerative processes are of major importance.

We studied markers for apoptosis and non-apoptotic cell death in ten different animal models for inherited retinal degeneration. These included human homologous models for autosomal dominant (P23H and S334ter transgenic rats) and autosomal recessive Retinitis Pigmentosa (rd1, rd2, rd10, Cngb1 KO, Rho KO mice), Lebers Congenital Amaurosis (RPE65 KO), and achromatopsia (cpfl1, Cnga3 KO mice).

We found that markers for non-apoptotic cell death, namely activation of calpain, HDAC and PARP, accumulation of cGMP and PAR, were increased during photoreceptor degeneration in all analyzed animal models. With the exception of the transgenic S334ter rat, all the studied animal models shared an astonishing scarcity or absence of markers for apoptosis (BAX upregulation and translocation onto mitochondria, cytochrome c leakage and activation of caspases-3 and -9).

Our work conclusively demonstrates the importance of non-apoptotic mechanisms in inherited retinal degeneration, and is directly relevant for potential therapeutic approaches. For most mutations, targeting cGMP signaling, epigenetic processes and calpain activity, appears far more promising than anti-apoptotic strategies. Our study explains the failure of past anti-apoptotic therapeutic attempts and at the same time provides a rational basis for novel neuroprotective interventions in inherited retinal degeneration.

16. Binding modes of SB-206553, a 5-HT2B/2C antagonist and a positive allosteric modulator of alpha7 nicotinic acetylcholine receptors

Reyes-Parada, M[1].,Moller, P[2].,Contreras, J[3].,Rojas, C[3].,Alzate-Morales, J[3].,Iturriaga-Vásquez, P[4].,[1]School of Medicine, Medical Sciences, University of Santiago de Chile.[2]Centro de Bioinformática y Simulación Molecular (CBSM), , Escuela de Ingeniería en Bioinformática, Universidad de Talca, Chile.[3]Centro de Bioinformática y Simulación Molecular (CBSM), Escuela de Ingeniería en Bioinformática , Universidad de Talca.[4]Departamento de Química, Facultad de Ciencias, Universidad de Chile. (Sponsored by Fondecyt Grants 1130185 (to MR-P), 11100177 (to JA-M) And 1100542 (to PI-V).)

Brain serotonin (5-HT) and nicotinic acetylcholine receptors (nAChRs) are implicated in several physiological processes and pathological conditions.

On the other hand, evidence from systems biology indicates that promiscuous drugs, *i.e.* those that act simultaneously at various protein targets, are clinically better in terms of efficacy, than those that act in a more selective fashion. However, the rational design of promiscuous compounds is a difficult task, particularly when the drugs are aimed to act at receptors with diverse structure, function and endogenous ligand.

In the present work, we determined the putative binding modes of SB-206553, a drug originally described as a 5-HT2B/2C antagonist and more recently as a positive allosteric modulator at α7-nAChR. To this end, we employed the crystal structures of the 5-HT2B receptor and the Ach binding protein, as templates to build homology models of the 5-HT2C receptor and the α7-nAChR,

probable binding modes of the drug and analyzed the main molecular interactions involved. Such an analysis serves to define the structural and molecular aspects underlying the high affinity showed by SB-206553, and will aid the rational design of novel compounds acting at both types of receptors.

17. Cambios transitorios en banda alfa reflejan alteración de la conectividad funcional en desórdenes severos de conciencia.

Rivera, G[1].,Egaña, J[1].,Díaz, V[2].,Maldonado, P[1].,[1]Fisiología, Medicina, Universidad De Chile.[2]Neurología, Medicina, Universidad De Chile. (Sponsored by Conicyt. Biomedical Neuroscience Institute)

El estado vegetativo es un desórden severo de conciencia (DSC) caracterizado por ausencia de respuestas consistentes, reproducibles y voluntarias frente a estímulos. Reportes anteriores muestran patrones variables de actividad electroencefalográfica evocada en respuesta a estímulos sensoriales. Esto se ha relacionado con diferentes capacidades para procesar estímulos externos debido a la indemnidad de regiones cerebrales específicas y/o sus conexiones con diferentes áreas cortico-subcorticales. Se propone que la injuria de los núcleos intralaminares talámicos y la pérdida de conexiones cortico-corticales son claves en la pérdida de la integración funcional de la actividad cortical que da cuenta de este estado. Una forma mediante la cual estas conexiones cortico-subcorticales contribuyen a la conectividad funcional es la modulación de la actividad en banda alfa frente a la presentación de estímulos sensoriales. Así, proponemos que cambios transitorios de alfa en respuesta a estos estímulos reflejan diferentes capacidades para integrar la actividad cortical. Utilizando tres pruebas oddball auditivas, analizamos los cambios en alfa (poder espectral/sincronía de fase) en un grupo de sujetos con DSC en respuesta a la presentación de estímulos infrecuentes de diferente complejidad. Nuestros resultados preliminares muestran que estos estímulos son capaces de inducir diferentes patrones de amplitud/fase en alfa, lo que está en línea con la evidencia actual y nuestra hipótesis que plantea que la pérdida de conectividad funcional determina esta condición, evidenciando distintas capacidades de procesamiento sensorial.

18. Chloride co-Transporter NKCC1 involvement in reduced excitability in an animal model of Schizophrenia

LORCA, E[1].,LOYOLA, S[1].,LARA, M[1].,PINO, D[1].,**Rojas, P[1]**.,[1]Biologia, Química y Biología, Universidad De Santiago De Chile.

Schizophrenia is one of the most common neurological diseases in human population, however there is no common hypothesis to explaining all its features. This is due in part to a inability of animal models to reproduce all the features observed in human patients. In this work we use Ketamine administration to generate a schizophrenia and psychosis-like animal model. Adult animals (7 weeks old) were injected intraperitoneally with of Ketamine (30 mgr/kgr) for five days. After two days locomotor activity showed to be significative higher than in control animals, as showed in other animal models. Because Hippocampus is one of the brain regions that showed alteration in this disease, Extracellular field recordings of the CA3-CA1 synapse were performed to evaluate the circuit excitability. In addition, Ketamine treated animals showed a decrease in the Input-Output relationship of Excitatory Post Synaptic Potentials (EPSP). In order to test the hypothesis that a decrease in inhibition can explain part of this pathology, we are assaying the function of the chloride co-transporter NKCC1, which modifies the excitation to inhibition balance due to GABA release. In summary, we validated the ketamine model by using both behavioral and electrophysiological methods and propose its usefullnes for studied of schizophrenia and psichosis-like disorders. FONDECYT 9403011, INNOVA-CORFO 1D12-xx078.

19. Prenatal fluoxetine alters the central chemoreception

Bravo, K[1].,Eugenín , J[1].,Llona , I[1].,[1]Biología, Facultad de Química y Biología., Universidad De Santiago De Chile. (Sponsored by Fondecyt 1090375, 1130874 And CONICYT Fellow.)

Altered level of serotonin (5-HT) during prenatal period generates respiratory abnormalities. Fluoxetine (specific 5-HT reuptake inhibitor) used during pregnancy has respiratory consequences in neonatal mice at P8 (P, postnatal age): the ventilatory response to hypercarbia is diminished. In the present work, we investigated if activation of chemosensitive nucleus of the brainstem is altered in fluoxetine exposed animals. Osmotic minipumps were implanted to CF-1 dams on day 5 of pregnancy to deliver fluoxetine (7 mg Kg-1 days-1) for up to 28 days. Mice without minipumps were used as control. The neural activation in response to hipercarbia was determinate by immunohistochemistry of c-Fos. In the nucleus of the solitary tract the number of activated neurons diminished in fluoxetine exposed animals. In contrast, no differences respect to control animals was observed in the raphe and hypoglossal nucleus. The number of serotoninergic neurons in raphe nucleus, determinated by immunohistochemistry of *Tryptophan hydroxylase* (*key enzyme in synthesis* of 5-HT), is not significantly different in neonates prenatally exposed to fluoxetine respect to control. Our results suggest that fluoxetine exposure during perinatal period alters development of central chemoreception in brainstem.

20. Nicotine acetylcholine receptor α4β2 mediates the increase in nicotinic cholinergic contribution to central respiratory chemoreception in mice neonates after prenatal nicotine-exposure.

Campos, M[1].,Eugenín, J[1].,[1]Biología, Química y Biología, Universidad De Santiago De Chile. (Sponsored by Jaime Eugenín León)

Prenatal nicotine exposure switches the cholinergic contribution to central chemoreception from muscarinic receptor- to nicotinic receptor-based mechanisms. In order to evaluate which nicotine acethylcholine receptor is involved, changes in fictive respiration induced by acidification of the superfusion medium (from pH 7.4-7.2) were recorded from brainstem-spinal cord preparations in the absence and presence of dihydro-β-erythroidine hydrobromide (DhβE), an alpha4 nAChR subtype antagonist, and methyllycaconitine citrate (MLA), an alpha7 nAChR subtype antagonist. DHβE but not MLA modified the response of fictive respiration induced by acidification in “en bloc” preparation of male, but not females neonatal mice exposed to prenatal nicotine (*p<0,5 Wilcoxon test). Our results indicate that nAChR subtype α4β2 is the main candidate to mediate the change from muscarinic to nicotinic cholinergic contributions in the chemosensory response observed in neonatal mice exposed to prenatal nicotine. Furthermore, our results suggest that nAChR subtype α4β2 participation occurs in a gender-dependent manner.

21. Humoral characterization of patients with multiple sclerosis diagnosis

Fundora, H[1].,Dorta Contreras, A[2].,[1]Inmunology, National Institute of Hygiene Epidemiology and Microbiology, Havana Medical University.[2]Labcel, Miguel Enriquez, Havana Medical University.

Introduction: The golden rule for multiple sclerosis diagnosis is the clinic diagnosis by expert`s criteria but the laboratory data are very important.

Objective: To determine the presence of immunoglobulin intrathecal synthesis, IgG oligoclonals bands and C3c fraction of complement system. To evaluate de sensibility of IgG oligloconals bands for multiple sclerosis diagnosis. To evaluate the relationship between intrathecal activation of complement system and multiple sclerosis evolution. To describe the intrathecal synthesis of neurotropic viruses antibody.

Methods: Albumin, IgG, IgA, IgM and C3c concentration in sera end cerebrospinal fluid were obtain by radial simple immunodiffusion. The oligoclonal bands of IgG were obtained by isoelectric focalization in agarosa gel. The MRZ reaction was determined by the antibodies index calculated starting from the concentrations of specific antibodies for each virus (measles, rubella and chicken pox-zoster).

Results and conclusions: In patientwith multiple sclerosis exists an intratecal immune response to expense of IgM with an important activation of the complement system. The sensibility of the detection of IgG oligoclonals bands for the positive diagnosis of multiple sclerosis ratifies this determination like a qualitative laboratory test of important value. The intensity of complement system intratecal activation is independent of the evolution time. The diagnosis possibility for MRZ positive reaction behaves in a different way in our country with regard to Europe.

22. Seasonal distribution and evolving forms of multiple sclerosis patients diagnosed from april 2004 to November 2007

Fundora, H[1].,Dorta Contreras, A[2].,Padilla Docal , B[2].,[1]Immunology, National Institute of Hygiene Epidemiology and Microbiology, Havana Medical University.[2]Labcel , Miguel Enriquez, Havana Medical University.

Introduction: Multiple sclerosis was first reported in Cuba in 1965. The most frequent appearance is observed in the first six months of the year.

Objective: To determine the seasonal distribution and evolving forms of multiple sclerosis patients diagnosed with the disease between April 2004 and November 2007.

Methods: Twenty-one patients with suspected multiple sclerosis and 42 outbreaks were studied. Patients were classified according to Lublin and Revingold´s criteria for clinical forms and according to McDonald.

Results and conclusions: Most patients were classified in outbreak and remission and only two patients classified as primary-progressive multiple sclerosis. The higher number of outbreaks occurred in the first two quarters of the year. It is recommended to study further weather variables that may be related to the emergence of these outbreaks in our environment.

23. Mannan binding lectin dynamic in cerebrospinal fluid.

Padilla Docal, B[1].,Dorta Contreras, A[1].,**Fundora, H[2]**., Reiber , H[3].,Christian , J[4]., [1]Labcel, Miguel Enriquez, Havana Medical University . [2]Immunology, National Institute of Hygiene Epidemiology and Microbiology, Havana Medical University. [3]Neurochemistry Laboratory, Neurochemistry Laboratory, University Göttingen. [4]Department of Biomedicine, Department of Biomedicine, University of Aarhus.

Background: Mannan-binding lectin (MBL), a protein of the innate immune response is attracting increasing clinical interest, in particularly in relation to its deficiency. Due to its involvement in brain diseases, identifying the source of MBL in CSF is important. Analysis of cerebrospinal fluid (CSF) can provide data that discriminates between blood-, brain-, and leptomeninges-derived proteins.

Methods: MBL was assayed in samples of CSF and serum with an ELISA, coated with anti MBL antibodies. Routine parameters such as albumin-, immunoglobulin- CSF/serum quotients, oligoclonal IgG and cell count were used to characterize the patient groups.

Results: MBL concentration in CSF was at least five-fold higher than expected for a molecular-size dependent passage from blood. In a QIgM/QAlb quotient diagram (Reibergram) 9/13 cases showed an intrathecal fraction in some cases over 80% of total CSF MBL concentration. The smaller inter-individual variation of MBL concentrations in CSF of the control group (CV = 66%) compared to the MBL concentrations in serum (CV = 146%) indicate an independent source of MBL in CSF.

Conclusions: MBL in CSF is predominantly brain-derived and all results pointed to the leptomeningeal cells as the source of the protein. This recognition of MBL in brain cells opens a new field of discussion about the function of the innate immune response in CNS in cases of acute and chronic neurological diseases.

