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Sociedad Chilena de Neurociencia

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XI Reunión Anual
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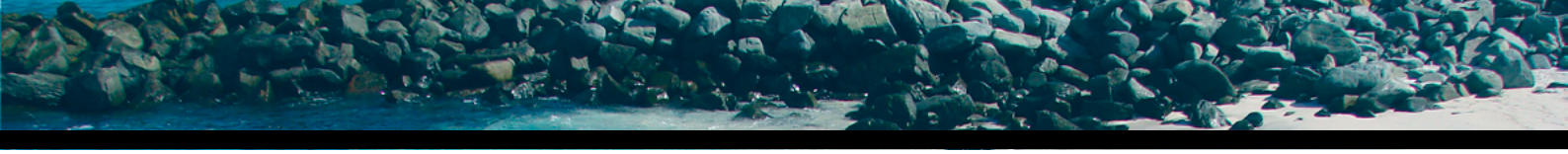
Congreso Conjunto
Coquimbo, Chile 2015

22 AL 25 DE SEPTIEMBRE 2015
Coquimbo, IV Región, Chile.
Hotel Enjoy Coquimbo



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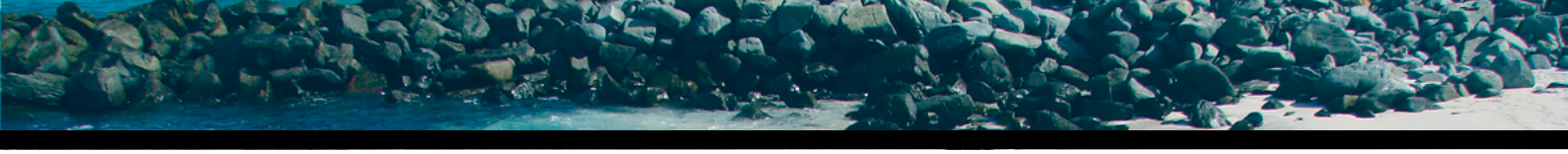

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Message from the Presidents

Dear colleagues and students,

We like to welcome you to the first joint conference of the Chilean Societies for Pharmacology, Physiological Sciences and Neuroscience held in this beautiful region of our country. In putting together this meeting we have taken advantage of our widely common interests, with a certain emphasis in drug addiction. We have chosen this place not only for its mystic beauty, but also to reinforce our commitment as scientists to transmit our endeavor to regions. As part of this pledge, several reach out activities will be conducted with young high school students on the main issue of addiction. This problem will also be discussed from various perspectives in a round table with the participation of relevant non-scientific community members.

We have made every effort to put together an ambitious program covering a variety of topics, with the hope that each one of you will find interesting aspects and will go back home with the feeling of having learned new things, as well as having had a great time. The meeting comprises the participation of very prominent invited foreign and local speakers, who will present us their current work in conferences and symposia. We want to underscore the Young Neuroscientist Symposium sponsored by the Chilean Society for Neuroscience, in which the speakers are advanced graduate students and postdoctoral fellows. We also have included two sessions of short oral presentations, and an special incorporation session with participants applying to become members of the Chilean Society for Pharmacology. There are also two poster presentations, which hold most of the works to be presented at this Joint Meeting and where the participation of students is particularly relevant.

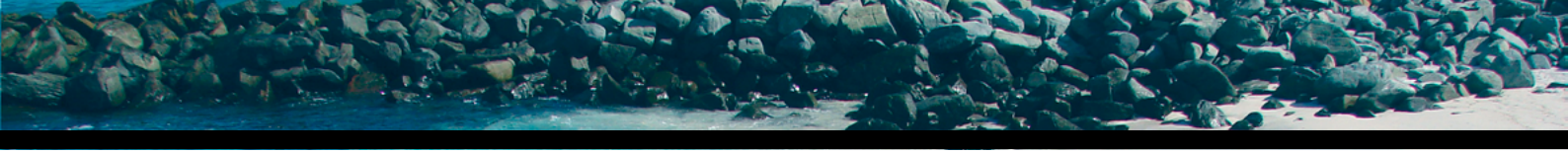
We are very satisfied by the number of people that are attending the meeting. Special attention was paid to the students, as our Societies are providing an important number of fellowships to allow them to take part of this meeting, adding to fellowships generously sponsored by the Comisión Nacional de Ciencia y Tecnología, CONICYT. Gladly, these efforts resulted in that nearly half the attendees are students.

The meeting represents a major financial endeavor for our societies, which would not be possible without the participation of several commercial companies sponsoring it. We like to thank Arquimed, Galénica, Lab-Tec, Loncotec, Sigma, Grupo Bios and Valquim and we encourage you to visit the stands where they show their products, and to attend a couple of technical talks the first day of the Meeting.

We anticipate counting with your presence in as many activities as you can, hoping this meeting will offer an opportunity for intense and fruitful interactions between established and young scientists and students. You are cordially invited to partake in the get together reception on Tuesday evening, and to have fun in the closing Dinner and Dancing on Friday. Use your leisure time on Thursday afternoon to visit the many interesting and unique places of this region of Coquimbo, Elqui Valley and nearby city of La Serena.

Have a good time!

Ramón Sotomayor Sociedad de Farmacología de Chile	Mauricio Boric Sociedad Chilena de Ciencias Fisiológicas	Juan Bacigalupo Sociedad Chilena de Neurociencia



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PROGRAM SUMMARY

	Tuesday Sep 22	Wesdnesday Sep 23
9:00 - 9:30		**Symposium (3)** Pharmacological modulation of neuronal and muscular nicotinic receptors **(J. Fuentealba) Salón Bahía 1**
9:30 - 10:00		
10:00 - 10:30		**Symposium (4)** Multidisciplinary approaches in the study of the brain: from genes to clinic. **(J. Sierralta) Salón Bahía 2**
10:30 - 11:00		
11:00 - 11:30		Coffee Break **Salón Bahía 3**
11:30 - 12:00		Plenary Lecture 2 C. TREVIÑO **Salones Bahía 1 y 2 Juntos**
12:00 - 12:30	Presentaions of Sponsoring Companies Talks Salón Bahía 2 **(12:00 - 13:30 hrs)**	
12:30 - 13:00		LUNCH **(Free Time)**
13:00 - 13:30		
13:30 - 14:00		
14:00 - 14:30	Registration **Salón Arena**	
14:30 - 15:00		Symposium (5) Young neuroscientists symposium **(A. Palacios) Salones Bahía 1 y 2 Juntos**
15:00 - 15:30		
15:30 - 16:00	Opening Remarks	
16:00 - 16:30	Opening Lecture **F. BEZANILLA Salones Bahía 1 y 2 Juntos**	
16:30 - 17:00		Break
17:00 - 17:30	Coffee Break **Salón Bahía 3**	
17:30 - 18:00		Coffee & POSTER SESSION **Salón Bahía 3**
18:00 - 18:30	**Symposium (1)** Physiology and pathophysiology of the renin-angiotensin-aldosterone system in the kidney **(A. González)** Salón Bahía 1. **Symposium (2)** New molecular targets for the treatment of alcoholism **(M. Rivera) Salón Bahía 2**	
18:30 - 19:00		
19:00 - 19:30		Plenary Lecture 3 R. de KLOET **Salón Bahía 1 y 2 Juntos**
19:30 - 20:00	Round Table Salones **Bahía 1 y 2 Juntos**	
20:00 - 20:30		**Asamblea socios** (Neurociencia) **Salón Bahía 1 / Oral Communications** (SOFARCHI) **Salón Bahía 2**
20:30 - 21:00	Welcome Cocktail **Foyer Ballroom**	
21:00 - 21:30		
21:30 - 22:00		
22:00 - 22:30		
22:30 - 23:00		

	Thursday Sep 24	Friday Sep 25
9:00 - 9:30	**Symposium (7)** Extrinsic and intrinsic signals that modulate brain fetal/neonatal programming **(P. Haeger) Salón Bahía 1**	**Symposium (9)** Aging & Neurodegenertion **(C. Hidalgo) Salón Bahía 1**
9:30 - 10:00		
10:00 - 10:30	**Symposium (8)** Physiological and structural insights of ion channels and membrane receptors **(C. Coddou)Salón Bahía 2**	**Symposium (10)** Neuropharmacology of stress, anxiety and depression. **(J. Bravo) Salón Bahía 2**
10:30 - 11:00		
11:00 - 11:30	Coffee Break **Salón Bahía 3**	Coffee Break **Salón Bahía 3**
11:30 - 12:00	Plenary Lecture 4 G. TORRES **Salones Bahía 1 y 2 Juntos**	Plenary Lecture 5 J.C. SAEZ **Salón Bahía 1 y 2 Juntos**
12:00 - 12:30		
12:30 - 13:00	LUNCH & POSTER SESSION **Salón Bahía 3**	LUNCH **(Free Time)**
13:00 - 13:30		
13:30 - 14:00		
14:00 - 14:30		**Symposium (11)** Pharmacological approaches for pathophysiological conditions associated with hypoxia and oxidative stress **(R. Castillo) Salón Bahía 1**
14:30 - 15:00		
15:00 - 15:30		**Symposium (12)** GABAergic, Memory and Stress **(J. Stehberg) Salón Bahía 2**
15:30 - 16:00		
16:00 - 16:30		Coffee Break **Salón Bahía 3**
16:30 - 17:00	Free Time	
17:00 - 17:30		Oral Presentations I **Salón Bahía 1** & II **Salón Bahía 2**
17:30 - 18:00		
18:00 - 18:30		
18:30 - 19:00		Plenary Lecture 6 A. GRACE **Salones Bahía 1 y 2 Juntos**
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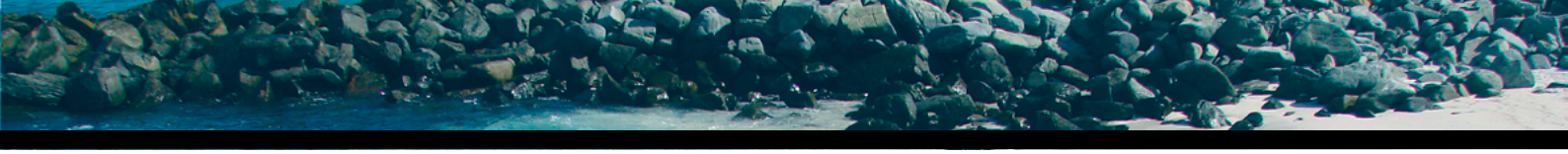
19:30 - 20:00		Concluding remarks
20:00 - 20:30		
20:30 - 21:00		Dinner & Dancing **Terraza Spa (21:00 - 01:00)**
21:00 - 21:30		
21:30 - 22:00		
22:00 - 22:30		
22:30 - 23:00		

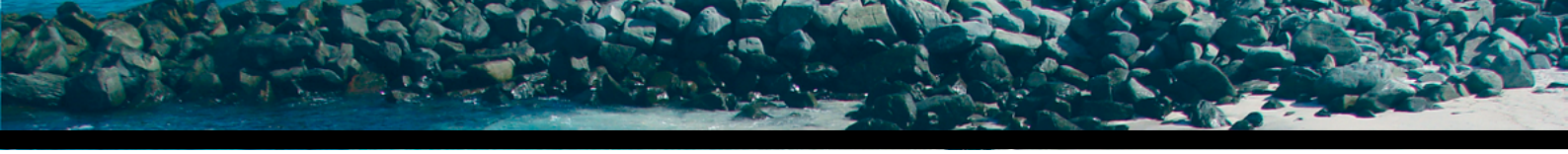


CONFERENCES

STIMULATING NEURONS WITH LIGHT AND GOLD

Bezanilla, Francisco[1]., Treger, Jeremy[2]., Carvalho-de-Souza, Joao[2]., Dang, Bobo[3]., Kent, Stephen[2]., Pepperberg, David[4]., [1]Biochemistry and Molecular Biology, professor, University of Chicago. [2]Biochemistry and Molecular Biology University of Chicago. [3]Chemistry University of Chicago. [4]Ophtalmology and Visual Sciences University of Illinois at Chicago.

Stimulation of neurons with light is a useful tool in understanding the circuitry of the nervous system. For example, it has long been known that pulses of infrared light (IR of wavelength ~ 2000 nm) are effective in exciting neurons and other excitable tissues. Recently we have found that the mode of action of IR pulses is via a rapid increase in temperature. The fast temperature increase changes the membrane capacitance (C) producing a current proportional to dC/dt, which depolarizes the membrane and initiates an action potential. This technique has two important drawbacks. First, it lacks localization of the delivered light energy, consequently is hard to aim at certain type of neurons and second IR of 2000 nm does not penetrate beyond 0.1 mm of tissue. We have sought to overcome these problems using visible light but still taking advantage of the same mechanism to excite the cells. Here we show that gold nanoparticles (20 nm in diameter) can be conjugated to high-avidity ligands for a variety of cellular targets. Once bound to a neuron, these particles transduce millisecond pulses of light into heat, via surface plasmon resonance, which changes membrane capacitance, depolarizing the cell and eliciting action potentials. The temperature increase does not exceed 2 C but it changes fast enough to induce a current proportional to dC/dt. Compared to non-functionalized nanoparticles, ligand-conjugated nanoparticles highly resist convective washout and enable photothermal stimulation with lower delivered energy and resulting temperature increase. Ligands targeting three different membrane proteins were tested; all showed similar activity and washout resistance. This suggests that many types of ligands can be bound to nanoparticles, preserving ligand and nanoparticle function, and that many different cell phenotypes can be targeted by appropriate choice of ligand. Combining optical detection of membrane potential and targeted gold nanoparticles we could stimulate and record from brain slices in an all-optical experiment. Our findings have applications as an alternative to optogenetics (without genetics) and potentially for therapies involving neuronal photostimulation. Supported by NIH grants EY023430, GM030376, and EY001792.



PHARMACOLOGY: TOOLS TO UNDERSTAND SPERM PHYSIOLOGY

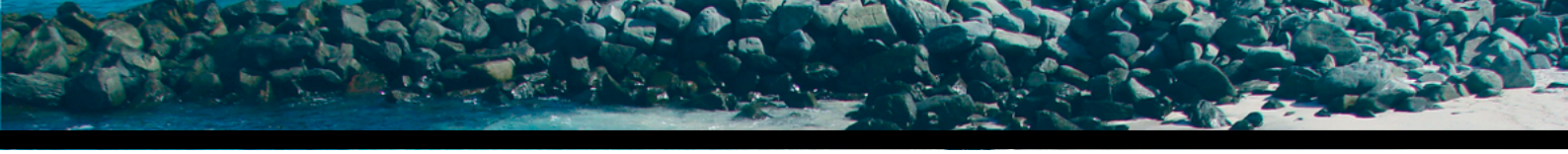
Trevino, Claudia[1]., Torres, Paulina[1].,Sánchez-Carranza, Oscar[1].,Darszon, Alberto[1].,López-González, Ignacio[1].,[1]Genética del Desarrollo y Fisiología Molecular Universidad Nacional Autónoma de México. (Sponsored by CONACyT 128566, PAPIIT IN204914 And The Alexander Von Humboldt Foundation)

Spermatozoa are uniquely equipped to reach, recognize and fuse with the egg. To perform these tasks, spermatozoa must be prepared to face constantly changing surroundings, and to overcome several physical barriers. Since they are basically transcriptionally and translationally silent, sperm cells rely profoundly on diverse signaling mechanisms to swim towards the egg,and to adjust to challenging environmental conditions. Deciphering these signaling pathways is of utmost importance to understand the process of fertilization and contribute to solve infertility problems or design novel contraceptive methods. Popular methodologies such as gene silencing or mutagenesis cannot be applied in sperm cells. Therefore to determine the molecular entities that participate in sperm functions, most of the studies rely on the use of drugs. We have special interest in two sperm specific ion channels whose absence produces sterility. One of them is a K^+ channel named Slo3, in mouse sperm Slo3 (mSlo3) is responsible for the plasma membrane hyperpolarization associated to capacitation (CAH), a prerequisite for fertilization. Recently, using flow cytometry, we reported that human spermatozoa also undergo CAH and our pharmacological studies in human sperm suggested the participation of at least two Slo family K^+ channels. Meanwhile, controversy exists as to whether Slo3, Slo1, or both, are expressed in human sperm. Since the specific pharmacological profile of heterologously expressed human Slo3 (hSlo3) K^+ channels had not yet been explored, we were unable to establish the precise identity of the involved K^+ channel(s). In the present study, we first determined the pharmacological profile of heterologously expressed human hSlo3 in Chinese Hamster Ovary cells, and then compared it with the human CAH pharmacological profile. We found that heterologously expressed hSlo3 possess a pharmacological profile that differs from that of mSlo3, consistent with species-specific differences observed among other sperm ion channels. While both the correlation analysis of hSlo3 currents and the CAH pharmacological profile confirmed the participation of hSlo3, they also suggest that additional K^+ channels may be involved in CAH, in particular Slo1 channels.