24. Respiratory rythm modulation by substance P is altered by prenatal nicotine exposure in neonatal mice

Olivares, M[1].,Beltran-Castillo, S[1].,Eugenín, J[1].,[1]Biología, Química y Biología, Universidad De Santiago De Chile.

The ventral respiratory column (VRC) is the main neural network in charge of generating the respiratory rhythm. An important modulation of the respiratory rhythm is exerted by Subtance P (SP) through neurokinin receptor 1 (NK1R). In fact, the destruction of NK1R-expressing neurons into VRC using SP/saporin conjugated leads to pathological breathing. We previously reported that prenatal exposure to nicotine decreases neonatal basal ventilation and central chemoreception, in part due to serotoninergic raphé neurons hypoactivity. Because of the large percentage of serotoninergic raphe neurons also contain SP, in this work we evaluated the effect of prenatal nicotine exposure upon the SP-induced changes of fictive respiration. An osmotic minipump delivering nicotine 60mg•Kg^{-1} was implanted s.c. into pregnant CF1 mice. Fictive respiration was recorded with suction electrodes from C4-C5 ventral roots in P0-P4 *"en bloc"* and directly over VRC neurons "slice" preparation. Superfusion with aCSF (30mM D-glucose, O2/CO2=95%/5%, pH 7.4, 28ºC) was followed by aCSF containing either SP (*en bloc*) or SP injected directly on VRC (slice). In both control and nicotine exposed mice, SP evoked a reversible dose-dependent increase of the respiratory frequency. The maximal frequency induced by SP (3µM en bloc, 1µM slice) was reduced from 273±30.8 % (n=5, control) to 177.1±30.4% (n=4, nicotine) and 318±3.2 (n=3, control) to 178±46.5 (n=2, nicotine) ($p<0.05$, Mann-Whitney test) respectively.

We concluded that prenatal nicotine reduces greatly the exogenously applied SP responsiveness, while a down-regulation of NK1R is presented as an open question. Supported by Fondecyt 1130874

25. A mutation in the S6 transmembrane helix uncouples voltage sensor activation to BK channel gating

Carrasquel-Ursulaez, W[1]., Contreras, G[2].,Sepulveda, R[3].,Aguayo, D[3].,González-Nilo, F[3].,González, C[2].,Latorre, R[2].,[1]Centro Interdisciplinario de Neurociencias de Valparaíso, , Universidad De Valparaíso.[2]CINV, , Universidad De Valparaíso.[3]CBIB, , Universidad Andrés Bello.

Large conductance Ca^{2+} and voltage-activated K^+ channels (BK) opening is mediated by a cross-talk of modular voltage and Ca^{2+} sensors. BK open probability is enhanced by increasing cytosolic Ca^{2+} concentration and/or depolarization. These stimuli activate sensors that are coupled by allosteric interactions to channel gating. Here, we report a point mutation of a phenylalanine (F380A) located in the S6 transmembrane helix that produces a large rightward shift of the open probability-voltage curve with minor changes in gating current. Interpretation of our results using an allosteric model, suggests that the F380A mutation modifies the channel closed-open equilibrium and uncouples voltage sensors from channel opening. Based on these functional studies, we propose a structural model that sheds light on the molecular nature of the coupling between the voltage sensor activation and pore opening. Moreover, the mutation F380A could be used for detailed voltage sensor studies in the presence of permeant cations.

26. External architecture of the large-conductance Ca^{2+} and voltage-activated K^+ (BK_{Ca}) channel revealed by a spectroscopic ruler.

Castillo, J[1].,Sanchez-Rodriguez, J[2].,Hyde, H[2].,Zaelzer, C[2].,Aguayo, D[1,3].,Kent, S[4].,Gonzalez-Nilo, F[1,3]., Bezanilla, F[1,2].,Latorre, R[1].,[1]Centro Interdisciplinario de Neurociencias de Valparaiso, , Universidad de Valparaiso.[2]Department of Biochemistry and Molecular Biology, , University of Chicago.[3]Center for Bioinformatics and Integrative Biology, , Universidad Andres Bello.[4]Department of Chemistry, , University of Chicago.

BKCa channels are involved in a large variety of physiological processes and regulatory β subunits are one of the mechanisms responsible for creating BKCa channel diversity, which is fundamental to the adequate function of many tissues. Despite the proven importance of this channel, little is known about its detailed structure. Here we disclose the external architectural intimacies of BKCa channels using lanthanide-based resonance energy transfer (LRET) as a molecular ruler to measure intra- and intermolecular distances. We introduced a genetically encoded lanthanide binding tag at different positions in the neighborhood of the first three (S0, S1, and S2) transmembrane helices of the α subunit and TM1 and TM2 helices of the β1 subunit. A fluorescent molecule of BODIPY-FL linked to a scorpion toxin, iberiotoxin (IBTX-BODIPY), was used as an acceptor for the LRET interaction with bound Tb^{3+}. These functional LBT-BKCa constructs were expressed in *Xenopus laevis* oocytes to carry out LRET experiments. Sensitized emission (SE) recordings from different LBT-BKCa positions had different kinetics indicating different relative positions of the donor for each construct. We analyzed SE records with a novel method [Hyde et al, *Structure* 20:1629 (2012)] that determines the position of LBT-tagged sites of BKCa to obtain a structural map of the transmembrane segments of a and β1 subunits. The methodology presented here gives us the first glimpses into the functional BKCa channel's external surface structure with and without the β1 subunit. Supported by Fondecyt grant 1110430 and NIH grants U54GM087519 and GM030376. CINV is a Scientific Millennium Institute.

27. Probing S4 re-arrangement during gating using optical tools

Kubota, T[1].,Lacroix, J[1].,Bezanilla, F[1]., **Correa, A[1].**,[1]Biochemistry and Molecular Biology, Biological Sciences Division, The University of Chicago.

Voltage Sensor Domains regulate ion channels and enzymes by undergoing conformational changes in response to changes in membrane potential. Voltage sensitivity relies on the transfer of gating charges located in the fourth transmembrane helix (S4) across the electric field. Gating charge transfer is believed to be associated with changes in the secondary structure of the S4 between alpha- and 3_{10}-helix, yet this conformational transition has not been detected yet. Because such transitions are expected to extend the distance between the two ends of the S4, we sought to identify changes in the distance between genetically-encoded spectroscopic probes (Lanthanide Binding Tag and mCherry) placed at the two ends of the S4 of Voltage-Sensitive Phosphatase (Ci-VSP) using LRET. We show that the S4 of Ci-VSP does not exhibit long-lived voltage-dependent changes in length, and that it most likely is alpha-helical. Short-lived changes of S4 length in this construct could not be detected using time-resolved FRET, indicating the unlikeliness of transient alpha to 3_{10} transitions. As the x-ray structure of the sodium channel from *Arcobactor butzlen*, NavAb, displays its whole S4 in a 3_{10} conformation, we substituted the native S4 segment of Ci-VSP by the NavAb S4. Our LRET results with the chimera show that the NavAb S4 most likely is alpha-helical and does not undergo long-lived alpha-3_{10} transitions. As gating currents were measured simultaneously, our results show that in these chimeric constructs the voltage-sensor operation does not require alpha to 3_{10} transitions of the whole S4. Supported by GM030376 and U54GM087519.

28. Turning a small into large conductance K-channel: How far can we go?

Diaz-Franulic, I[1].,Navarro, N[2].,Naranjo, D[2].,[1]Programa de Doctorado En Neurociencia, Facultad de Ciencias, Universidad De Valparaíso.[2]Departamento de Neurociencia, Facultad de Ciencias, CINV / Universidad De Valparaíso. (Sponsored by David Naranjo)

Potassium channels are membrane proteins that allow passage of K+ ions across the hydrophobic core of the membrane. The extremely conserved sequence in the residues lining the selectivity filter (TTVGYGD) discriminate between ions having similar radii. Despite of this conservation, closely related potassium channels display differences of up to two orders of magnitude in their K+ ion transport rates. The substitution of Pro475 by Asp or Gln increases Shaker K-channel unitary conductance, in 100 mM K+, by 8- and 4-fold, respectively. To determine how these point mutations affect channel conductance so dramatically we introduced additional negatively charged residues a) to positions aligning with the “glutamate rings” that electrostatically control conductance in BK channels (positions 476 and 479 in Shaker) and b) to the inner cavity to test how pore-occupancy affects the single channel conductance. Then, we measured the single channel conductance in a wide range of symmetrical [K+]. We also estimated the mutant’s internal radii of capture by adding sucrose to increase the viscosity of the internal solution. Here we show that a) extra Asp residues at the inner cavity increase Shaker single channel conductance, b) as in MthK, but unlike BK channels, electrostatics at the inner mouth is very important for conductance, and c) replacement of Pro475 by Asp/Gln did not modify pore dimensions. We suggest that conduction mechanisms are preserved in large and small conductance K-channels where pore occupancy increases single channel conductance, but pore dimensions set a smaller upper limit of K+-transport rate of small conductance K-channels.

29. Bisindolylmaleimide and calphostin C, two PKC inhibitors increase the activity of connexin-43 hemichannels by oxidation.

Vega, J[1,2].,Sáez, J[1].,[1]Departamento de Fisiologia, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile.[2]Labopratorio de Fisiología Experimental, Instituto Antofagasta, Universidad De Antofagasta.

Introduction: Hemichannels are located at the cellular surface of most mammalian cells. They are formed by connexin or pannexin proteins and regulated by phosphorylation/desphosphorylation mechanism. Our objective was to determine the regulation of connexin-43 (Cx43) hemichannels by protein kinase C (PKC) inhibitors.

Methods: To determine changes in hemichannel activity we measured dye uptake (ethidium) in real time. The HeLa cells stably transfected with Cx43 or Cx43-mutated in an intracellular cysteine (Cys-less Cx43) were exposed to a PKC inhibitors such calphostin C, bisindolylmaleimide, and chelerythrine. In some experiments, cells were pre-incubated with a reducing agent dithiothreitol (DTT).

Results: It was found that incubation with calphostin C or bisindolylmaleimide increased dye uptake, an effect that was blocked by lanthanum ions (La3+). However, chelerytrine did not significantly affect the dye uptake. Preincubation with DTT drastically reduced (~50%) the dye uptake induced by PKC inhibitors, suggesting the involvement of reactive oxygen derived species (ROS). Also, Cys-less Cx43 reduced ~50% the effect induced by bisindolylmaleimide and ~90% by calphostin C. Discussion: PKC inhibitors induce a rapid increase in plasma membrane permeability mediated by Cx43 hemichannels, a phenomenon that involved desphosphorylation and oxidation of Cx43.

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30. K^+ translocation by the giant squid axon Na^+/K^+ ATPase

Castillo, J[1].,Basilio, D[1].,Latorre, R[1].,Bezanilla, F[1].,Holmgren, M[1].,[1]Laboratorio de Fisiologia Celular, Montemar, , Universidad De Chile.

The Na^+/K^+ pump is a membrane protein which plays a fundamental role in maintaining the Na^+ and K^+ electrochemical gradients in animal cells. When internal and external Na^+ is absent the pump can only undergo K^+ translocation reactions. At equilibrium, the distribution of the different protein conformations depends on the rate constants of each step leading to K^+binding and unbinding. If some of these rate constants are voltage-dependent, sudden changes in membrane electric potential will shift the equilibrium. In those translocation reactions K^+ has to travel a fraction of the membrane electric field generating a transient current signal. Here K^+pump currents were measured under voltage clamp conditions using the giant axon of the Humboldt squid, which due to its large diameter (1 -1.5 mm) allows the detection of these kind of measurements. By using H_2DTG, a reversible inhibitor of the squid Na^+/K^+ pump, we were able to obtain H_2DTG-sensitive transient currents in response to voltage jumps in K^+/K^+ conditions. Kinetics of these transient currents shows two main components. These two kinetic components are uncoupled and the origin of the fast component remains uncertain. However, charge displacement distribution and the slow rate constant show a clear dependence on the K^+ external concentration revealing that the entrance of the K^+ to the Na^+/K^+ pump from the external side is a voltage-dependent step.

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31. Differences in excitability of ventricular myocytes from neonatal and adult rats

Oshiyama, N[1].,Bassani, J[1,2].,Bassani, R[2].,[1]Department of Biomedical Engineering, School of Electrical and Computer Engineering, University of Campinas.[2]Center for Biomedical Engineering, , University of Campinas.

Background: Although differences have been shown in the expression pattern of Na^+ channel isoforms in neonatal and adult ventricular myocardium, possible differences in cell excitability have not been investigated yet. Here we show developmental changes in Na^+ current (I_{Na}) associated with altered cardiomyocyte excitability.

Methods: Membrane potential (E_m) and I_{Na} were measured in ventricular myocytes from neonatal (5 days) and adult (3-6 months) Wistar rats with the whole-cell patch-clamp configuration during stimulation with current (4 ms duration) and voltage pulses (-70 to +70 mV, 200 ms duration, V_{hold}= -80 mV; Ca^{2+} and K^+ currents were blocked), respectively. The I_{Na} steady-state activation curve (i.e., the fractional Na^+ conductance vs. E_m) was described by the Boltzmann equation. Data were compared with Student's t test.

Results: Compared to adults, the I_{Na} activation curve in neonates was ~2 times steeper, but shifted to the right by ~10 mV ($p< 0.001$). This change was associated with increase in threshold depolarization (29.9 ± 4.4 vs. 17.0 ± 2.8 mV, $p< 0.03$) and in the stimulus intensity required to trigger an action potential (AP) (15.6 ± 4.2 vs. 5.8 ± 0.4 pA/nF, $p< 0.04$), although diastolic E_m was not significantly different. Neonates also exhibited lower membrane depolarization rate (dV/dt_{max}) during the phase zero of the AP (43.8 ± 3.3 vs. 115.0 ± 19.7 V/s, $p< 0.01$).

Conclusion: Neonatal cardiomyocytes are less excitable than adult cells, which can be explained by differences in Na^+ channel activation properties, possibly due to altered Na^+ channel isoforms expression during development.