STRESS AND THE BRAIN: FROM ADAPTATION TO DISEASE

De Kloet, Ron[1]., [1]Endocrinology and Metabolic Disease, Medicine, Leiden University Medical Center. (Sponsored by Royal Netherlands Academy Of Arts And Sciences)

Cortisol and corticosterone (CORT), secreted from the adrenals as end product of the hypothalamus-pituitary-adrenal (HPA) axis, have a profound action on structure, plasticity and function of specific brain circuits underlying stress adaptation. This action exerted by the stress hormones is mediated by two receptor systems: glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) that modulate slow gene transcription as well as fast non-genomic responses. MR has a principal localization in limbic circuitry including hippocampus and amygdala; GR is expressed, unevenly, in every brain cell, particularly if involved with regulation of the stress response. MR and GR operate in complementary manner during three different phases of the stress response. First, the onset of the stress reaction when MR mediates CORT action on appraisal of novel information and emotional reactivity. Second, the termination characterized by CORT promoting behavioural adaptation and memory storage. Third, the basal phase when ultradian and circadian oscillations of CORT permit recovery and growth, while maintaining the brain's responsivity to stress. Upon imbalance of MR:GR-regulated limbic-cortical signaling pathways the initiation and/or management of the neuroendocrine stress response is compromised. At a certain threshold this may lead to a condition of HPA axis dysregulation and impaired behavioural adaptation, which can enhance susceptibility to stress-related psychopathology. Such imbalance can be inherited through genetic variation of the receptors, or is imposed by stressful experiences, notably in early life, that moderate gene expression by epigenetic processes: DNA methylation and/or histone acetylation. In chronic stress models of human psychopathology we observed in hippocampal neurons a large genomic reorganization that is revealed upon acute challenge with CORT and is characterized by activation of intracellular signaling pathways involved in chromatine organization and epigenetic processes. In behavioural realm such stressful conditions shift resources from amygdala – hippocampus/prefrontal cortex to amygdala- striatal pathways, that underlie more simple habit behaviour for coping purposes. This switch can be prevented by manipulating the MR, supporting the evidence that this CORT receptor is a prominent target to promote stress resilience. De Kloet ER (2014) From Receptor Balance to Rational Glucocorticoid Therapy. *Endocrinology* 155: 2754-2769.



NEW SIGNALING MECHANISMS IN THE ACTIONS OF AMPHETAMINE

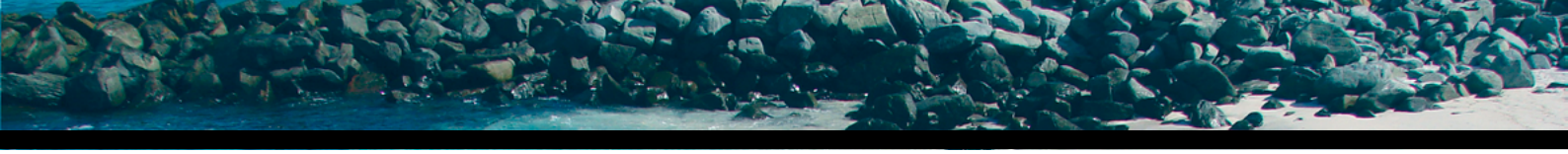
TORRES, G[1]., [1]Pharmacology, Associate Professor, University of Florida.

The dopamine transporter (DAT) regulates extracellular dopamine (DA) levels and signaling via uptake and efflux. Addictive psychostimulants such as amphetamine increase extracellular DA levels in motivational and reward brain areas by targeting DAT. Examining the basic mechanism(s) that affect DAT efflux is critical for understanding both fundamental aspects of DA regulation and for clinical intervention in DA-related brain disorders associated with the therapeutic use and abuse of psychostimulants. Several studies have revealed a plethora of protein-protein interactions influencing DAT distribution and activity; suggesting that the fine-tuning of DA homeostasis occurs via an elaborate interplay of multiple mechanisms. We recently reported that βγ subunits of G proteins bind directly to the C-terminus (residues 582-620) of DAT to down-regulate uptake activity. Here we report that the novel DAT/ Gβγ interaction also promotes DA efflux through DAT. Specifically, activation of Gβγ subunits using mSIRK increased DA efflux through DAT using both heterologous cells and primary neurons in culture. This effect was blocked in the presence of gallein, a Gβγ inhibitor. Likewise, a TAT-peptide containing the Gβγ interacting domain of DAT blocked the ability of mSIRK to induce DA efflux, suggesting the effect of Gβγ on efflux is a result of a direct interaction with the transporter. Based on these data, we hypothesized that Gβγ may also be involved in the actions of amphetamine. In similar efflux experiments, amphetamine induced a dose-dependent increase in DA efflux in both heterologous cells and primary neurons in culture. More importantly, amphetamine-induced efflux was blunted in the presence of either gallein or the TAT-peptide containing the DAT interacting domain. Furthermore, inhibition of Gβγ with gallein also attenuated the amphetamine-induced locomotor activity *in vivo*. Collectively, our data suggest that the direct interaction of Gβγ subunit with DAT has an important role in both physiological and amphetamine-induce DA efflux.

GAP JUNCTION CHANNELS AND HEMICHANNELS: FROM FUNDAMENTAL SCIENCE TO THEIR INVOLVEMENT IN INFLAMMATORY RESPONSES OF CHRONIC HUMAN DISEASES

Sáez, Juan C[1],. [1]Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago-Chile and Instituto Milenio, Centro Interdisciplinario de Neurociencias de Valparaíso, Valparaíso, Chile.

Most cells of the animal kingdom express gap junction channels (GJCs), which form membrane specializations found at cell interfaces called gap junctions. GJCs allow transfer of ions and small molecules between contacting cells. In mammals, they are formed by proteins called connexins and their physiological function is to coordinate metabolic and electrical responses among members of cell communities; they also play a relevant, but less studied functional role as cell-cell adhesion structures. More recently, a new form of membrane channels, termed hemichannels (HCs; half of GJ channel) have been found in the cell surface of most cells where they transiently communicate the intra and extracellular compartments; also, they can be formed by pannexins (unrelated to connexins). Now days, HCs are recognized as cell membrane pathways for releasing autocrine and paracrine cell signals as well as for transferring metabolites. Gap junction channels and HCs present distinct unitary current events, permeability features and regulatory properties. In several inherited human diseases loss of gap junctional communication and/or gain of HC function have been shown to be directly related to the pathological condition. Inhibition of HCs prevents cell degeneration most likely due to the reduction of Ca^{2+}influx that promotes inflammasome activation. Accordingly, selective HC blockers are effective in reducing cellular dysfunction and degeneration in animal models of chronic human diseases of different etiology, including muscle atrophy, Alzheimer's disease and epilepsy. Consequently, selective HC blockers could become a new generation of anti-inflammatory agents to reduce tissue dysfunctions in chronic diseases.



THE CIRCUITRY OF DOPAMINE SYSTEM REGULATION AND ITS DISRUPTION IN SCHIZOPHRENIA AND DEPRESSION

Grace, Anthony[1]., [1]Departments of Neuroscience, Psychiatry and Psychology University of Pittsburgh, PA, USA.

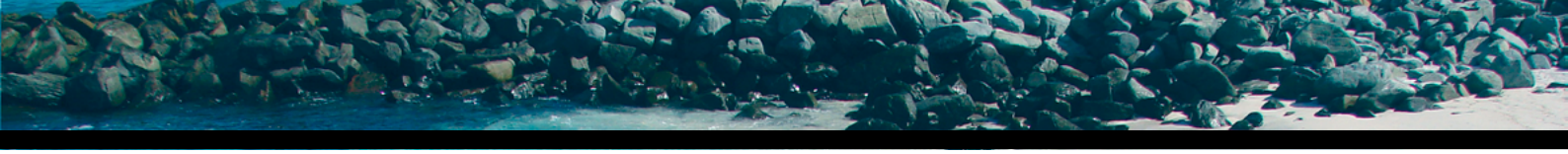
The dopamine-containing neurons of the midbrain have been implicated in a broad array of psychiatric disorders, ranging from schizophrenia to drug abuse and depression. However, studies seem to indicate that it is not the dopamine neurons themselves that are responsible for these pathological states, but instead the disorders appear to arise due to a disruption of dopamine neuron regulation by afferent inputs. Dopamine neurons recorded in vivo are known to exhibit multiple functional activity states, including baseline tonic firing and phasic activation in response to salient stimuli. Phasic burst firing is believed to be the behaviorally relevant "signal" of the dopamine neuron, whereas the level of tonic discharge represents the "gain" or the level of amplification of this signal. This tonic gain is differentially regulated by multiple brain regions, including the hippocampus, the amygdala, and the prefrontal cortex. Disruptions in these regions can interfere with the normal tonic/phasic balance within the dopamine system. Electrophysiological and behavioral studies in animal models of psychiatric disorders, as well as and human imaging studies in patients, suggest that this disruption may underlie the pathological state of the dopamine system that is present in psychiatric disorders. Specifically, we found that hippocampal hyperactivity in schizophrenia may be responsible for the hyperdopaminergic state of psychosis, whereas prefrontal cortical-amygdala overdrive diminishes reward-related dopamine neuron activity leading to anhedonia in depression. This type of information can contribute both to a better understanding of the pathophysiology of major psychiatric disorders, as well as glean insights into novel avenues of treatment and potentially in preventing the emergence of these disorders.

ROUND TABLE

ALCOHOL Y CEREBRO ADOLESCENTE, UNA MIRADA BIO-PSICO-SOCIAL

FINANCIA: Chilean Chapter of the Society for Neuroscience USA
Coordina: María Estela Andrés

Participantes: Katia Gysling, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile.
Paulo Egenau, Director Ejecutivo de la Fundación Paréntesis.
Carlos Ibáñez, Psiquiátra de la Unidad de Adicciones, Clínica Psiquiátrica Universitaria, Universidad de Chile.



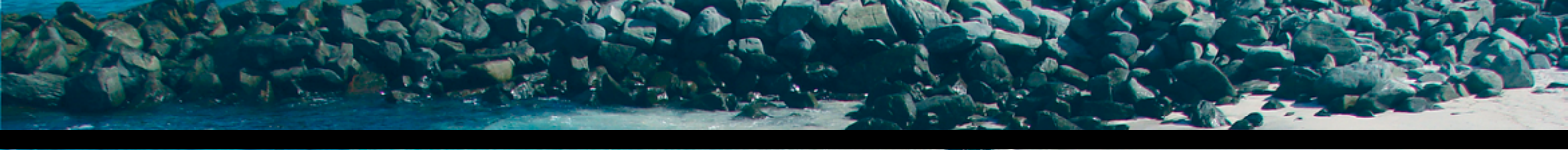
SYMPOSIA

SYMPOSIUM
PHYSIOLOGY AND PATHOPHYSIOLOGY OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN THE KIDNEY: FROM CELLS AND ANIMAL MODELS TO HUMAN DISEASE
Chair: Alexis González

REGULATION OF RENIN IN THE RENAL COLLECTING DUCT CELLS

Gonzalez, Alexis A[1]., [1]Instituto de Quimica, Facultad de Ciencias, Pontificia Universidad Católica De Valparaíso. (Sponsored by FONDECYT 11121217)

Renin-angiotensin system (RAS) plays a major role in the physiological control of blood pressure and fluid volume. RAS dysregulation is also responsible for the development of hypertension. Beside the systemic RAS, which is mainly controlled by the production and release of renin from the juxtaglomerular (JG) cells in the kidney, the RAS is also present in tubular segments of the nephron. In particular, renin has been detected in renal principal collecting duct (CD) cells. Although the regulation of JG renin is well known, the mechanism by which renin is regulated in the CD and its potential role in contributing to RAS activation and distal sodium reabsorption have not been investigated. In M-1 CD cell line, Ang II increased cAMP levels, renin mRNA expression, prorenin and renin protein abundance and renin activity in culture media. This effect was prevented by PKC inhibitor calphostin C, PKC-alpha dominant negative and by PKA inhibition. Forskolin-induced increase in cAMP and renin expression was prevented by calphostin C. PKC inhibition and Ca2+ depletion impaired Ang II-mediated CREB phosphorylation and renin upregulation. Adenylate cyclase 6 (AC) siRNA remarkably attenuated the Ang II-dependent upregulation of renin mRNA. Physiological activation of AC with vasopressin increased renin expression in M-1 cells. The results suggest that the Ang II-dependent upregulation of renin in CD cells depends on PKCα, which allows the augmentation of cAMP production probably via AC6 and activation of cAMP/PKA/CREB pathway. This is a novel mechanism responsible for the regulation of local RAS in the distal nephron.



ROLE OF 11B-HYDROXYSTEROID DEHYDROGENASE-2 IN ENDOCRINE ARTERIAL HYPERTENSION

Carvajal, Cristian[1]., [1]Endocrinology, Medicine, Pontificia Universidad Católica De Chile. (Sponsored by This Work Was Supported By Chilean Grants: FONDECYT 1130427 (CFB) And 1150427 (CCM), CORFO 13CTI-21526-P1 And The Millennium Nucleus On Immunology And Immunotherapy P09/016-F (ICM).)

Arterial Hypertension (AH) is a multifactorial condition that mostly affects renal, cardiac and vascular tissues. Here aldosterone is highlighted as key player in both physiological and patho-physiological conditions. Similar to aldosterone, cortisol can bind to mineralocorticoid receptor (MR) in epithelial tissues and non-epithelial tissues with similar affinity and could trigger the same hypertensive effects. This is particularly relevant in human physiology since concentrations of circulating cortisol are 1,000-2,000 times higher than aldosterone, the natural agonist of MR. In normal conditions, activation of MR by cortisol does not occur, because action of 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) enzyme, which inactivates efficiently cortisol (F) to cortisone (E), avoiding the binding of F to MR. In 11βHSD2 deficiency, AH occurs due to agonist action of cortisol over MR, which produces in renal tissue an increase in sodium re-absorption, and a vascular response that finally increase the blood pressure. We have shown that AH associated to partial or severe 11βHSD2 deficit could be more frequent than expected. We studied the 11βHSD2 deficiency by a sensitive LC-MS/MS determination of both F and E, and we found a high F to E ratio, with normal levels of cortisol tetrahydrometabolites. It suggested a high prevalence of 11βHSD2 enzyme disorders that are undiagnosed, which could reach up to 15% of the general hypertensive population. This prevalence associated to low frequencies of HSD11B2 genetic alterations, led us to postulate that other factors and epigenetic modifications, such as promoter CpG methylation and specific miRNA in HSD11B2 gene, could influence dynamically the onset and progression of the hypertensive disease. In this respect, we have recently proposed and studied the association of 11BHSD2 with aging, salt intake and epigenetic modifications.

ROLE OF THE IMMUNE SYSTEM IN ANGIOTENSIN AND ALDOSTERONE DEPENDENT HYPERTENSION

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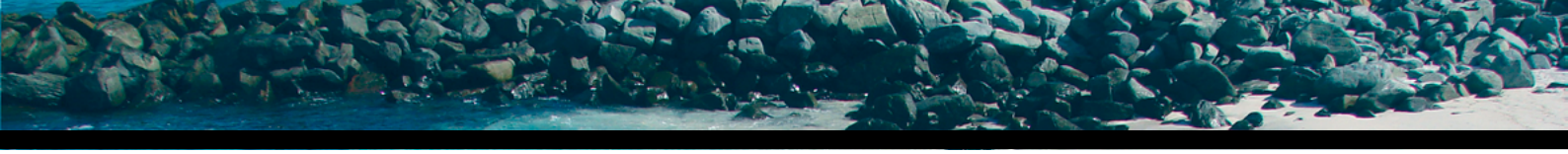
Hypertension is a major cardiovascular risk factor that induces tissue damage, morbidity and mortality. In the clinical context, in the vast majority of hypertensive patients the cause for the development of high blood pressure is unknown. However, it is known that the inadequate activation of the Renin-Angiotensin–Aldosterone System (RAAS) cause hypertension and target organ damage, characterized by oxidative stress, low-grade chronic inflammation, hypertrophy and fibrosis in kidney and in the cardiovascular tissue. Furthermore, the pharmacological blockade of the RAAS is effective to lower blood pressure in hypertensive patients. Previous data have suggested that the activation of the adaptive immune responses in the development of hypertension/tissue damage. The state of the art concerning the role of the immune system in the development of hypertension and target organ damage will be presented. The data supporting the activation of adaptive immune response due to experimental RAAS-dependent hypertension will be presented. The available evidence indicate the activation of lymphocyte T helper 1 (Th1), Th17 immune responses and the down regulation of Treg in RAAS-dependent hypertension. More recently, the use of genetically modified mice has allowed to investigate the role of of Dendritic Cells (DC), myeloid cells that orchestrate the immune response, in the initiation of hypertension and the establishment of low-grade inflammation and fibrosis . The results indicate that DCs (CD11cHi cells) are necessary for hypertension development/maintenance and tissue damage due to the hyperactivity of the RAAS. These effects possibly are independent of adaptive immunity activation.