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32. CaMKIIN-mediated modulation of synaptic function in the olfactory cortex.

Fernández, G[1].,Sanhueza, M[1].,Palma, V[1].,[1]Departamento de Biología, Facultad de Ciencias, Universidad De Chile.

CaMKII is the most abundant protein in the central synapses and is crucial for learning and memory processes. Despite having been widely studied, little is known about its regulation. CaMKIIN is an endogenous protein that has been shown to specifically inhibit CaMKII activity. The development of peptides based on the inhibitory region of CaMKIIN (CN peptides) has revealed a possible function of this protein in the regulation of synaptic strength. Experiments in hippocampal slices have shown that CN peptides persistently reduce synaptic transmission through an effect on postsynaptic CaMKII. The piriform olfactory cortex is an emergent sensory cortex model with high endogenous levels of CaMKIIN. There are two main types of excitatory neurons in this cortex, pyramidal and semilunar cells, with specific morphologies and connectivity patterns that make them a valuable system to study CN effects on synapses with very different but well-described properties. We found that, as in the hipocampal CA1, CN peptide decrease basal synaptic transmission in the piriform cortex. Paired-pulse experiments in whole-cell configuration indicate that transient incubation of slices with CN peptide causes persistent presynaptic modifications that are different for the two cell types. On the other hand, preliminary recordings of miniature excitatory postsynaptic currents (mEPSC) suggest that these incubations can also affect the distribution of the mEPSC amplitudes in a cell-specific way. These two observations imply that CaMKIIN could modulate synaptic function in piriform cortex by affecting the activity of both pre- and postsynaptic CaMKII.

33. Regulation of M-current and neuronal excitability by the neuronal-specific glucocorticoid-regulated kinase 1.1

Miranda, P[1].,Gonzalez Montelongo, R[2].,Armas Capote, N[3].,Lamas, J[4].,Alvarez De La Rosa, D[1].,**Giraldez, T[5]**.,[1]Physiology, , University of La Laguna.[2]Physiology-Research Unit HUNSC, , University of La Laguna.[3]Research Unit-HUNSC, , University of La Laguna.[4]Physiology, , University of Vigo.[5]University Hospital NS Candelaria-Research Unit, , University of La Laguna.

The M-current formed by tetramerization of Kv7.2 and Kv7.3 subunits is a neuronal voltage gated K^+ conductance that controls resting membrane potential and cell excitability. In *Xenopus laevis* oocytes, an increase in Kv7.2/3 function by the serum- and glucocorticoid-regulated kinase 1 (SGK1) has been reported. We now show that the neuronal isoform of this kinase (SGK1.1), with distinct subcellular localization and modulation, up-regulates the Kv7.2/3 current in *Xenopus* oocytes and mammalian HEK293 cells. In contrast to the ubiquitously expressed SGK1, the neuronal isoform SGK1.1 interacts with PIP_2 and is distinctly localized to the plasma membrane. An SGK1.1 mutant with disrupted PIP_2 binding sites produced no effect on Kv7.2/3 current amplitude. SGK1.1 failed to modify the voltage dependence of activation and did not change activation or deactivation kinetics of Kv7.2/3 channels. These results suggest that the kinase increases channel membrane abundance, which was confirmed with flow cytometry assays. To evaluate the effect of the kinase in neuronal excitability we generated a transgenic mouse (Tg.sgk) expressing a constitutively active form of SGK1.1 (S515D). Superior cervical ganglion (SCG) neurons isolated from Tg.sgk mice showed a significant increase in M-current levels, paralleled by reduced excitability and more negative resting potentials. SGK1.1 effect on M-current in Tg.sgk SCG neurons was abrogated after muscarinic receptor activation. Transgenic mice with increased SGK1.1 activity also showed diminished sensitivity to kainic acid-induced seizures. Altogether, our results unveil a novel role of SGK1.1 as a physiological regulator of the M-current and neuronal excitability.

34. Ezh1 and Ezh2 regulate dendritic arborization through differential control of psd-95 gene expression in hippocampal neurons.

Henriquez, B[1,2].,Bustos, F[2,3].,Aguilar, R[2,3].,Montecino, M[2,3].,Van Zundert, B[2].,[1]Biology and Chemistry Department, Faculty of Science, San Sebastian University.[2]Center for Biomedical Research, Faculty of Biological Sciences and Faculty of Medicine, Andres Bello University.[3]FONDAP Center for Genome Regulation, , Andres Bello University. (Sponsored by Postdoctoral FONDECYT 3110138 (BH), CONICYT 24110099 (FB), CONICYT 24120931 (RA), FONDECYT 1101012 (BvZ), FONDAP 15090007 (MM).)

Polycomb-Group proteins (PcG) Ezh1 and Ezh2 are key epigenetic regulators that can silence genes through H3K27me3. Little is known about the role of PcG in controlling neuronal plasticity associated genes. We analyzed the expression of PcG proteins during hippocampal development, their contribution in transcriptional regulation of the PSD-95 gene, a critical neuron plasticity regulator, and dendritic arborization. mRNA and protein levels were analyzed by qRT-PCR and western-blot, respectively, at distinct development stages: rat hippocampal tissue (from E18 to P30) and hippocampal cultures (from 2 to 20 DIV). Over-expression and knock-down assays were realized using shRNA-lentiviral particles, electroporation by AMAXA® and transfection by Neuromag-Magnetofection™. Chromatin Immunoprecipitation (ChIP) assays were realized in hippocampal tissue and quantified by qPCR. We report that Ezh1 and Ezh2 are expressed in neurons and astrocytes: Ezh2 expression is high during development and then declines, whereas Ezh1 expression persists. Reduced PSD-95 gene transcription correlates with an enrichment of Ezh2 at the PSD-95 gene promoter, while depletion of Ezh2 leads to increased transcription of the PSD-95 gene. Interestingly, increased PSD-95 gene transcription is accompanied by the presence of Ezh1 and RNA Polymerase II complexes at the PSD-95 gene promoter, while knock-down of Ezh1 protein reduces PSD-95 transcription in mature neurons. Analyzing the dendritic architecture revealed that, Ezh2 knock-down produces decreases in the number of secondary and tertiary branches. Our findings indicate that Ezh1 and Ezh2 have antagonistic roles on the PSD-95 gene promoter during hippocampal neuron maturation, suggesting an important role for PcG proteins in developmental-dependent neuronal plasticity.

35. A single N terminus gate in a Voltage-activated potassium channel acts on each of the four subunits in the pore.

Holmgren, M[1].,Venkataraman, G[2].,Srikumar, D[3].,[1]NINDS, Investigator, NIH.[2]NINDS, Student, NIH.[3]NINDS, Assistant, NIH.

Voltage-activated potassium (K_V) channels are potassium selective integral membrane proteins formed by the assembly of four homologous subunits. In response to a membrane depolarization, K_V channels open allowing K^+ to permeate. In some members of is the site of action in the pore specific to the subunit to which the bound N terminus belongs? s, sustain depolarization leads to inactivation caused by an N terminus gate. In shaker K_V channels, the first ~20 amino acids at the NH_2 terminus of the protein are essential in enabling it to act as a gate. The tip of the NH_2 terminus interacts with residues in the intracellular cavity of K_V channels, blocking the permeation of K^+. By nature of their tetrameric architecture, inactivating K_V channels have four N terminus gates and a set of four sites of action in the intracellular cavity. Yet, N-type inactivation is produced by the binding of only one N terminus gate. Is the site of action in the pore specific to the subunit to which the bound N terminus belongs? To address this question we have developed a concatemer shaker K_V channel and studied the energetics of inactivation of a natural RNA editing site in the intracellular cavity that substantially disrupt inactivation when all subunits are edited. Our results suggest that the single inactivation gate has all the freedom to bind in the intracellular cavity of the channel.

36. Synaptotagmin-C2B Domain and Complexin Reduce the Asynchronous Activation of the Evoked Release

Quiroz-Manriquez , E[1]., **Jorquera, R[1].**, [1]Neuroscience Department, School of Medicine, Universidad Central del Caribe, Bayamón, PR 00956, USA.

Nerve-evoked vesicle fusion has been kinetically separated in two components, synchronous and asynchronous. The Ca^{2+}-sensor Synaptotagmin binds the SNARE-complex and drive fusion. Complexin co-evolve with Synaptotagmin, bind the complex and speed the exocytosis. Synaptotagmin controls the Ca^{2+}-dependency of quantal release and synchronicity where Complexin cooperate in this function. Neuronal disruption of Synaptotagmin leaves an asynchronous release with a slight Ca^{2+}-cooperativity suggesting the presence of an asynchronous Ca^{2+}-sensor. The asynchronous sensor is thought to mediate the asynchronous releases observed during burst of activity, low Ca^{2+} or replacing Ca^{2+} by Sr^{2+}. Here we investigated the asynchronous release at the Drosophila glutamatergic synapses. We monitor the differential effects of Ca^{2+}, Sr^{2+} and Cd^{2+} in the evoked release modifying the expression of Synaptotagmin and Complexin at the nerve terminals. In addition, we explored the C2A and C2B Ca^{2+} binding domains mutants of Synaptotagmin. Our data shows that the asynchronous/synchronous release depends inversely with the level of Synaptotagmin and Complexin. Moreover, C2B but not C2A domain of Synaptotagmin reduces the asynchronous/synchronous release. Our data suggests that Complexin may be increasing the accessibility of Synaptotagmin for the SNARE complex by the C2B domain reducing the asynchronous/synchronous release. The Cd^{2+}-sensibility was consistent with the Ca^{2+}-dependency in the release probability discarding major alteration in calcium influx

37. Characterization of intrinsic properties of CA1 pyramidal neurons in organotypic hipocampal slices

Karmelic, D[1]., Palma, V[1]., Sanhueza, M[1]., [1]Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

Correlation-based plasticity processes, such as long-term synaptic potentiation, generate positive feedback loops, intrinsically destabilizing for neuronal networks. This is prevented by a set of slower plasticity phenomena, termed 'homeostatic plasticity', acting in opposition to the changes in neural activity. These mechanisms may include a global scaling of the synaptic inputs of a cell and adjustments of the intrinsic excitability through modification of different conductances.

Hippocampal slices are among the most relevant models in neurobiology, and in their cultured form allow chronic and genetic manipulations, fundamental in order to study the mechanisms of long-term plastic phenomena. Synaptic homeostatic plasticity of organotypic hipocampal CA1 pyramidal neurons has been described in response to chronic inactivity, but it may not account for the robustness in the long-term firing rate observed *in vivo*. We therefore decided to study the homeostatic adaptation of the intrinsic excitability of CA1 principal cells in cultured slices.

As a first step to describe such plasticity, we implemented the interface organotypic culture system, achieving the recapitulation of known structural and physiological characteristics of the slices. We then characterized the intrinsic properties of 8-11-days-*in-vitro* CA1 pyramidal neurons. Using whole-cell recording, we measured the firing threshold and the spiking frequency in response to different stimulation currents as direct excitability indicators. In order to assess the conductances that could underlie excitability modifications, we analyzed the action potential parameters. Chronic inactivation experiments and lentiviral manipulation of the expression of candidate regulatory proteins –such as CaMKII– are currently underway.
Financial support: CONICYT scholarship and FONDECYT (1110237).

Posters II

38. Temperature depennce of action potential finring in snail central neurons

PINO, D[2]., PEÑA, N[2]., ESPINOZA, M[2]., CONTRERAS, D[2]., MUÑOZ, F[1]., PERTUSA, M[2]., MORALES, B[2]., **Rojas, P[2]**., [1]NEUROBIOLOGY, , COLUMBIA UNIVERSITY.2Biologia, Química y Biología, Universidad De Santiago De Chile.

Antarctic organisms have undergo a series of adaptations which allow them to live at water temperatures ~-2°C, and perform all physiological functions as similar taxa at warmer temperatures. In order to understand the adaptations underwent by the nervous system in the process of action potential generation, we studied the temperature dependence of extracellular field activity in two mollusk living in different thermal environments: the land snail *Helix pomatia*, and the antarctic limpet *Nacella concinna*. *N. concinna* specimens were collected in Fildes Bay (62°12'0" 58°57'51"), Antarctica. Extracellular field potentials were recorded in the limpet cerebral ganglia from -2 to 15°C, having a maximum peak between 4 to 8 °C. Activity decreased at higher temperatures and in some experiments stops at 15°C. In *H. pomatia* ganglia the extracellular activity was almost inexistent below 10 °C and showed a peak between 15 to 22 °C, decreasing to almost zero over 32°C. These differences in neuronal activity can be explained by the differences in the set of voltage dependent ion channels, their temperature dependence of activation, or both. Because K+ channels are determinants in setting the firing patterns, we wanted to study how these channels are involved in the adaptation of neuronal activity and generation of firing pattens. In isolated ganglia the rhythmic activity is affected by the A-type K+ channel blocker 4-Aminopyridine, showing that this conductances is important for setting firing patterns and a possible candidate for the adaptation to low temperature. Funded by INACH RT_15-12.

39. Role of Chloride co-Transporter NKCC1 in excitability of Dentate Gyrus in chronic epilepsy

LARA, M[1]., LOYOLA, S[1]., MANTELLERO, C[1]., LORCA, E[1]., PERTUSA, M[1]., MORALES, B[1]., **ROJAS, P[1]**., [1]Biologia, Química y Biología, Universidad De Santiago De Chile.