ROLE OF THE PRORENIN RECEPTOR IN THE MODULATION OF THE INTRATUBULAR RAS

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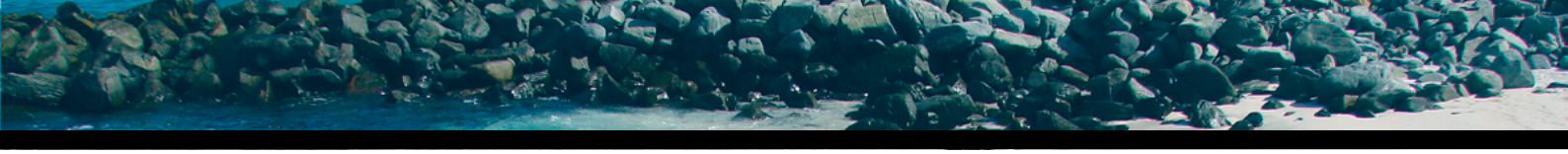
Augmentation of renin synthesis and activity in the collecting ducts provides a novel pathway for intrarenal and intratubular Ang II formation due to the presence of angiotensinogen substrate and angiotensin converting enzyme in the nephron. The binding of the prorenin receptor (PRR) to renin or prorenin enhances renin activity and fully activates the biologically inactive prorenin, respectively. The PRR are augmented in the renal inner medulla and urine of Ang II hypertensive rats. Renin and the PRR may interact to increase intratubular Ang II formation in the distal nephron. The local interaction of prorenin and PRR also stimulates intracellular signals that may contribute to the development and progression of hypertension. However, the demonstration that the PRR is also able to up-regulate COX-2 in inner medullary collecting duct (IMCD) cells, raises the question whether the PRR plays a dual role in the regulation of blood pressure. Ang II increases soluble PRR (sPRR) secretion through an AT1 receptor and furin-mediated mechanism in IMCD cells. In Ang II-infused rats for 2wk during the early phase of Ang II-dependent hypertension, the PRR stimulates COX-2 and subsequently PGE2 in the renal medulla, which attenuates the vasoconstrictor effects of Ang II on renal hemodynamics. In contrast, after 2w of Ang II infusion, increased sPRR distal luminal secretion occurs along with decreased PRR abundance on collecting duct plasma membranes. The evidence reflects that the complexity between short and long term modulatory effects of the PRR depend on the status of the RAS activation in the collecting duct.

SYMPOSIUM
NEW MOLECULAR TARGETS FOR THE TREATMENT OF ALCOHOLISM
Chair: Mario Rivera

NEW ROLES FOR MU-OPIOID RECEPTORS IN THE PHARMACOLOGICAL EFFECTS OF ETHANOL

Rivera-Meza, Mario[1,2]., Urra, Jonathan[1]., Berríos/Cárcamo, Pablo[1]., Herrera-Marschitz, Mario[1,2]., Quintanilla, María Elena[1]., [1]Programa de Farmacología Molecular y Clínica, Facultad de Medicina, Universidad De Chile. [2]Millenium Scientific Initiative Biomedical Neuroscience Institute. (Supported by: FONDECYT #11130241: BNI P09-015-F.)

Relapse to alcohol use is the major problem in the treatment of alcoholism, while the anti-relapse medications available have only limited clinical efficacy. Animals that have chronically consumed ethanol and are deprived of it for long periods show a great increase in their alcohol intake (binge) when its access is re-allowed. This binge-drinking behavior, termed the alcohol deprivation effect (ADE), constitutes an accepted animal model of alcohol relapse. The biological and neurochemical bases of ADE are not known; however animal studies have showed that ADE binge-drinking results from an enhancement of the rewarding properties of ethanol. It has been reported that animals displaying a higher expression of opioid receptors show increased levels of alcohol intake. However, recent reports indicate that chronic ethanol intake can induce a desensitization of mu-opioid receptors, likely by receptor synthesis inhibition or by receptor uncoupling-internalization process. A subsequent rebound in opiate receptor levels or membrane re-externalization could conceivably be involved in the development of the ADE in which ethanol is withdrawn for prolonged periods. Animal studies have showed that acetaldehyde, the first metabolite of ethanol, is a motivational and reinforcing molecule. In the brain, ethanol-derived acetaldehyde can condense with dopamine to generate salsolinol. This compound is self-administered intracranially by animals, suggesting that salsolinol is the resulting molecule mediating the rewarding effects of acetaldehyde and ethanol. Recent evidence indirectly suggests that salsolinol may exert its action through an opiate mechanism, however there are no studies showing that acetaldehyde or salsolinol acts directly on opioid receptors. In this presentation we will report *in vivo* and *in vitro* studies aimed at determining *(i)* whether an upregulation of mu-opioid receptor is involved in the development of the ADE binge-drinking in UChB alcohol-preferring rats and *(ii)* whether ethanol-derived salsolinol mediates its effects through an opioid receptor mechanism. These studies will help to determine the role of the opiate system in the development of ADE binge-drinking and may be of value in the development of new therapeutic strategies against alcoholism.



SALSOLINOL, AN ETHANOL METABOLITE, EXERTS MOTIVATIONAL EFFECTS LEADING TO INCREASES IN ETHANOL INTAKE

Quintanilla, M[1,2,3]., Rivera-Meza, Mario[3]., Berríos, Pablo[3]., Bustamante, Diego[3]., Morales, Paola[3]., Cassels, Bruce[4]., Herrera-Marschitz, Mario[3]., Israel, Yedy[3]., [1]Programa de Farmacología Molecular y Clínica, Facultad de Medicina, Universidad De Chile. [2]Programna de Farmacología Molecular y Clínica, Facultad de Medicina, UNiversidad de Chile. [3]Departamento de Farrmacología Molecular y Clínica, Facultad de Medicina, Universidad de Chile. [4]Departamento de Química, Facultad de Ciencia, Universidad de Chile.

In the brain, racemic salsolinol [(RS)-Sal] is generated by the condensation of acetaldehyde (generated from ethanol) with dopamine. Previous reports have shown that (*RS*)SAL is self-infused by rats into the posterior ventral tegmental area (VTA) at significantly lower concentrations than those of ethanol and acetaldehyde, suggesting that (*RS*)SAL is a most active product of ethanol metabolism. In the present studies with UChB rats bred for their alcohol preference we initially assessed the effect of (*RS*)SAL injected repeatedly intra-VTA (30.0 pmol/0.2 µl) or intraperitoneally (10 mg/kg) on: (i) place preference, (ii) locomotor activity and (iii) ethanol intake. Results showed that (*RS*)SAL injected either into the VTA or administered intraperitoneally led to (i) conditioned place preference, (ii) locomotor sensitization and (iii) a marked increase in ethanol intake, which was suppressed by naltrexone. Salsolinol was detected in the brain extracellular fluid following the systemic (i.p.) administration of 10 mg/kg of (*RS*)SAL, suggesting that (*RS*)SAL is able to cross the blood brain barrier. In subsequent studies we separated and injected the enantiomers (*R*) SAL or (*S*)SAL (30 pmol/1.0 µl) into the VTA of naïve rats to study whether one or both SAL enantiomers is/are responsible for the motivated behavioral effects, sensitization and increase in voluntary ethanol intake. These studies showed that repeated administration of (*R*)SAL led to (i) conditioned place preference, (ii) locomotor sensitization and (iii) marked increases in binge-like ethanol intake. Conversely, (*S*)SAL did not influence any of these parameters. Overall, data indicate that (*R*)SAL administration to rats stereospecifically induces motivational and behavioral sensitization effects, and leads to marked increases in intoxicating ethanol intake.