Granule cells of the dentate gyrus (GCDG) are the gatekeepers of the tri-synaptic hippocamapal circuit in part by an intrinsically low cell excitability, which is pathologically increased in Temporal Lobe Epilepsy. Alterations in the delicate balance between excitation and inhibition in the nervous system is involved in several neurological conditions. g-amino butyric acid (GABA) is the most important inhibitory neurotransmitter in the nervous system, however excitatory effects have been observed in GCDG in epileptic patients. This is produced by an outward flux of chloride ions, whose pathological intracellular concentration is mediated by the activity of the Sodium Potassium co-Transporter 1 (NKCC1), which accumulates chloride in the cell. The expression of this transporter is very low in mature brain, showing a decrease in expression for born to virtually no detection at P18. In light of this evidence, we wanted to study how intracellular chloride levels and NKCC1 expression alter the circuit excitability in mature GCDG, in order to understand the role of this co-transporter in both normal and epileptic neurons for a possible use as anti-epileptic drug. Here we show by extracellular field potentials recordings of acute hippocampal slices of adult animals that application of the selective NKCC1 inhibitor Bumetanide produces a decrease in circuit excitability in synaptic and population spike signals of both control and epileptic animals. In summary, these results show that NKCC1 is functionally expressed in mature neurons, and careful investigation of its function should be performed before its use as anti-epilpetic drug. Funding by FONDECYT 1130904, INNOVA-CORFO 12IDL1-16051.

40. Multiple whole-cell patch clamp to study state transitions in the awake mouse

Flores, F[1,2,3]., Kodandaramaiah, S[4]., Wickersham, I[3]., Forest, C[5]., Brown, E[1,2,3,6,7]., Boyden, E[3,4,8,9]., [1]Department of Anesthesia, Critical Care and Pain Medicine, , Massachusetts General Hospital. [2]Anesthesia, Critical Care and Pain Medicine, , Harvard Medical School. [3]Department of Brain and Cognitive Sciences, , Massachusetts Institute of Technology. [4]Media Arts and Sciences Deaprtment, , Massachusetts Institute of Technology. [5]Woodruff School of Mechanical Engineering, , Georgia Institute of Technology. [6]Institute for Medical Engineering, , Massachusetts Institute of Technology. [7]Division of Health Sciences and Technology, , Harvard - MIT. [8]Department of Biological Engineering, , Massachusetts Institute of Technology. [9]McGovern Institute for Brain Research, , Massachusetts Institute of Technology. (Sponsored by Pedro Maldonado Arbogast)

Observing membrane currents and potentials in vivo is fundamental to understand neural processing, pathophysiology of brain disorders, and drug actions in intact brains. When recorded in vivo, synaptic currents and potentials provide a measure of network activity, and a correlate to the features observed in the scalp EEG, which is routinely used in research and clinical practice. Also, most neurons do not spike very often or at all, and therefore, extracellular recordings under-represent the extent of neural activity that can be observed. The current gold-standard for precise measurement of synaptic events is whole-cell patch clamp. A number of technical challenges, however, have made this technique difficult to perform in vivo, limiting the insights to be obtained from it. Some of us have recently introduced an automated method to perform whole-cell recordings from neurons in the living brain (S.B. Kodandaramaiah et al, Nature Methods 2012; details and updates at http://autopatcher.org). Now we have extended that technique to perform whole-cell recordings in multiple neurons of the awake mouse at once. The multipatching robot achieves stable whole cell recordings from pairs and triplets of neurons for as long as 60 minutes. We have performed in vivo multipatching combined with EEG/LFP recordings to study the transition from awake into sedated states, and the relationships between synaptic events and the EEG features observed during the sedated state produced by different classes of anaesthetic drugs.

41. The functional incorporation of Tyr-coumarin into proteins synthesized by HEK293T cells

Steinberg, X[1].,Galpin, J[3].,Goodchild, S[2].,Ahern, C[3]., Brauchi, S[1].,[1]Instituto de Fisiología, Facultad de Medicina, Universidad Austral De Chile.[2]Life Science Centre, Cardiovascular Research Group, University of British Columbia.[3]Carver College of Medicine, Molecular Physiology and Biophysics, University of Iowa. (Sponsored by Fondecyt 1110906, MecesupAUS0704)

The generation of novel tools for the observation of natural phenomenons it's a constant (pre)occupation for researchers of all areas. Therefore engineering of new methodological approaches capable of alter the biochemical properties of proteins *in vivo* will be always beneficial for the broad fields of natural sciences. The use of un-natural amino acids (UAAs) into proteins had emerged as a promising strategy to manipulate, with unbeatable precision, protein structure *in vitro* and *in vivo*. One of the strategies for UAA incorporation is one that implicate the use of an evolved aatRNA/aaRS pair able to recognize specifically the UAA for its introduction into a TAG codon mutant on living systems. Our laboratory proposed to engineer a tRNA/RS pair for the incorporation of a version of tyrosin, modified with the coumarin fluorophore (Tyr-coum) into proteins synthesized by mammalian cells. To engineer the eukariotic RS we used a bacterial version as template. Rational design was guided by molecular modeling and mutations were incorporated by site-directed mutagenesis. The efficiency of incorporation was evaluated by single cell microscopy. Our work show that the seven point mutations made over the tyrosil-tRNA synthetase are enough to modify the enzyme performance, now allowing the functional incorporation of Tyr-coum into TAG mutant proteins synthesized by HEK293T cells. Single molecule imaging was obtained and evaluated. One of the mayor projections of this work is related with the acquisition of structural information from coumarin's fluorecence fluctuations on living systems with high signal-to-noise ratio.

42. Comparing the characteristics of the transcriptional complexes formed by the family of CoREST proteins

Barrios, A[1].,Gómez, A[1].,Sáez, J[1].,Toffolo, E[2].,Battaglioli, E[2].,Andrés, M[1].,[1]Department of Cell and Molecular Biology, Faculty of Biological Sciences, Pontificia Universidad Católica De Chile.[2]Department of Biotechnology and Translational Medicine, , University of Milan.

CoREST (CoREST1) was initially identified as a transcriptional co-repressor associated to REST transcription factor to repress neuronal gene expression in non-neuronal tissue. Mammal genomes contain and express three different CoREST genes. CoREST1 is encoded by *rcor1* gene and the paralogues, *rcor2* and *rcor3* encode CoREST2 and CoREST3 proteins, respectively. Although the three proteins present a high sequence identity, little is known about CoREST2 and CoREST3 transcriptional activities or functional partnerships. Is also unknown whether they are similarly or differentially expressed in time and tissues. The purpose of this work is to evaluate the functional properties of CoREST proteins and their pattern of expression in adult rat brain. Reporter gene experiments in cell lines show that all CoREST proteins behave as transcriptional repressors; however CoREST2 and CoREST3 have significantly lower transcriptional repressor capacity than CoREST1. Interestingly, while CoREST3 shows a similar interaction with histone deacetylases HDACs1/2 as CoREST1; CoREST2 interacts weakly with HDACs1/2. In line with these data, CoREST2 repressor activity is not inhibited by the HDAC inhibitors SAHA and trichostatin A, while a complete reversion of CoREST1 and CoREST3 repression is observed in the presence of these HDACs inhibitors. Accordingly, CoREST2 immunoprecipitates present significant less HDAC activity *in vitro* compared with CoREST1 and CoREST3. All CoREST proteins are expressed in adult rat brain. In conclusion, our data support the possibility that CoREST proteins form transcriptomes with different repressor capacity.

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43. Binding mechanism of the potent and selective blocker A1899 to four K_{2P} channels in light of the new crystal structures

Gonzalez, W[1].,Martinez, G[2].,Poblete, T[2].,Alzate-Morales, J[2].,[1]Centro de Bioinformática y Simulación Molecular, Facultad de Ingeniería, Universidad De Talca.[2]Centro de Bioinformática y Simulación Molecular Universidad de Talca. (Sponsored by Carlos González León)

Two-pore domain potassium (K_{2P}) channels are expressed in the central nervous system. These proteins form dimers and each subunit has two pore-forming loops (P1, P2) and four transmembrane domains (TM1-TM4). Both pore regions (P1, P2) have the conserved TXGXG motif, known as the selectivity filter, a structure characteristic for all selective potassium channels, where X denotes a hydrophobic amino acid. K_{2P} channel family consists of 15 proteins divided into six subfamilies based on sequence similarity and functional resemblance. They are involved in the maintenance of the resting potential, regulation of excitability, sensory transduction, metabolic regulation and neuroprotection. K_{2P} channels are related with several pathologies in humans (Ex: TASK-1 channel is an important modulator of multiple sclerosis) therefore, they represent an important target for study. K_{2P} channels are blocked by a variety of compounds, despite, there are only a few documented examples of potent and selective blockers for these channels. A1899 (2′-[(4-methoxybenzoylamino) methyl] biphenyl-2-carboxylic acid 2,4-difluorobenzylamide) is reported as a specific and highly potent blocker of the channels TASK-1 and TASK-3, particularly the former. In this study, we describe the binding mechanism of A1899 to four K_{2P} channels: TASK-1, TASK-3, TASK-2 and the recently crystallized TRAAK channel, from a molecular dynamics perspective and detailing the differences for each ligand-channel interactions using MM/GBSA method in agreement with reported experimental data. Acknowledgment: Anillo ACT-1104

44. Angiotensin II AT_2 receptors localized at immunoreactive neurons in the inferior colliculus at developmental stages.

Arce, M[1].,Sànchez , S[1].,Fuentes, L[2].,Ciuffo, G[1].,[1]BIOLOGIA MOLECULAR, QUÍMICA, BIOQUÍMICA Y FARMACIA, UNIVERSIDAD NACIONAL DE SAN LUIS.[2]FARMACOLOGIA, QUÍMICA, BIOQUÍMICA Y FARMACIA, UNIVERSIDAD NACIONAL DE SAN LUIS. (Sponsored by IMIBIO-SL CONICET)

The classical effects of Angiotensin II (Ang II) are well-known but new evidences involve this peptide in development and organogenesis. It can also modulate neuronal activity in brain regions involved in cognition, motor control and sensory integration. Ang II recognizes two receptor subtypes, named as AT_1 and AT_2, based on pharmacological basis. The Inferior Colliculus (IC) is a critical midbrain station for the auditory processing, involved with motor activities related to audition. Previously, we studied Ang II receptor localization by binding autoradiography in midbrain and hindbrain of newborn rats at different stages (P0, P8 and P15). Specific binding at the IC increased with age, being maximal at P15 with prevalence of AT_2 receptors, in agreement with higher AT_2 receptor mRNA expression at this stage.

The aim of this work was to study the localization of AT_2 receptor in the IC in P15 animals by immunofluorescence assays and to identify cellular types.

Immunofluorescence staining with anti AT_2 receptor antibody and Anti β III –Tubulin antibody (specific neuronal marker) recognize the same neurons, thus indicating the AT_2 labeled cells are neurons. Immunoreactive neurons also exhibited selective Ang II AT_2 binding by autoradiography. The pattern of Ang II AT_2 receptor´s expression in the IC encompasses the pattern of the brainstem maturation and connection to the cerebral system. Since IC was involved in the the auditory processing and scape behavior, we can assume that AT_2 receptors might play a potential role in organogenesis and the establishment of neuronal circuits.

45. Mechanical transduction in olfactory and pheromone sensory neurons shares components of their respective chemotransduction pathways

Blanchard, K[1]., Villar, P[1]. ,Cuevas, A[1]., Bacigalupo, J[1].,[1]Department of Biology, Faculty of Sciences, University of Chile.

In the olfactory epithelium (OE), olfactory-sensory neurons (OSNs) transduce airborne odorants to electrical signals conveyed to the olfactory bulb (OB). OSNs project chemosensory cilia containing G-protein coupled odorant receptors to the nasal cavity. Odorant binding triggers a signaling cascade, where adenylyl cyclase (ACIII) generates cAMP that opens Ca^{2+}-permeable cyclic nucleotide-gated channels (CNGs); Ca^{2+} gates Ca^{2+}-activated Cl- channels (CaCCs), generating the receptor potential. A Na^{+}/Ca^{2+} exchanger (NCX) and a plasma membrane Ca^{2+}-ATPase (PMCA) remove the Ca^{2+}. The OB exhibits Θ-range oscillations synchronized with respiration. Mechanical perturbation of the cilia evokes responses similar to the odor responses. ACIII blockage or CNGA2 knockout abolish odor and mechanical responses, and uncouple OB oscillations from respiration. These observations suggest that OSNs mechano and chemotransduction use a common signaling pathway (Grosmaitre et al., 2007). We investigated whether CaCC and NCX also participate in the mechanical responses to Ringer puffs. Blocking CaCC with niflumic acid reduced mechanical responses in single OSNs (loose patch) and field potential recordings (electroolfactogram, EOG). NCX Inhibition slows down EOG relaxation in odor and mechanical responses. We also investigated if pheromone-sensory neurons (PSNs) from the vomeronasal organ are mechanosensitive as well. In effect, these neurons synergistically responded to both pheromone and mechanical stimulation. The two cell types adapt to both stimuli. PSNs mechanical responses are inhibited by blocking phospholipase-C (U73122), key to pheromone transduction. Our results are consistent with the notion that in each cell type the chemo-and mechanical transduction are mediated by the same respective pathways.

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46. Contralateral-noise suppression of cochlear responses is enhanced by electrical microstimulation of the auditory cortex

Aedo, C[1].,León, A[1].,Dragicevic, C[1].,Terreros, G[1].,**Delano, P[1,2].**,[1]Fisiología y Biofísica, Medicina, Universidad de Chile.[2]Otorrinolaringología, Medicina, Universidad De Chile.

The auditory efferent system is constituted by two main neural circuits: (i) descending projections from the auditory cortex to the inferior colliculus and to the superior olivary complex (SOC) and (ii) olivocochlear fibers originated in the SOC and directed to the cochlear receptor. The olivocochlear function can be assessed by measuring a brainstem reflex constituted by auditory-nerve fibers, cochlear nucleus and olivocochlear neurons. This reflex is activated by contralateral acoustic stimulation and produces a reduction in the amplitude of ipsilateral cochlear responses. Here, we used anesthetized chinchillas (n=6) to investigate the influence of auditory-cortex descending projections in the magnitude of the olivocochlear acoustic reflex. Cochlear electrical responses were recorded using a round-window electrode (CAP: compound action potential of the auditory nerve and CM: cochlear microphonics), before, during and after contralateral noise stimulation, in response to tones of different frequencies (1-8 kHz) and levels (60-90 dB SPL). In addition, the effects of contralateral noise stimulation on cochlear responses were studied simultaneously to auditory cortex electrical microstimulation (MS) (2-50 μA, 32 Hz rate). In four experiments, cortical MS produced an increase of the suppressive effect of contralateral noise stimulation (-3.0 to -5.0 dB) on CAP responses to 2-4 kHz tones. In addition in some cases, cortical MS changed the polarity of the efferent reflex from CAP increase to decrease. These results demonstrate that the activation of the descending projections from the auditory cortex to olivocochlear neurons enhances the suppressive effects of the olivocochlear reflex. Supported by FONDECYT 1120256.

47. Heterogeneous distribution of mitral/tufted cells of the Accessory Olfactory Bulb in *Octodon degus* (Rodentia: Hystricognathi)

Delgado, S[1].,Fernández, P[1]., Mpodozis, J[1]., [1]Biologia, Ciencias, Universidad De Chile.

The Vomeronasal System (VNS) is involved in the modulation of social and reproductive behaviors in mammals. The sensory epithelium of this system is located at the Vomeronasal Organ (VNO), and contains “olfactory like” neurons expressing different kinds of semiochemicals receptors. Projections of these neurons target the Accessory Olfactory Bulb (AOB), where they segregated into two subdomains: the rostral region (rAOB) and the caudal region (cAOB). Some Caviomorphs rodents exhibit large size differences between both subdomains. In *Octodon degus*, the rAOB doubles the volume of the cAOB, and also features more and larger glomeruli. At present, it is not know whether these heterogeneities involve also the secondary (Mitral/tufted, M/T) projection cells this system. We then performed a stereological study of the number and density of cells in the Mitral Cell Layer (MCL) of the AOB of Octodon degus. For this purpose, the AOB of four adult individuals of O. degus were processed according standard protocols for Nissl staining. Morphometric measures of both AOB sub domains were then obtained using Neurolucida system and StereoInvestigator software. We found that the rAOB had 55% more cells in the mitral cell layer that the cAOB (rAOB=7,933.44±2,876.74; cAOB=4,397.91±1,311.58). The volume of the rAOB was also 37% larger (rAOB=0.0000829±0.0000204 cm^3; cAOB=0.0000306±0.0000124 cm^3), while the cell density in both subdomains was similar (about 100.000 neurons/um^3). These data suggest that the anatomical segregation of sensory properties expressed at the AOB subdomains is translated to the telencephalic stage of sensory system.

48. Cholinergic Neurotransmission Links Chemosensory Cells To Neurogenic Inflammation In The Nasal Cavity

Finger, T[1,3]., Saunders, C[2,3]., Christiensen, M[1,4]., Tizzano, M[1]., [1]Cell & Devel. Biology, Sch. of Medicine, Univ. Colorado Denver.[2]Cell & Devel. Biology, Sch. Medicine, Univ. Colorado Denver.[3]Neuroscience, Graduate School, Univ. Colorado Denver.[4]Department of Bioscience, , Aarhus University (DENMARK). (Sponsored by National Inst. Health, NIDCD)

Solitary chemosensory cells (SCCs) are specialized epithelial chemosensors of the airways which respond to irritants via the canonical bitter taste transduction cascade (T2R receptors, Gα-gustducin and TRPM5). When stimulated, SCCs trigger peptidergic nociceptive nerve fibers causing alteration of respiratory rate indicative of trigeminal activation. Direct chemical excitation of trigeminal pain fibers can result in neurogenic inflammation in the surrounding epithelium. In the current study, we test whether activation of nasal SCCs can trigger local inflammation leading to mast cell degranulation and plasma leakage into the epithelium. The prototypical bitter compound, denatonium caused significant plasma extravasation and mast cell degranulations in wild type mice but not in mice with genetic deletion of elements of canonical taste transduction cascade, demonstrating that activation of SCCs can lead to local inflammation. Ablation of peptidergic trigeminal fibers by resinoferatoxin prevented the SCC-induced nasal inflammation, indicating that SCCs evoke inflammation only via neural activity and not by release of local inflammatory mediators. Additionally, blocking nicotinic acetylcholine receptors prevents SCC-mediated neurogenic inflammation showing the necessity for cholinergic transmission from SCC to nerve fiber. Finally, we demonstrate neurogenic inflammation depends on activation of the neurokinin 1 (NK-1) receptor suggesting that release of substance P, the natural ligand for NK-1, from the nerve fibers triggers both the degranulation of mast cells and the changes in endothelial cells leading to plasma leakage. Taken together, these results demonstrate that SCCs utilize nicotinic cholinergic neurotransmission to trigger peptidergic trigeminal nociceptors and link SCCs to the traditional neurogenic inflammatory pathway.

49. Unconventional photoreceptors in the inner nuclear layer of the avian retina

Hernandez, A[2]., Ramirez, J[2]., Nasi, E[1]., **Gomez, M[2,3]**., [1]Instituto de Genetica, Ciencias, Universidad Nacional de Colombia.[2]Biologia, Ciencias, Universidad Nacional de Colombia.[3]Neurofisiologia, Biofisica, Centro Internacional de Fisica. (Sponsored by Colciencias Grant 222852128276)

The discovery of melanopsin-expressing intrinsically photoresponsive retinal ganglion cells (ipRGCs) paved the way for understanding light regulation of non-visual functions, like the pupillary reflex and circadian rhythms entrainment. The scarcity of mammalian ipRGCs, however, is a hurdle for studying melanopsin signaling. In the avian retina melanopsin also expresses in the outer nuclear layer. We used the chicken embryo as a model system to investigate its functionality. A polyclonal antibody was raised against both chicken melanopsin isoforms, validated by Western blot, and utilized to corroborate melanopsin distribution in the retina; strong immunoreactivity occurred in areas that likely include horizontal, bipolar, and some amacrine cells. To assess possible intrinsic photosensitivity of identified neurons, retinas were enzymatically dissociated. Morphologically well-preserved neurons were obtained, and their physiological viability was tested with whole-cell recording. Because the PLC cascade has been implicated in light-signaling by melanopsin in other systems, calcium changes were monitored in cells loaded with fluorescent indicators; both imaging and higher-sensitivity photomultiplier measurements were utilized. A population of bipolar and horizontal cells showed substantial increases in Ca fluorescence upon illumination. The light responses exhibited a long latency and a slow time course, spanning minutes. Electrophysiological recordings revealed in some cases a small light-evoked inward current (tens of pA) with a similar time course. In few additional instances, light could reversibly modulate voltage-gated currents. These observations indicate that intrinsic photosensitivity amongst retinal neurons is not confined to rods, cones, and some ganglion cells. The physiological role of these novel light sensors remains to be investigated.

50. Searching for the molecular basis of light transduction in microvillar photoreceptors of mollusks and chordates

Gonzalez, K[1].,Galindo, C[1].,Nasi , E[2,3]., Gomez, M[1,3]., [1]Departamento de Biologia, , Universidad Nacional de Colombia.[2]Instituto de Genetica , , Universidad Nacional de Colombia .[3]Cellular Dynamics Program, , Marine Biological Laboratory.

Animal visual opsins have been grouped, based on sequence analysis, into a few categories which closely follow the main morphological classes of photoreceptors. In particular, 'R-opsins' comprise a large group of light-sensing molecules found in visual receptors of arthropods and mollusks, and in circadian receptors of vertebrates. They presumably couple via a Gq to the phosphoinositide cascade; however, downstream effector mechanisms remain controversial, and conflicting evidence has been obtained from different species. The molecular underpinnings of this signaling pathway have been studied systematically only in *Drosophila*, raising questions about possible species divergence, and highlighting the need for a wider phylogenetic coverage. To help fill this gap, we have begun identifying by PCR putative light-signaling molecules in R-type photoreceptors from widely separate phyla: the microvillar photoreceptors from the eye of a mollusk (the scallop) and the neural tube of a primitive chordate (amphioxus). The transcriptome of the scallop retina and the amphioxus genome provided a rational framework for primer design, complementing homology-based criteria. We initially focused on two key phototransducing elements, the light-regulated enzyme and potential light-activated ion channels. Amplifications and RACE extensions using scallop retina cDNA yielded two PLC-b isoforms, while a partial PLC clone has been obtained in amphioxus. From a bioinformatics search, several amphioxus TRP orthologs were selected as candidates for the light-sensitive conductance, and three isoforms were cloned from neural tube cDNA. Antibodies against peptides from the predicted sequences and ribo-probes are being utilized to localize their expression by immunohistochemistry and *in situ* hybridization, respectively.

51. TRPM5 channel in the main olfactory system is necessary for pheromonal-induced prolongation of the estrous cycle.

López, F[1].,Delgado, R[1].,López, R[2].,Bacigalupo, J[1].,Restrepo, D[2].,[1]Biología, Facultad de Ciencias, Universidad de Chile.[2]Department of Cell and Developmental Biology, Rocky Mountain Taste and Smell Center, , University of Colorado Anschutz Medical Campus.

Mature female mice, grouped in the absence of a male stimulus, exhibit a suppressed estrous cycle (the Lee-Boot behavioral effect). Previous studies by Ma, Miao and Novotny (Biol. of Reprod. 59:1317, 1998) indicated that 3,5-dimethylpyrazine (DMP) elicits this pheromonal behavior. Unpublished data of our lab show that the subpopulation of olfactory sensory neurons (OSNs) that expresses the calcium activated cation channel TRPM5 (transient receptor potential channel M5), are specialized to pheromones like DMP and are unresponsive to environmental odorants, and OSNs expressing TRPM5 target a subset of glomeruli innervated by mitral cells sending axons to the medial, vomeronasal amygdala (Thompson et al., J Comp Neurol.1;520(8):1819-30, 2012). Here we show that mice with TRPM5 deficiency whose OSNs show substantially decreased response to DMP display decreased induction of estrous suppression elicited by housing multiple female mice per cage. We also show that only a very small number of vomeronasal sensory neurons (VSNs) express TRPM5 and the TRPM5-expressing VSNs do not respond to DMP at the low concentration (12.5 nM) that the main olfactory epithelium sensory neurons (OSNs) respond to. These data strongly support the involvement of TRPM5 in pheromonal transduction in the cilia of OSNs from the main olfactory system TRPM5. Supported by: FONDECYT 1100682 (JB), CONICYT fellowship (FL), DC04657 (DR), DC006070 (DR), TW007920 (JB and DR).

52. Role of TRPM8 channels in the hypersensitivity to cold of corneal primary sensory neurons induced by axonal damage

Piña, R[1].,Ugarte, G[1].,Pino, R[1].,**Madrid, R[1].**, [1]Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile.

Injury of the sensory fibers innervating the cornea is frequently followed by dysesthesias, ocular dryness and changes in thermal sensitivity, among other sensory alterations. The molecular and cellular mechanisms underlying the changes in the transduction capability and encoding of nervous impulses in response to axonal damage of the different neuronal subtypes innervating this tissue, are still poorly understood. In the cornea, both the responses to cold and the ongoing electrical activity of the exquisitely temperature-sensitive cold sensitive neurons (CSNs) are strongly dependent on the functional expression of the cold- and menthol-sensitive channel TRPM8. CSNs of the cornea act not only as cold thermoreceptors but also as the humidity detectors of the ocular surface, and their role in the sensory alterations in response to axonal damage remains unclear. Usingcalcium imaging and patch clamp techniques in retrograde labeled corneal neurons dissociated from trigeminal ganglia, we studied the modifications in thermal- and chemical-sensitivity of CSNs that result from the controlled injury of corneal sensory axons in mice. We found that controlled surgical injury of corneal fibers resulted in an increase in both the percentage of CSNs, and their sensitivity to cold and menthol, with only slight differences in the mean temperature threshold compared to control corneal sensory neurons. Our results suggest that cold hypersensitivity of the eye surface, induced by surgical damage of corneal nerve fibers, could be linked to an increase in the functional expression of TRPM8 channels in corneal primary sensory neurons.

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53. Role of I_{KD} current in painful hypersensitivity to cold induced by chronic peripheral nerve injury

Ugarte, G[1].,Restrepo, C[1].,Gonzalez, A[1].,Pertusa, M[1].,Madrid, R[1].,[1]Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile.

Cold allodynia is a common form of neuropathic pain after axonal damage of somatosensory neurons. The cellular and molecular mechanisms underlying this sensory alteration are poorly understood. Cold-sensitivity of primary somatosensory neurons is determined by a balance between TRPM8 channels, the main molecular entity responsible for the excitatory cold-activated current, and Shaker-like Kv1.1-1.2 potassium channels, the molecular counterpart of I_{KD}, an excitability brake current. We studied the role of I_{KD} in painful hypersensitivity to cold after nerve damage, using chronic constriction injury (CCI) of the sciatic nerve as a model of axonal damage-induced cold allodynia in mice, in combination with calcium imaging and patch clamp techniques in dissociated neurons from L3-L5 dorsal root ganglia. We found that the population of cold-sensitive neurons (CSNs) is increased after injury, and that the mean cold threshold of CSNs is shifted to higher temperatures. The pharmacological suppression of I_{KD} shifted the threshold of control CSNs to higher temperatures, a shift that is strongly reduced in CSNs from CCI animals. Mean I_{KD} current density is reduced in CSNs from CCI animals compared to sham mice, with no differences in TRPM8-dependent current density. The electrophysiological characterization of CSNs suggests an increase in the population of neurons with nociceptive-like phenotype after nerve injury. Our results suggest a recruiting of primary sensory neurons of nociceptive routes due to the reduction of I_{KD} current density in response to axonal damage, providing a mechanism for cold allodynia.

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54. Linking solutions via cluster methods for a conductance-based mathematical model of cold thermoreceptor

Maidana, J[1].,Olivares, E[1].,Orio, P[1].,[1]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso.