Supported by FONDECYT grants #1130012, #11130241, and #1120079 and the Millennium Scientific Initiative BNI P09-015-F

PERINATAL LEAD EXPOSURE AND INCREASED ETHANOL INTAKE: THE ACETALDEHYDE CONNECTION

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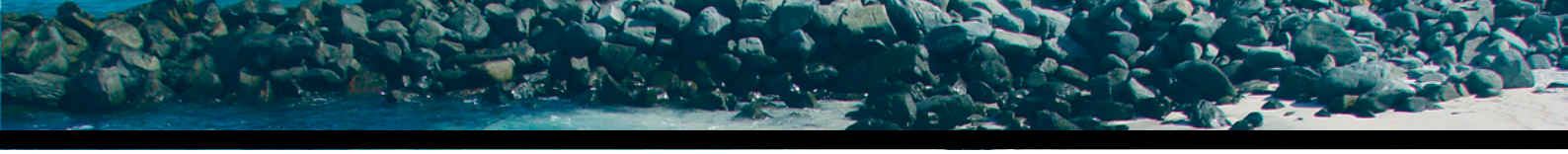
Emerging data reveal that developmental exposure to environmental neurotoxicants induces differential reactivity to challenging events later in life, including drug addiction. We have demonstrated that low level lead (Pb) exposure (220 ppm in drinking water) during gestation and lactation enhances the motivational and stimulants responses to ethanol in periadolescent male pups. In an attempt to unravel the neurobiological basis of these differences, and based on our own and other authors' evidences, we have attributed a critical role to brain ethanol metabolism as a probable mechanism for its reinforcing effects. It is noteworthy that given the reported ADH low activity in the brain, catalase catalyses central ethanol oxidation to acetaldehyde, whereas ALDH favors acetaldehyde oxidation to acetate. We thus first demonstrated that catalase activity modifications by pharmacological activation (3 nitropropionic acid, 20 mg/kg s.c.) or inhibition (1,2,4 aminotriazole, 250 mg/kg i.p.) resulted in parallel changes in chronic ethanol intake and self-administration in the Pb-exposed animals. Moreover, a shRNA anticatalase lentiviral vector microinfused in ventral tegmental area reduced ethanol intake selectively in Pb-exposed animals both, at the beginning of the free-choice test as well as after stable ethanol intake levels were reached. On the other side, brain acetaldehyde accumulation by pharmacological ALDH inhibition (cyanamide, 0.3 mg i.c.v.) enhanced ethanol intake and resultant locomotion, although predominantly in control animals, while a trend was evident in the Pb-exposed group. These results contrast with *peripheral* acetaldehyde accumulation by systemic cyanamide administration (25 mg/kg i.p.), a pharmacological approach employed in clinical practice to dissuade ethanol consumption that resulted in a decrease in both, ethanol intake and locomotion predominantly in Pb-exposed animals. Overall, these data reveal the importance of brain acetaldehyde accumulation in the enhanced ethanol responses exhibited in animals exposed to environmental Pb levels. They also provide further evidence and open up new avenues in the implication of brain metabolism in ethanol reinforcing and stimulant effects. Supported by: FONCyT, SECyT and CONICET.



IS SALSOLINOL THE FINAL EFFECTOR OF ETHANOL REINFORCEMENT?

Berríos-Cárcamo, Pablo[2,1]., Rivera-Meza, Mario[2,1]., Quiroz, Gabriel[3]., Zapata-Torres, Gerald[4]., Iturriaga-Vásquez, Patricio[3]., Reyes-Parada, Miguel[5]., Herrera-Marschitz, Mario[2,1]., Israel, Yedy[2]., Quintanilla, Maria Elena[2]., [1]Institute of Biomedical Sciences, Millenium Institute BNI, Faculty of Medicine, University of Chile.[2]Molecular and Clinical Pharmacology Program, Faculty of Medicine, University of Chile.[3]Departamento de Ciencias Químicas y Recursos Naturales, Facultad de Ingeniería y Ciencias, Universidad de la Frontera, Temuco, Chile.[4]Molecular Graphics Suite, Department of Inorganic and Analytical Chemistry, Faculty of Chemical and Pharmaceutical Sciences, University of Chile.[5]Escuela de Medicina, Facultad de Ciencias Médicas, Universidad de Santiago de Chile, Santiago, Chile. (Sponsored by Acknowledgments: Supported By CONICYT-PCHA/Doctorado Nacional/2013-21130865, And FONDECYT (1120079, 11130241, 1130012, 1110263).)

The reinforcing effect of ethanol, defined as the property of a drug to promote behaviors that favors its consumption, is a major contributor of ethanol dependence. Ethanol is reinforcing via local activation of the brain reward system. The formation of acetaldehyde, the primary ethanol metabolite, has been shown to be required for ethanol to be reinforcing, activating dopaminergic neurons in the ventral tegmental area (VTA). Acetaldehyde can condense with dopamine to form racemic (*R*/*S*) salsolinol, which has been proposed to be the substance that exerts the ethanol reinforcing effect. For salsolinol to be accepted as the final effector of ethanol reinforcement it needs: (i) to be formed in the VTA after an acute ethanol exposure; (ii) to reproduce the ethanol reinforcing effect; (iii) to be a potent reinforcer, over the efficacy of ethanol or acetaldehyde; (iv) to impair ethanol reinforcement if salsolinol synthesis is prevented; and (v) to be reinforcing by a direct mechanism of action (not be further metabolized). Evidence shows that: (i) only a non-enzymatic pathway for salsolinol synthesis has been thoroughly characterised. A salsolinol synthesis rate from the concentrations of synaptic dopamine and acetaldehyde derived from ethanol, can be estimated to generate salsolinol 10^{-8} to 10^{-7} M. (ii) & (iii) Salsolinol administration into the VTA promotes the release of dopamine in the nucleus accumbens, induces a conditioned place preference, and is self-administered by rats into the VTA at lower concentrations (10^{-7} M) than acetaldehyde (10^{-5} M) or ethanol (10^{-2} M). (iv) Currently, the effect of salsolinol cannot be prevented due to lack of a specific inhibitor. Nevertheless, when dopaminergic neurons obtained from the VTA have been pharmacologically deprived of dopamine (one of the salsolinol substrates) ethanol and acetaldehyde are unable to activate these neurons, while salsolinol can. (v) (*R*)-salsolinol administration in the VTA enantioselectively induces a conditioned place preference, which implies an interaction with a protein target. Salsolinol is an agonist of μ-opioid receptors and binds to the dopamine transporter, both mechanisms could generate its reinforcing effect. In summary, while there is no conclusive proof that salsolinol is responsible for the ethanol reinforcing effect the evidence discussed here suggest that salsolinol is involved.

SYMPOSIUM
PHARMACOLOGICAL MODULATION OF NEURONAL AND MUSCULAR NICOTINIC RECEPTOR: IMPACT ON SYNAPTIC FUNCTION
Chair: Jorge Fuentealba

CHOLINERGIC REGULATION OF NEUROINFLAMMATION AND NEUROPROTECTION: IMPLICATION OF THE SIGNALING PATHWAY A7NACHR/NRF2/HO-1

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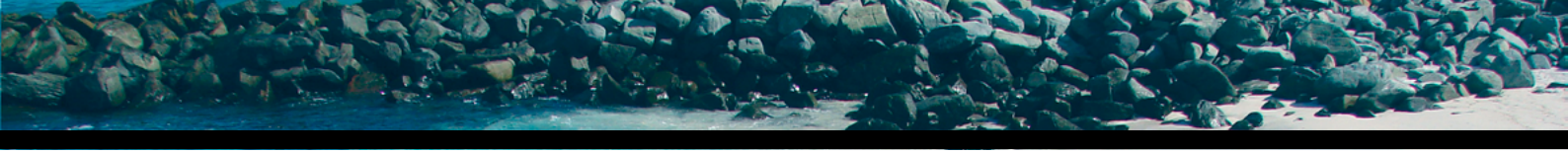
nAChRs are shown to afford neuroprotection and to regulate inflammation, in particular via the α7 nAChR activation in macrophages which regulates the 'cholinergic anti-inflammatory pathway'. The transcription factor Nrf2 (nuclear factor-erythroid 2-related factor 2) is a master regulator of redox homeostasis, it controls the expression of phase 2 enzymes that act in a cytoprotective manner against oxidative stress, including heme oxygenase-1 (HO-1).The aim of this study was to evaluate the implication the α7nAChR/Nrf2/HO-1 pathway in two disease models: (i) *a neurodegenerative model* based on subchronic oxidative stress (rat hippocampal organotipic cultures –OHCs-exposed to lipopolysaccharide and antimycin-A; LPS/AA) and (ii) *brain ischemia models* (OHCs exposed to oxygen and glucose deprivation and *in vivo* stroke model).

OHCs exposed for 4 days to antimycin-A (0.1 μM) and lipopolysaccharide (1 ng/ml) caused low neurotoxicity on their own, measured as propidium iodide fluorescence in CA1 and CA3 regions. However, their combination caused a greater detrimental effect, in addition to mitochondrial depolarization, overproduction of reactive oxygen species and Nox4 overexpression. Subtoxic antimycin-A per se increased ROS and mitochondrial depolarization, although these effects were significantly higher when combined with subtoxic LPS. More interesting was the finding that exposure of OHCs to the combination of subtoxic concentrations of LPS/AA triggered aberrant protein aggregation, measured as thioflavin S immunofluorescence. The α7 nicotinic receptor agonist, PNU282987, prevented the neurotoxicity and the pathological hallmarks observed in the LPS/AA subchronic toxicity model (oxidative stress and protein aggregates); these effects were blocked by α-bungarotoxin and tin protoporphyrin, indicating the participation of α7 nAChRs and HO-1 induction.