Cold sensitive nerve endings respond to changes in temperature with transients changes in mean firing rate (dynamic response). When adapted to a steady temperature, they display a rhythmic ongoing firing pattern that can be tonic or bursting, regular o irregular, depending on the temperature (static response). Based on a previous model that reproduces the static response (Huber & Braun, 1998), we have developed a model that in addition displays dynamic response. To adjust the free parameters of the new model, we implemented an evolutionary optimization algorithm, which selected parameter sets that displayed a dynamic firing rate response. 160 independent runs of the fitting algorithm yielded different parameters sets that were subject to visual selection. We selected a subset of 63 parameter combinations which had an appropriate behavior. To characterize the performance of this parameter subset, we simulated static and dynamic responses and compared them to a set of experimental data. We also performed a clustering of the parameter sets based on five clustering techniques of the R cran package ClValid. Five sets of parameters were selected as the best when compared to physiological recordings. Clustering shows that two of them have similar features while the other three are shown to be more distant in the parameter space. Support: Fondecyt 1130862 and ACT-1113. CINV is a Millenium Science Institue (Ministerio de Econoía, Chile)

55. Learning of gaze strategies resolves dynamic environment constraints of a visuomotor task

Mariman, J[1]., Burgos, P[1]., Maldonado, P[1,2]., [1]BNI, Medicina, Universidad De Chile.[2]Fisiologia y biofisica, Medicina, Universidad De Chile. (Sponsored by Iniciativa Científica Milenio (CENEM))

Previous studies show that skillful subjects use anticipatory eye movements to predict the trajectories of task's objects during sports or daily activities, which suggests that this behaviour would require a learning process. However, the mayority of learning paradigms involves statics environments. Our aim was to describe the gaze strategies used during a visuomotor task in a dynamic environment and their changes as part of a learning process. We recorded eye movements of fourteen subjects, during one-day practice (twenty five blocks) of a videogame consisting in catching targets displaying three different types of a downward accelerate curve trajectory. The joypad movement was artificially rotated sixty degrees counterclockwise. This was a kinematic-motor alteration that required to be learned. We found a quick increase in subjects performance since the third block for all trayectories. Gaze strategy was preferably a combination of saccadic and smooth pursuit eye movements. Both temporal and eye movements preference increase along the learning. Anticipatory strategy regarding target displacement was an early and optimized gaze behaviour, and showed significative correlations with the general performance. Results confirm previous finding about predictive component of gaze behaviour during visual exploration, and show clearly that anticipatory strategy resolve the dynamic constraints of the environment. Moreover, the strategy changes over time suggest that the optimization of eye movements control is a critical component of the visuomotor skill, allowing an effective information extraction to deal with dynamic environments. Thus, the adquisition of internal models for the control of eye movements seems consistent with our observations.

56. Analysis of neural oscillations in the olfactory system of rainbow trout (*Oncorhynchus mykiss*).

Olivares, J[1]., Orio, P[2]., Schmachtenberg, O[1]., [1]Centro Interdisciplinario de Neurociencias de Valparaíso, Facultad de Ciencias, Universidad De Valparaíso.[2]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad De Valparaíso. (Sponsored by Financiado Por FONDECYT 1120513 Y CINV Milennium Institute)

In the present study, we aboard the analysis of olfactory processing in the telencephalon of the rainbow trout, by means of parallel extracellular electrophysiological recordings of the electroolfactogram (EOG) and local field potentials (LFPs) to answer: Where are olfactory oscillations generated and how are they transmitted? Which are their basics features? Is there any specificity in responses to different odorants? Our results indicate that the source of oscillations is within the olfactory bulb, inducing telencephalon sectors into phase with frequencies around 13 Hz (Fourier analysis). EOG and LFP amplitude increases with increasing stimulus intensity, but no changes were observed in the oscillatory frequencies. The average length of the responses in the telencephalon differ, being: Bile salts = 4.1 s; skin extract = 5.1 s; Amino acids = 6.9 s; Finger´s = 7.6 s (averages of 41 records, N = 10), which possibly represents characteristics of each odorant.

57. Stereological and immunohistochemical analysis of excitatory and inhibitory innervation of the trigeminal, facial and ambiguous motor nuclei of the mouse.

Oñate, A[1].,Faunes, M[1].,Fernández, S[1].,Henny, P[1]., [1]Anatomía Normal, Facultad de Medicina, Pontificia Universidad Católica De Chile. (Sponsored by Comisión Nacional De Ciencia Y Tecnología De Chile, Through FONDECYT (N° 11100433) And ANILLO (N° ACT-1109) Grants To P.H., And The Escuela De Medicina De La Pontificia Universidad Católica Through An Interdepartamental Grant Awarded To P.H. (Dept. Anatom)

Orofacial muscles are involved in vital functions, such as mastication, swallowing, whiskering and production of vocalizations. In humans, they are also involved in speech. Jaw muscles, facial muscles and pharynx, larynx and oesophagus muscles are respectively controlled by the trigeminal, facial and ambiguous brainstem motor nuclei. Neurons of these branchimotor nuclei exhibit various patterns of activity and susceptibility to pathological conditions. The aim of this study is to characterize the glutamatergic and GABAergic/glycinergic innervation of motoneurons of these nuclei. We used adult C57BL/6 male mice. Using immunohistochemistry for the glutamate vesicular transporters VGluT1 and VGluT2, and for the glycine and GABA vesicular transporter VIAAT, we quantified the number of VGluT1+, VGluT2+ and VIAAT+ varicosities throughout the trigeminal, facial and ambiguous motor nuclei, utilizing a stereological method, the Optical Fractionator. We also quantified the proportion of varicosities that directly apposed somatic compartments. Our preliminary results show that in the trigeminal motor nucleus, of a total estimate of ~790,000 varicosities, ~28 % are VGluT1+, ~41 % are VGluT2+ and ~31 % are VIAAT+. In the facial motor nucleus, of a total estimate of ~4,600,000 varicosities, ~2 % are VGluT1+, ~49 % are VGluT2+ and ~49 % are VIAAT+. The results indicate that the inhibitory input to the trigeminal motor nuclei is lower than to the facial motor nuclei, which can be accounted by the low proportion of VGluT1+ input to the facial nucleus. Current work is being carried out to determine the number and proportions of these synaptic inputs to the ambiguous nucleus, as well as within each nuclei specific subdivisions.

58. Characterization of the Early-Visual Processing in the Retina Using Motion Cloud.

Ravello, C[2].,Escobar, M[1].,Hurtado, J[2]., Astudillo, A[1]., Araya, J[2]., **Palacios, A[2]**., [1]Departamento de Electronica, , Universidad Tecnico Federico Santa Maria.[2]Centro Interdisciplinario de Neurociencia de Valparaiso, , Universidad de Valparaíso.

The *Octodon degus* is a diurnal rodent that rely on their visual system to achieve their critical behavioral tasks, so is a remarkable model to study the early-visual processing, specifically the retina. Only recently natural stimulus has been introduce in the characterization of the visual system. However, it turns to be challenging the precise control of statistical properties of natural images to be used in visual science. An alternative option is to use Motion Clouds (MC), a set of stimulus preserving some of the properties of natural scenes found in nature. Here we use an array of multi-electrodes (256 electrodes, 20Kz) to record from a population of retina ganglion cells (Gc) in response to variation in motion direction, speed and spatiotemporal frequency tuning. We have evaluated the response of an entire population of Gc for different speeds (ranging from 5º/s up to 300º/s) and we found that the speed response of Gc is far to be homogeneous. For instance, in some cells showing activity modulated by speed, the activity increases with the speed up to a peak, then decay. Maintaining a constant speed of 40º/s we also varied the spatial frequency obtaining a response curve. The characterization of Gc behavior in terms of population coding using MC represent a first step forward the understanding on the capacity of the early visual processing at the retina level.

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59. Ganglion Cells Omissions Responses in Rodent Retina: Wavelength and Natural Stimulus Dependency.

Pizarro, M[1].,Escobar, M[2].,Nettle, C[3]., Hurtado, J[1]., Araya, J[1]., **Palacios, A[4]**., [1]Centro Interdisciplinario de Neurociencia de Valparaiso, , Universidad De Valparaíso.[2]Departamento de Electronica, , Universidad Técnica Federico Santa María.[3]Departamento de Electronica, , Universidad Tecnico Federico Santa Maria.[4]Centro Interdiciplinario de Neurociencia de Valparaiso, Ciencia, Universidad De Valparaíso.

The retina is organized in successive nuclear and synaptic layers, and respond to complex light stimulus with a rich repertoire of behaviors. The retina output is formed by a diversity of ganglions cells with axons conveying action potentials to the brain. Recently it was pointed out that the retina was able to support predictive responses to movement or flashes. In particular, the retina is able to respond to the omission of a single flash from an accurate sequence of short flashes. However, any wavelength dependency of the response is unknown. Here we tested the retina of adult *Octodon degus*, a diurnal dichromate (λ_{max} 360 nm UV and λ_{max} 505 nm green rodent, using an array of multi-electrodes (256 electrodes, 20Kz) able to measure field and action potentials. First a series of short dark or light flashes (40ms) of λ370 or λ500 nm was presented where sometimes a flash cycle is omitted with a certain probability. Second we present a series of short moving approaching and stops natural scenes stimulus sequences, where we study the retinal response to the violation of the expectative-moving forward versus stop- in the sequence. In this natural scenarios some ganglion cells also presented a predictive behavior when the stimulus failed the expectation. The analysis suggests that *degus* retina displays ganglion cells that respond to the omission in simple and more complex ways. This non-standard behavior is wavelength dependent and also related to the statistics content of the input stimulus.

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60. Using orthologous channels to localize molecular determinants involved in chemical and thermal sensitivity of TRPM8 ion channel

Rivera, B[1]., Madrid, R[1]., **Pertusa, M[1]**., [1]Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile.

TRPM8 is the main molecular entity responsible for detection of cold in the somatosensory system. This channel is activated by cold temperatures, voltage and cooling compounds such as menthol. It has been reported that there are major differences in temperature activation threshold and sensitivity to chemical agonists among different TRPM8 orthologs, coincident with they body temperature. Chicken TRPM8 (cTRPM8) displays larger sensitivity to cold and menthol than mouse ortholog (mTRPM8), suggesting that non-conserved regions in cTRPM8 could be able to tune the responses of the channel to cold and menthol. To identify protein domains in cTRPM8 associated with their enhanced sensitivity, we performed chimeras exchanging N- and C-termini between mouse and chicken orthologs of TRPM8, and we assessed their responses to cold and menthol using calcium imaging. We found that the transference of N-terminal domain of cTRPM8 to mTRPM8 (N-ter-cM8/mM8) is sufficient to get the characteristic features of the chicken ortholog, which include responses to cold and menthol larger than in mTRPM8, and a 5oC shift in the temperature threshold of activation to warmer temperatures. On the other hand, chimeric channels that consist of N-terminal and six transmembrane domains of cTRPM8 with the mouse c-terminus (mM8/C-ter-cM8) displayed a largely impaired response to cold combined with a large cTRPM8-like menthol response. These results suggest that both N- and C-terminal domains contain molecular determinants with a relevant role in the thermal and chemical sensitivity of TRPM8 channels.

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61. Role of N-terminal domain on thermal sensitivity of TRPM8 ion channel

Pertusa, M[1].,Gonzalez, A[1].,Hardy, P[1].,Salas, J[1]., Madrid, R[1].,[1]Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile.

TRPM8, a calcium-permeable cation channel activated by cold, cooling compounds such as menthol and voltage, is the main molecular entity responsible for detection of cold temperatures in the somatosensory system. The N-terminal domain of TRPM8 consists of 693 aminoacids and little is known about the contribution of this intracellular domain to the function and regulation of this channel. In order to evaluate the role of N-terminus, we performed different deletions and chimeras with other members of the TRP family, and we evaluated the activity of these mutants using calcium imaging and patch clamp techniques in transfected HEK293 cells. We identify two different regions into the N-terminal domain that contribute to the activity, proper folding and assembly of TRPM8. We found that the deletion or substitution of the first forty aminoacids yield ion channels with higher responses to cold and menthol. These changes include a shift in the temperature threshold of activation towards higher temperatures and a shift in the dose-response curve to menthol to lower concentrations. We also observed that the region encompassing positions 40 to 60 is a key element in the proper folding and assembly of TRPM8; deletions inside this region rendered nonfunctional channels that are retained in the endoplasmic reticulum. Our results suggest an important contribution of the initial region of the N-terminus in the setting of the thermal and chemical sensitivity and the proper assembly and tetramerization of this polymodal ion channel.

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62. Evaluation of the odorant potency of human sweat components using electro-olfactogram in the rainbow trout, oncorhynchus mykiss.

Valdés, J[1]., Ponce , D[1]., Olivares, J[1]., Schmachtenberg , O[1]., [1]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad De Valparaíso. (Sponsored by Financiado Por: FONDECYT 1120513 Y CINV Millennium Institute)

Salmonids detect the presence of predators using visual and chemical cues. Chemicals released by human skin constitute an extraordinarily potent odor for salmonids. To date, it is unknown which or what combination of human skin-derived components are the most potent odorants. Using the electro-olfactogram on live anesthetized rainbow trout, we studied the olfactory potency of the 11 principal constituents of a standardized human sweat. We found that lactic acid and the vitamins thiamine and riboflavin have the highest olfactory potency, being detected at a concentration as low as 50 nM. Pyruvic acid and glucose were detected at concentrations above 130 nM and 5.4 µM, respectively, while acetic acid and urea elicited responses only above 10 µM. An artificial human sweat (AHS) with 25 of its natural constituents was prepared to simulate the effects of natural sweat, but little cross-adaptation was found between the two. Our data identify lactic acid, thiamine and riboflavine as novel potent odorants for salmonids, and show that these compounds constitute the most effective individual odorants among the principal human sweat components. However, the overall odor potency of human sweat is the result of a complex mixture with many active ingredients.