OHCs exposed to oxygen and glucose deprivation (OGD), elicited cell death, measured by propidium iodide and MTT staining. PNU282987, after OGD, reduced cell death, ROS production and TNF release. This was associated with induction of HO-1 expression; an effect reversed by the α-bungarotoxin and by tin protoporhyrin IX (SnPP). The protective effect of PNU282987 was lost in microglia-depleted OHCs as well as in OHCs from Nrf2 deficient vs. wild type mice, an effect associated with suppression of HO-1 expression in microglia. Administration of PNU282987 1 h after induction of photothrombotic stroke *in vivo* reduced infarct size and improved motor skills in Hmox1lox/lox mice, that express normal levels of HO-1 but not in LysMCreHmox1$^{\Delta/\Delta}$ in which HO-1 expression is inhibited in myeloid cells, including the microglia.

In conclusion, activation of the α7nAChR induces Nrf2 and HO-1 to regulate neuroinflammation and oxidative stress affording neuroprotection.



MODULATORS OF ACETYLCHOLINE RECEPTOR CLUSTERING IN THE MATURATION OF THE NEUROMUSCULAR JUNCTION: A PHARMACOLOGICAL APPROACH.

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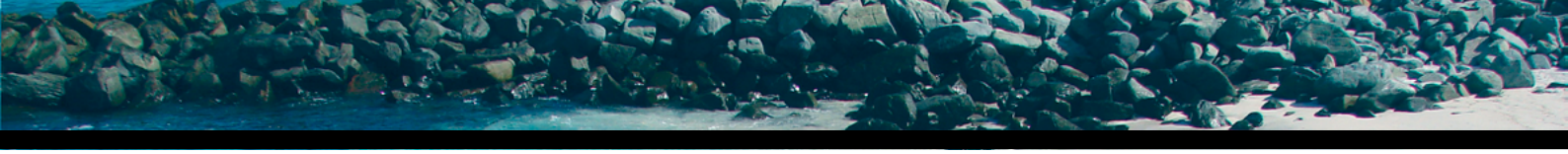
Introduction: Clustering of acetylcholine receptors (AChRs) in discrete regions of the muscle sarcolemma is a hallmark of postsynaptic differentiation at the neuromuscular junction (NMJ). During postnatal maturation of this synapse, pro- and anti-clustering signals are believed to sculpt the postsynaptic morphology. Considering that signaling pathways triggered by ACh/Cdk5 and Wnt ligands modulate the embryonic formation of the NMJ, we have focused in studying weather they could regulate NMJ maturation through a pharmacological approach. **Materials and Methods:** C2C12 myoblasts were differentiated onto laminin matrices to induce the formation of complex postsynaptic structures, which were stained with α-bungarotoxin after single or combined treatments with the ACh agonist Carbachol, the Cdk5 inhibitor Roscovitine, and the Wnt activator Lithium. The intracellular effectors ß-catenin, p35 and nestin were detected by immunocytochemistry and Western blot. **Results:** Whereas Carbachol and lithium induced a total disaggregation of AChRs, Roscovitine induced and increased clustering area, evidenced by the presence of fragmented postsynaptic structures, a characteristic feature of aged NMJs. Roscovitine was unable to rescue the loss of AChR clusters triggered by lithium. In turn, Roscovitine treatment increased the ACh/Cdk5 effector p35 in cytosolic and membrane-associated fractions, whereas lithium decreased the total amount of p35, even in the presence of Roscovitine. **Discussion**: The results of our pharmacological approach suggest that ACh/Cdk5 and Wnt pathways could functionally interact to regulate AChR clustering and, thus, the maturation of the NMJ.

EFFECTS OF NICOTINIC MODULATORS ON THE NEUROTRANSMITTER RELEASE, IMPACT ON SYNAPTIC FUNCTION

GANDIA, L[1]., NANCLARES, CARMEN[1]., COLMENA, INES[1]., [1]DEPARTAMENTO DE FARMACOLOGIA Y TERAPEUTICA UNIVERSIDAD AUTONOMA DE MADRID. (Sponsored by Ministerio De Economía Y Competitividad (MINECO, SAF2013-44108-P) And FUNDACION EUGENIO RODRIGUEZ PASCUAL)

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated channels expressed in the central and peripheral nervous systems, where they play important regulatory neural functions, such as neurotransmitter release and are implied in a variety of physiological and pathological responses such as anxiety, pain, nicotine addiction, and cognitive functions. nAChRs are pentameric complexes and their diversity arises from the multiple combinations of subunit assembly. A large number of nAChR subunits has been identified: there are ten α subunits (α1-α10), four β subunits (β1-β4), one γ, one δ and one ε subunit. The muscle nAChR is composed of two α1 subunits and one β, δ and ε (or γ in the embryonic receptor) subunits. In neuronal nAChRs, two α and three β subunits, in various combinations, can produce different receptor subtypes; At the central nervous system, the α4β2* receptor subtype form the major nAChR subtype, while at the peripheral nervous system, nAChRs formed by α3, β4 and α5 subunits appear to be predominant. The α7* nAChR is expressed on neurons and non-neuronal cells, as well as in immune cells, thus suggesting its possible role in brain immunity, inflammation, and neuroprotection in addition to its well known role in learning and memory. Furthermore, these receptors are highly permeable to calcium, implicating them as significant modulators of intracellular signaling and neurotransmitter release from neurons. In this presentation we will focus on the pharmacological modulation of α7 nAChRs with novel positive allosteric modulators (PAM) that improve the endogenous cholinergic neurotransmission without directly activating α7 nAChRs. Different compounds have been reported as PAM of α7 nAChR, including ivermectin, galantamine, 5-hydroxyindole (5-HI) or PNU-120596. According to their pharmacological profiles (effects on current responses, reactivation of desensitized α7 nAChRs, augmentation of ACh window current, and agonist concentration-response characteristics) the existence of at least two subtypes of α7 nAChR PAMs has been proposed. We have tested the effects of a type I (5-HI) and a type II (PNU-120596) PAM on neurotransmitter release in hippocampal neurons in culture. We have found that both compounds increased GABA release in response to the selective activation of α7 nAChRs, with no effects on glutamate release. These results suggest that these compounds might be therapeutically useful in processes such as schizophrenia in which a decrease in the number of α7* nAChRs (particularly in the hippocampus) has been described. α7 nAChRs agonists and PAMs are being also tested for the treatment of processes such as Alzheimer's disease, autism, or pai



EFFECTS OF NEW ALKALOIDS FROM NATURAL SOURCES THAT MODULATE THE NICOTINIC RECEPTOR IN NEURONAL AD MODELS.

Fuentealba, J[1]., Araya, Juan[1].,Silva-Grecchi, Tiare[1].,[1]Fisiologia, Ciencias Biologicas, Universidad De Concepción. (Sponsored by Fondecyt Iniciacion 1109091; Fondecyt 1130747)

Effects of new alkaloids from natural sources that modulate the nicotinic receptor in neuronal AD models.

Jorge Fuentealba[1,2], Juan Araya[1], Tiare Silva-Grecchi[1]

[1]Laboratory of Neuroactive Compound Screening, [2]Center for Advanced Research in Biomedicine (CIAB-UdeC); Physiology Department, Faculty of Biological Sciences, University of Concepción, Concepción, Chile.

Alzheimer´s disease (AD) is a progressive and deadly neurodegenerative disorder that has no cure. It has been established that soluble oligomers (~56 kDa) of the beta amyloid peptide have a fundamental role in the development of this pathology, which has been strongly associated with deterioration in cholinergic neuronal transmission (cholinergic theory). Furthermore, some currently used treatments modulate the cholinergic tone in the central nervous system, but they have not been effective at stopping the disease. Neuroprotective properties of nicotine in AD treatment have been described through its effect on α7 subtype nicotinic receptors (nAChR) and the further activation of the PI3K/Akt/Bcl-2 survival pathway. This evidence presents the possibility to examine α7 agonists as a new possible therapy for AD, which does not possess secondary and adverse effects that come from nicotine use.

New quinolizidinic alkaloids from the *Fabaceae* family were obtained to evaluate their possible action on α7 nAChR and possible neuroprotective effects in a neuronal AD model. We used cellular viability, Ca^{2+} microfluorimetry, electrophysiology and western blot techniques in neuronal primary cultures and cell lines to evaluate the effects of the extracts on Aβ-induced toxicity. The protective effect of these alkaloids was observed in rat cortical neurons, but not in cells that did not express α7 nAChR, such as HEK293T. Additionally, a recovery in the Aβ-induced decrease in synaptic activity frequency in the neuronal network was observed. Our results suggest that quinolizidinic alkaloids exert neuroprotective effects against Aβ toxicity through modulation of α7 nAChR, which could represent the starting point for characterization of new molecules from natural sources with anti-AD activity.