63. The chemosensory cilia of olfactory sensory neurons internalize glucose from the mucus and metabolize it by glycolysis to use as energy source

Villar, P[1].,Reyes, J[2].,Bacigalupo, J[1]., [1]Biología, Ciencias, Universidad De Chile.[2]Química, Ciencias, Pontificia Universidad Católica De Valparaíso.

The chemosensory cilia of olfactory sensory neurons (OSNs) are long and thin structures (~ 60x0.2 µm) and lack internal membranes. A cAMP pathway couples odor binding to membrane receptors with cyclic nucleotide-gated channels opening. The cilia use high ATP levels during chemotransduction, demanded by used by adenylyl cyclase, ATPases and kinases. Our estimates of resting ATP level, ATP diffusion and consumption suggest that nearest mitochondria, at the base of the cilia, are incapable of sustaining chemotransduction along the entire cilium under intensive stimulation. There are glucose transporters in the supporting cells (SCs), suggesting a directional movement through the epithelium towards its apical surface (Nuñez-Parra et al, Chem Senses 36:771, 2012). We hypothesize that SCs release glucose to the mucus from where the cilia incorporated it, and metabolize it to ATP under situations of high energy demands. To test this we detected glycolytic enzymes by immunoblotting in ciliary membranes. We determined cilia and knob accumulation of a fluorescent glucose analog applied to mucosal side of intact olfactory epithelia and to isolated OSNs, suggesting the apical presence of a glucose transporter. By immunocytochemistry we show ciliary localization of this transporter in dissociated rat and toad OSNs. Field recordings (electroolfactogram) revealed that inhibition of glycolysis or oxidative phosphorylation impairs the odor responses. These results suggest that olfactory cilia possess two complementary ATP sources, knob oxidative phosphorylation and ciliary glycolysis. FONDECYT 1100682 (JB), DI/VRIEA/PUCV (JGR)

64. Pannexin 1 participates in chronic pain in rats

Bravo, D[1].,Retamal, J[1].,Ibarra, P[1].,Hernández, A[1]., Pelissier, T[2].,Constandil, L[1].,[1]Biología, Química y Biología, Universidad De Santiago De Chile.[2]ICBM, Medicina, Universidad De Chile. (Sponsored by FONDECYT 1120952)

Chronic pain is a serious public health problem, affecting millions of patients around the world, mainly because pharmacological interventions have shown poor results, with low successful treatments and undesirable side effects. Thus, we investigated the role of a new participant in this process, pannexin 1. This membrane channel has been described as a crucial element in many pathological conditions related with neuronal and glial hyperexcitability, such as epilepsy, stroke and neuroinflammation. However, the role of pannexin 1 in chronic pain has never been studied. Hence, the aim of this investigation was describe the participation of pannexin 1 in this condition. In a model of chronic pain (neuropathy) in rats, we studied the expression of pannexin 1 in the lumbar spinal cord, with western blot. Then, we observed the variations in hyperalgesia in rats, with the paw pressure test, after an intrathecal injection of 10panx, a peptide that selectively blocks Pannexin 1, at different concentrations. Additionally, we studied the variations in spinal wind up, in vivo, after intrathecal injection of 10panx. Wind up is strongly related with NMDA receptor activation. Effectively, we found that pannexin 1 is widely expressed in lumbar spinal cord. Moreover, we found that after 10panx administration, hyperalgesia diminished in a dose-dependent manner. We also observed a 50% diminution of the spinal wind up activity, strongly suggesting an interaction among NMDAR and pannexin 1. Therefore, pharmacological blockage of pannexin 1 modifies hyperalgesia and spinal nociceptive transmission in neuropathic rats, thus demonstrating, for the first time, the participation of this membrane channel in chronic pain.

65. Patch clamp recordings of Ca^{2+}–dependent Cl- channelsfrom chemosensory cilia of dissociated vertebrate olfactory receptor neurons.

Delgado, R[1].,Bacigalupo, J[2].,[1]Biología, Facultad Ceincias, Universidad De Chile.[2]Biologia, Facultad de Ciencias, Universidad De Chile. (Sponsored by FONDECYT 1100682)

Olfactory sensory transduction takes place in the chemosensory cilia of olfactory sensory neurons (OSNs). It involves a cyclic AMP signaling cascade that mediates a depolarizing receptor potential, resulting of the opening of an unselective cation cyclic nucleotide-gated channel (CNG) and a Ca^{2+}-activated Cl- channel (ClCC).The ClCCis activated by Ca^{2+} entering through CNG. The CNG has been well characterized at macroscopic and single-channel levels. However, reported ClCC properties derive from noise analysis of the macroscopic current, as single-channel recordings from olfactory cilia are not available. The main ClCC is thought to be ANO2 (1.2 pS, $K_{0.5}$= 0.4 µM), although ClCCs of other two subfamilies also express in the cilia: Best-2 (0.26 pS; $K_{0.5}$= 1.8 µM; Pifferi et al, 2006) and ClCa4l (González-Silva et al., 2013), suggesting that they may also be implicated. ClCa4l has not been characterized electrophysiologically. We recorded multi-channel currents of three different ClCCs from inside-out patches excised from individual cilia oftoad, rat and mice dissociated OSNs,using high resistance patch-clamp electrodes (>50 Mohm). We recordedchannels withconductances of 11 and 22 pS ($K_{0.5}$= 0.38) in toad; 19 and 42 pS ($K_{0.5}$= 0.5) in rat and 56 and 96 pSin mice, all blocked by niflumic acid (20 µM). We also observe a Ca^{2+} -induced increment in basal noise likely due to unresolved events generated by a low conductance ClCC, which we are currently investigating.

66. Diacylglycerol activates the light-dependent channels TRP and TRPL in the photosensitive microvilli of Drosophila melanogaster photoreceptors

Delgado, R[1].,Muñoz, Y[2].,Peña Cortés, H[3].,Givalisco, P[4].,Roth , A[4].,Bacigalupo, J[4].,[1]Biología, Facultad de Ciencias, Universidad de Chile.[2]Biolgía, Facultatd de Ciencias, Universidad De Chile.[3]Biotechnology Center D. Alkalay Federico Santa Maria.[4]Molecular Plant Physiology Max Planck Institute, Golm, Germany. (Sponsored by FONDECYT 1100730 (RD), CONICYT (YM))

Drosophila light-dependent channels (TRP, TRPL) reside in the light-sensitive microvilli of photoreceptor's rhabdomere. In phototransduction, photoactivated rhodopsin triggers a signaling cascade involving phospholipase-C, which cleaves phosphatidylinositol bisphosphate (PIP_2) generating IP_3, diacylglycerol (DAG) and a proton. DAG is phosphorylated to phosphatidic acid by DAG kinase (DGK) and may also be turned into polyunsaturated fatty acids (PUFAs) by a lipase. It is debated which is the channel activatorof TRP and TRPL: PIP_2, DAG, H^+ or PUFA, while IP_3 has been discarded. We tested each candidate in inside-out TRP-expressing rhabdomeric patches using lipids, mutants and pharmacology. In patches excised under illumination TRP was constitutive active, but could be turned off with millimolar ATP. In contrast, the channel was closedin patches excised in darkness, but could be open with DAG and subsequently closed by ATP.The ATP effect was abolished by inhibiting DGK and absent in rdgA (DGK mutant). This evidence reveals that excised patches retain DGK. DAG activated TRP under a DAG-lipase inhibitor, suggesting that PUFAs are not involved. PIP_2 had no affect and acidification (pH 6.4) irreversibly activated TRP, inconsistent with a regulatory role on the channel. In addition, we studied the lipid profile of a rhabdomere-enriched membrane preparation derived from light- and dark-adapted eyes, by gas-chromatography-Mass-spectrometry. Remarkably, we detected a light-dependent increment in DAG (C34:1). Altogether, the results strongly support DAG as the TRP/TRPL activator andare inconsistent a similar role of PUFAs PIP_2 and H^+.

67. Structural insight into the gating mechanism of nicotinic-receptor channels

Gonzalez-Gutierrez, G[1]., Grosman, C[1]., [1]Molecular and Integrative Physiology, , University of Illinois at Urbana-Champaign.

ELIC and GLIC, two bacterial nicotinic receptor-like channels, are remarkably amenable to both structural and functional studies. This observation, along with the notion that the overall structure and functional properties of these channels are very similar to those of their metazoan counterparts, amply justifies the use of ELIC and GLIC as models for the entire superfamily. At the level of the transmembrane pore, the structural model of ELIC shows a prominent constriction around the 16' position of the M2 alpha-helices, whereas that of GLIC displays a much wider, semi-conical cavity with helices tilted away from the long axis of the pore. As a result, the structural models of ELIC and GLIC are often considered to represent the closed-channel and open-channel conformations, respectively, of all members of the superfamily, a notion that assumes that the end states of the gating conformational change are conserved across orthologs. Thus far, however, attempts to capture the different physiologically relevant conformations of each of these channels in well-diffracting crystals have failed. Here, we used X-ray crystallography to characterize a number of mutations that favor specific conformations of the same channel as assessed electrophysiologically, in a membrane-embedded environment. Our results provide novel structural insight into gating mechanisms in this superfamily of neurotransmitter-gated ion channels and, at the same time, raise questions about allosteric mechanisms and the validity of proton-gated channels as models of channels gated by the binding of neurotransmitter molecules.

68. Tuning a mathematical model of dynamic response in cold-sensitive nerve endings: role of TRPM8 and other conductances

Herrera, G[1].,Maidana, J[3].,Olivares, E[1].,Flores, K[3].,Madrid, R[2].,Orio, P[3]., [1]Programa de Doctorado en Ciencias, Mención Neurociencia, Ciencias, Universidad De Valparaíso. [2]Departamento de Biología, Facultad de Química y Biología, Universidad De Santiago De Chile. [3]Centro Interdisciplinario de Neurociencia de Valparaíso, Ciencias, Universidad De Valparaíso.

The site of thermal transduction of cold-sensitive receptors is the morphologically unspecialized ending of sensory axons. Cold-sensitive nerve terminals (CSNT) encode steady temperatures with regular, rhythmic temperature-dependent firing patterns that range from irregular tonic to regular bursting (static response). During abrupt temperature changes, CSNTs show a dynamic response, transiently increasing their firing frequency on temperatures decreases and silencing upon temperature increases (dynamic response). To date, mathematical models that simulate the static response based on two depolarizing/repolarizing pairs of membrane ionic conductance have been published. These models fail to reproduce the dynamic response of CSNTs under conditions of abrupt changes of temperature. We improved this model by including TRPM8 as a temperature-dependent conductance. Computer simulations show that it appropriately reproduces the dynamic response of CSNT, while preserving the static response behavior experimentally observed in cold sensitive nerve terminals from mouse cornea. We are further characterizing this model and its parameter dependency, and found that the TRPM8 conductance is essential to display a dynamic response. In agreement with experimental results, TRPM8 is also needed for the ongoing activity in the absence of stimulus (i.e. neutral skin temperature). Also, by systematic variations of the depolarizing/repolarizing conductance densities, we have identified their relevance in the CSNT impulse discharge patterns. This allowed us to further refine our model to adequately reproduce the response to a variety of temperature protocols reported in wet experiments. Support: Fondecyt 1130862 & 1131064, ACT-1113. CINV is a Millenium Sciente Institute (Ministerio de Economía, Chile)

69. Non-stationarity of the scalp-recorded auditory steady state response evoked by amplitude-modulated acoustic signals in rats

Prado-Gutiérrez, P[1]., Martínez-Montes, E[1]., Castro-Fariñas, A[1]., Morgado-Rodriguez, L[1]., Diez, T[1].,Velarde, E[1].,[1]Basic Neuroscience Cuban Neuroscience Center.

Based on the classical additive model for the evoked potential generation, the decrease of amplitude that occurs over the sequential averaging of sweeps of auditory steady state recordings has been exclusively attributed to the cancelation of noise. However, this behavior might also reflect the non-stationary response of the ASSR generators. For testing this hypothesis, the time course of the ASSR amplitude was thoroughly characterized in rats. Responses were obtained in eight adult animals stimulated with tones of 8 kHz modulated in amplitude at 115 Hz and presented at 30, 50 and 70 dB SPL. Results show that the amplitude of the ASSR exponentially decreases during the first seconds of stimulation, independently of the background noise cancelation. Higher decreases of amplitude were obtained as the stimulus intensity increased. Introducing a delay between the stimulus onset and the beginning of the averaging reduces the effect of the non-stationarity on the response amplitude estimated after the completion of the averaging. These results provide evidences to develop more realistic models of the ASSR generation and might have implications for audiometric tests designed to objectively assess the supra-threshold hearing in subjects who cannot provide reliable results in the psychophysical trials

70. On the use of chemical modification to determine connexin hemichannel topology and function.

Tong, X[1].,Lopez, W[1].,Liu, Y[1].,Harris, A[1]., **Contreras, J[1]**., [1]Pharmacology and Physiology, New Jersey Medical School, Rutgers University.

Connexin hemichannels can release transmitters (ATP, glutamate) that play key paracrine signaling roles, but the properties of the connexin pore are still poorly understood. Cysteine-scanning mutagenesis, combined with thiol-reagent modification, is a powerful methodology for identifying pore-lining and gating residues. However, in connexin hemichannels this approach has yielded controversial data. We performed cysteine-scanning mutagenesis of several residues within the connexin26 (Cx26) pore, followed by chemical modification using methanesulfonate (MTS) reagents. Strikingly, we observed that modification with MTS reagents, at different pore lining residues, was reversed within minutes of reagent washout. This reversal should not occur unless reducing agents have access to the site. We therefore tested whether the connexin pore allowed cytosolic glutathione, a well-known endogenous cytosolic reductant, to access the sites. We found that inhibition of gamma-glutamylcysteine synthetase, which generates a precursor of glutathione, using buthionine sulphoximine, decreased cytosolic glutathione in Xenopus oocytes and drastically reduced reversibility of MTS modification. Furthermore, we found that cysteine modification by maleimide reagents, which cannot be reversed by glutathione, was not reversed under the same conditions. Using reconstituted hemichannels in a liposome-based transport-specific fractionation assay, we confirmed that homomeric Cx32, Cx26, Cx30 and heteromeric Cx26/Cx32 and Cx26/Cx30 hemichannels are permeable to glutathione. These results suggest that: (a) connexin hemichannels may mediate physiological glutathione release in diverse cell types; (b) maleimide-based modification is the more appropriate approach to perform chemical modification to study structure-function of connexin channels, and other channels and transporters that are permeable to large molecules, such as pannexin channels.

71. *dlg* Regulates Postsynaptic Glu-Receptors and Presynaptic Ca^{+2}-Channel *in Drosophila*.

Astorga, C[1].,Ramirez, M[1].,Delgado, R[2]., Jorquera, R[3]., Sierralta, J[1].,[1]ICBM, Facultad de Medicina, Universidad De Chile.[2]Biología, Facultad de Ciencias, Universidad De Chile.[3]Department of Neuroscience, , Universidad Central del Caribe, PR. (Sponsored by FONDECYT 1120684 And ICM P09-015F)

The DLG-MAGUK subfamily of proteins plays a key role in the structure and function of the synapse. Discs-large (*dlg*) is the only gene of this subfamily in *Drosophila*, originating two protein products, DLGA and DLGS97. In mammals DLG proteins have been implicated in the clustering of postsynaptic glutamate receptors (GLUR). In *Drosophila* DLG also plays a role in the regulation of the size of GLUR clusters. Here for the first time we study the differential contribution of DLGA and DLGS97 to the basal function and plasticity of the larval neuromuscular junction. Miniature current analysis reveals that dlgS97 and double mutants, but not dlgA mutants display increased quantal size. Morphological measurements, indicate bigger GLUR clusters in double mutants and dlgS97 confirming the role of DLG in postsynaptic function and the differential contribution of DLG isoforms. Additional analyses of nerve-evoked release indicates that all mutants compared with control display reduces quantal release, increased facilitation and decreased depression, indicating a reduced release probability. These results, together with the finding of a reduced external calcium sensitivity in the release probability and an abnormal distributions of cacophony calcium channel unveil an undescribed presynaptic function of DLG.

72. An endogenous CaMKII inhibitor as a possible regulator of synaptic strength and plasticity in the hippocampus

Pino, G[1].,Vera, J[1]., Vergara, C[1]., Sanhueza, M[1]., [1]Laboratorio de Fisiología Celular, Facultad de Ciencias, Universidad De Chile.

Calcium/calmodulin-dependent kinase II (CaMKII) is the most abundant protein in hippocampal glutamatergic synapses and its activation, autophosphorylation and binding to the GluN2B subunit of the NMDA receptor are necessary for the induction of long-term potentiation (LTP). A specific CaMKII inhibitor named CaMKIIN, is present in CaMKII-containing cells and its expression is transiently increased after learning. In addition to inhibit CaMKII activity, CaMKIIN interferes with the normal kinase interaction with GluN2B. Peptides derived from CaMKIIN (CN peptides) have provided insights about the possible physiological role of this endogenous inhibitor. Transient application of these peptides persistently depresses transmission and reduces basal CaMKII-GluN2B binding in hippocampal slices. Moreover, subsequent LTP induction is enhanced, and LTP can be reinduced in synapses previously potentiated until saturation. To further investigate these phenomena, we transiently preincubated hippocampal slices with CN or a control peptide (2 h, and 2 h of washing). In the synaptoneurosomal fraction derived from the slices we quantified the total and phosphorylated amount of synaptic CaMKII and of glutamate receptor subunits, by Western Blot. We also evaluated differences in the miniature synaptic currents (minis), by patch-clamp recording. Our results show a significant CN-induced decrease in the GluA1 and GluN1 subunits of AMPA and NMDA receptors, respectively, and in the amount of CaMKII phosphorylation at threonine 286. Moreover, preliminary experiments suggest a decrease in minis amplitude. We propose that CaMKIIN could be acting as a regulator of synaptic strength and subsequent plasticity after learning-induced plastic changes in the hippocampus.

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73. Calcium signaling in muscle cells from patients with RyR mutations causing Malignant Hyperthermia.

Figueroa, L[2].,Kraeva, N[1].,Manno, C[2].,Edwards, J[2].,Riazi, S[1].,**Ríos, E[2]**.,[1]Malignant Hyperthermia Unit (MHIU), , University of Toronto.[2]Molecular Biophysics and Physiology, , Rush University.

Malignant Hyperthermia, MH, is a hereditary sub-clinical condition characterized by events of uncontrolled release of calcium, deadly if untreated. 60% of MH cases are due to mutations of the RyR1 calcium release channel. A puzzling property of these mutations is their phenotypic variability. We are comparing calcium signals in cells from “MHS” patients (positive for 2 standard tests) with those from normal donors (MHN). Subjects are genotyped for the proteins of interest. *Gracilis* muscle biopsies are received overnight from MHIU and studied acutely with voltage clamp and calcium imaging, or used to generate cultures. Myotubes were imaged for $[Ca^{2+}]_{cyto}$ (fluo-4) and V_m (di-8-ANEPPS). In response to brief field stimuli, normal donors had brief action potentials (t ~15 ms) and a monophasic Ca transient, which in rare occasions was followed by a slow wave. Cells from a MHS patient with the mutation G341R always had a 2-phase response —small initial transient followed by a large propagated wave. Two MHS patients with positive reaction to only one of the 2 tests had Ca transients with intermediate features. E Jaimovich and associates have shown that slow Ca release occurs through IP3 receptors. We are currently using pharmacology and local photorelease of IP3 to explore the possibility that IP3-mediated Ca signals are altered and cause some of the phenotypic variability in familiar MH. These approaches should allow a characterization of cellular signaling defects in multiple mutations, linked to MH and other muscle diseases. *Supported by the NIH (USA) and University of Toronto.*

74. Relevance of scavenger receptor a (SR-A) for the inflammatory activation of microglia and astrocytes in alzheimer's disease.

Cornejo, F., Ramírez, G., von Bernhardi, R. Department of Neurology, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile. flcornej@uc.cl

Neuroinflammation is involved in damage mechanisms of Alzheimer's disease. Its main effectors are glial cells, especially microglia and astrocytes, which undergo several changes during this process. Among them, it has been shown that class A Scavenger Receptor (SR-A) expression, which is observed in both abovementioned glial cells, increases in response to inflammatory stimulus. The aim of this study was to evaluate if SR-A has an active role in the inflammatory activation of Glia. We evaluated the role played by SR-A in glial cell activation assessing production of NO by Griess assay, cytokine levels by ELISA, and expression of activation identity markers by immunolabeling and activation of signaling pathways by western blot. In primary glial cultures from wild type ($SR\text{-}A^{+/+}$) and knockout mice for SR-A ($SR\text{-}A^{-/-}$), it was found that lipopolysaccharide (LPS)-stimulated $SR\text{-}A^{-/-}$ astrocytes release more NO than $SR\text{-}A^{+/+}$ astrocytes, effect that was associated with an extended activation of ERK1 signaling pathway. In contrast, evaluation of LPS-induced cytokines production, only $SR\text{-}A^{+/+}$ astrocytes released IL1β. The abolition of IL1β release appeared to be associated with the inhibition of activation of JNK and p38 signaling, and to the delayed activation of IκB/NFκB pathway in response to LPS. Microglia from $SR\text{-}A^{-/-}$ mice failed to show activation-associated morphological changes and were also unable to secrete IL1β, although they showed an increased release of NO. Our results suggest that SR-A participates in the activation of specific inflammatory responses by astrocytes and microglia, and the activation of signaling pathways involved in the production of soluble mediators.

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75. Methylphenidate amplifies long-term plasticity in CA1 of hippocampus via a polysynaptic mechanism.

Contreras D.[1], Carreño M.[1] , Zeise M [2], Morales B[1]. [1]Depto. de Biología, Fac. Química y Biología, Universidad de Santiago de Chile, [2]Escuela de Psicología, Fac de Humanidades, Universidad de Santiago de Chile. darwin.contrerasp@usach.,cl

Methylphenidate (MPH) is a psychostimulant used widely in the therapy of the Attention Deficit/Hyperactivity Disorder and recently has become also a drug of abuse. Our and other laboratories have demonstrated that MPH modifies synaptic plasticity in the hippocampus. However, the cellular and molecular mechanisms involved are still unknown. We investigated the effects of MPH on LTP in hippocampus slices. 3-4 weeks old Sprague-Dawley rats were decapitated under halothane anesthesia, and hippocampus slices (400 µm thick) were prepared. LTP was induced and recorded in CA1 by applying a theta burst stimulation (TBS, 5 trains, 100 Hz) at the Schaeffer collaterals. Superfusion of hippocampal slices during 20 min with MPH, increased in a dose-dependent manner the magnitude of LTP from 134.6±1.2 % (controls) to 137.5 ± 2.8 7% (3nM; n=3,3; $p>0.05$), 163.4±10.4% (50nM; n=5,7; $p<0.05$), 194.3±5.8 (5 µM; n=6,8, $p<0.01$), and 196.4±4.2% (50 µM; n=4,4; $p<0.01$). The paired-pulse curves remained unchanged after perfusion with MPH, suggesting that the effect of MPH does not involve modifications of presynaptic components. The increase induced by MPH was inhibited by 5µM timolol, β-adrenergic blocker, from 194.3±5.8% (TBS+5µM MPH) to 152.7±1.66% (TBS+5µM MPH+Timolol); n=4,4; $p<0.01$). Interestingly, LTP increase was also inhibited by 5 µM of SCH23390, D1/D5 blocker, from 192±7% (TBS+5µM MPH) to 151.3±0.9% (TBS+MPH+SCH23390; n=3,3; $p<0.01$). Both effects probably are postsynaptic because the paired-pulse curves remains unchanged. These results suggest that MPH increases LTP in a dose-dependent manner involving β-noradrenergic and D_1/D_5 receptors through a polysynaptic mechanism.

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76.

Mariqueo T[1,2], Agurto A[1], Murath P[1], Fernandez E[1] and Aguayo LG[1]
[1]Department of Physiology. University of Concepción, Chile,
[2]Programa de Doctorado en Farmacología, Universidad de Chile.

Here we investigated the pre and postsynaptic effects of ethanol on glycinergic currents by using whole cell recordings of C57BL/6J spinal neurons in culture. We found that ethanol increased the frequency of miniature inhibitory postsynaptic currents. This effect involves presynaptic calcium release from internal stores since incubation with a calcium chelator, BAPTA-AM and an inhibitor of Phospholipase C (PLC) prevented ethanol actions. Similar blockade was obtained by incubation with Rp-cAMP, an inhibitor of adenyl cyclase (AC). At the post synaptic level, ethanol increased the decay time constant of spontaneous mIPSCs (30±10% above control) in a group of neurons only (n=13/26 cells). On the other hand, glycine activated recordings were consistently potentiated (69±13% above control), which suggests that ethanol modulates synaptic and non-synaptic receptors in a differential fashion. Supporting the role of G protein modulation on ethanol responses we studied the effects of a non-hydrolyzable GTP analog (GTP-γ-S). GTP-γ-S increased the decay time constant in a group of neurons (28±12%, n=11/19 cells) and potentiated the glycine activated Cl- current in all neurons examined (86±31%). Confocal microscopy studies showed that α1 GlyRs, Gβ and Piccolo colocalized in 40% of synaptic receptors. In summary, this study shows that ethanol increases presynaptic glycine release through activation of PLC and AC dependent pathways. Additionally, co-localization of Gβγ and GlyRs might account for the diversity of ethanol sensitivity at the post synaptic level.

Keywords: glycinergic mIPSCs, spinal neurons, ethanol modulation

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77. *In vitro* and *in vivo* modulation of the inflammatory activation of glial cells by bone marrow mesenchymal stem cells.

Soler, B; Gimpel, J; von Bernhardi, R.
Laboratory of Neuroscience, Neurology Department, School of Medicine, Pontificia Universidad Católica de Chile.
bernarditasoler@gmail.com

Introduction: In Alzheimer disease (AD), glial cell dysregulation is associated with a chronic neuroinflammatory condition, which could be responsible for Aβ accumulation, oxidative stress, synaptic dysfunction, and neurodegeneration.
As a possible treatment for AD, we are studying bone marrow mesenchymal stem cell (MSC) and propose that MSC modulate glial cells response to inflammation and Aβ in AD reducing their cytotoxicity.

Methods: Glial cells were stimulated with LPS for 24 h and then exposed to MSC conditioned media (MSC-CM) for 24 h. MSC under control condition and exposed to 1 µg/ml lipopolysaccharide (LPS) or 10 ng/ml interferon γ were assessed. We evaluated the effect of MSC-CM on Aβ phagocytic and the secretion of IL1β and TNFα by glia. We injected MSC in the hippocampus of AD model mice and evaluated the secretion of cytokines, Aβ levels and inflammation glia markers after 10 days.

Results: Secretion of TNFα and IL1β was reduced by 80% in presence of MSC-CM. Phagocytosis of Aβ by glia increased 5-fold at LPS stimulated conditions, and 30-fold when LPS-treated glia were exposed to LPS-treated MSC-CM. Mouse receiving a stereotaxic injection of MSC in the hippocampus showed a significant reduction in secretion of IL1β and TNFα and Aβ load, and an increase in glial cells CD68 and GFAP markers.

Conclusion: MSC-CM changed the activation pattern of glia, increasing phagocytosis of Aβ and reducing secretion of inflammatory cytokines. Our results suggest that cytotoxicity is reduced by MSC both *in vitro* and *in vivo*.

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