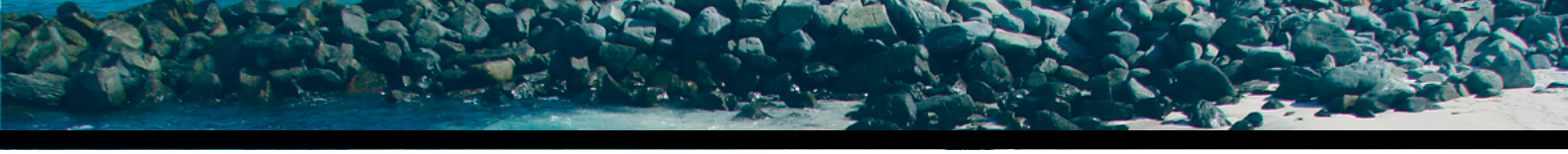
Fondecyt iniciacion 1109091; Fondecyt 1130747

SYMPOSIUM
MULTIDISCIPLINARY APPROACHES IN THE STUDY OF THE BRAIN: FROM GENES TO CLINICS
Chairs: Jimena Sierralta-Pedro Maldonado

THE ENDOPLASMIC RETICULUM AND PROTEIN TRAFFICKING IN AXONS

Couve, A[1,2]., [1]Program of Physiology and Biophysics, Institute of Biomedical Sciences (ICBM), Universidad de Chile. [2]Biomedical Neuroscience Institute (BNI) Universidad de Chile.

Neurons are responsible for the generation and propagation of action potentials that constitute the fundamental mechanism for information transfer. They are large and polarized cells with dendrites and axons constituting their major functional domains. Axons are thin and long specializations that mediate the conduction of electrical impulses. Regulation of the axonal proteome is key to generate and maintain neural function. Although fast and slow protein transports have been known for decades, newly identified mechanisms to control the abundance of axonal proteins based on local biosynthesis and processing have emerged in recent years. We speculate that early secretory organelles in the axon provide a functional route for trafficking and, in combination with local protein synthesis of membrane and secreted proteins, an alternative mechanism to regulate the axonal proteome. Using complementary in vitro and in vivo experimental models in rats and Drosophila we explore the presence and function of the endoplasmic reticulum and other early local secretory organelles in the axon. In Drosophila we investigate the role of neuronal atlastin in locomotion and axonal protein trafficking. In mammalian peripheral neurons we evaluate the role of early secretory organelles in the delivery of sodium channels. Funded by CONICYT USA2013-020, Fondecyt 1140617 and ICM P-09-015F.



CHEMOKINE SIGNALING PAVES THE WAY FOR THE INITIAL TRAJECTORY OF HABENULAR AXONS.

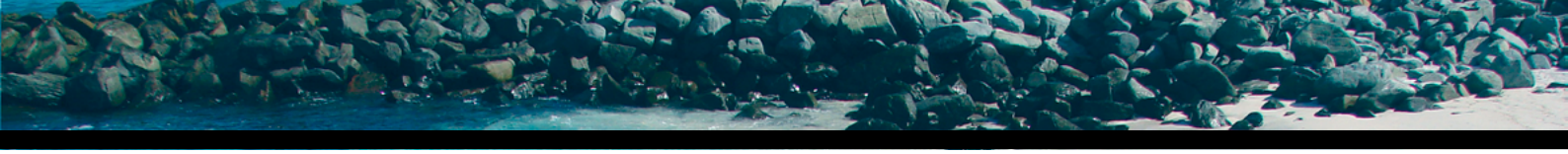
Guerrero, Nestor[2,1]., Meynard, Margarita[2,1]., Armijo, Lorena[2,1]., Rojas-Rivera, Diego[2,1]., Palma, Karina[2,1]., Colombo, Alicia[2].,Reig, German[2,1]., Hetz, Claudio[2,1]., Concha, Miguel[2,1]., [1]BNI Biomedical Neuroscience Institute. [2]ICBM, Facultad de Medicina, Universidad de Chile. (Sponsored by Grant Sponsors: FONDECYT (1120558) And The Scientific Millennium Initiative (P09-015-F).)

One key step in neural development is the establishment of connectivity between neuronal groups, a process that is based on the interaction between the axonal growth cone and its environment. Besides the classical axon guidance systems that comprise semaphorin-, netrin-, slit- and ephrin-based environmental ligands, signals that are essential for cellular chemotaxis such as the chemotactic cytokines (chemokines) also participate in axon guidance. For instance, the stromal cell-derived factor-1 (Cxcl12), a chemokine known to control proliferation and migration of progenitor cells through the receptor Cxcr4, also works as an axon guidance cue in various contexts. In the embryonic zebrafish forebrain, *cxcr4b* transcripts are detected in the habenulae (Hb) at the time of efferent axonal projection suggesting a role of Cxcr4/Cxcl12 signaling in guiding Hb axons. During development, these axons organize as a bilateral pair of fascicles known as fasciculi retroflexus that extend ventrocaudally in direction of the ventral midbrain. In this study, we asked whether Cxcr4/Cxcl12 signaling played an *in vivo* role in guiding Hb axons to the ventral midbrain in zebrafish, and if this role involved the interaction with other axon guidance cues. We visualized Hb-IPN connectivity in larvae of the double mutant-transgenic *cxcr4-/-;puo4f1-hsp70:GFP* and *cxcl12-/-;puo4f1-hsp70:GFP*, using *in vivo* imaging, anti-acetylated tubulin immunostaining, lipophilic dye tracing, focal electroporation, and morpholino oligonucleotide knock-down of *robo3a/b*. We found that Hb axons devoid of Cxcr4a/Cxcl12b signaling fail to exit the Hb from its normal ventral point, show convoluted trajectories, excessive branching, and project ectopically to the contralateral Hb and the antero-dorsal forebrain. Importantly, these defects are completely rescued by functional abrogation of *robo3a*, which is expressed at high levels by Hb neurons, suggesting that Cxcr4a/Cxcl12b antagonizes Robo3a-dependent repulsive cues normally present posterior and ventral to the Hb. We observed that Cxcr4b biochemically interacts with Robo3, and that both receptors become internalized from the cell membrane in the presence of Cxcl12. We thus propose that Cxcr4/Cxcl12 antagonizes Robo3-mediated repulsion through removal of repulsive protein complexes from the surface of the growth cone. Our results provide novel insights of how growth cones integrate potentially conflicting cues and navigate through repulsive environments.

STUDY OF THE LOCALIZATION, TRAFFIC AND FUNCTION OF SYNAPTIC PROTEINS USING *DROSOPHILA* NEUROMUSCULAR JUNCTION AS MODEL SYSTEM.

Sierralta, Jimena[1]., Astorga, César[1].,De Gregorio, Cristian[1].,Delgado, Ricardo[1].,Andrés, Couve[1].,[1]Biomedical Neuroscience Institute, Facultad de Medicina , Universidad De Chile. (Sponsored by ICM-P-09-015F/FONDECYT 1120684/ CONICYT-USA 20130020)

Drosophila is a powerful system to study neuronal function due to the diversity of molecular and genetic tools and the feasibility to do *in vivo* studies. In particular the study of the synaptic function in *Drosophila* is facilitated by the use of the larval neuromuscular junction (NMJ) as a model system. The NMJ is an accessible glutamatergic synapse that shares several similarities with vertebrate glutamatergic synapses, such as similar complement of receptors and scaffold proteins, and a regulated glutamate release similar to central synapses. Even more, accessible axons allow the *in vivo* study of the traffic of synaptic proteins along the axons by the use of GFP-labeled proteins. We will present work on the analysis of the function of two highly conserved genes. *atlastin*, the second most common gene mutated in hereditary spastic paraplegias (HSP) a genetic disorder characterized by length-dependent axonopathy of the corticospinal tracts. Atlastin, is localized to the tubular ER, where it catalyzes the homotypic fusion of ER membranes. Genetic manipulation of Atlastin expression in *Drosophila* motorneuron associates to locomotor defects of the larvae and adult flies. In this HSP model the organization of the ER and Golgi apparatus in motor neuron`s axons show altered morphologies as well as the synaptic function evaluated by the evoked currents in the muscle. The second gene we have studied is the gen *dlg* a scaffold protein that in vertebrates has been associated to the anchoring of the glutamate receptors (GLUR) in the postsynaptic membrane and their activity dependent insertion or removal at the synapse. In addition to the postsynaptic defects found in *dlg* mutants that associate not only the cluster of the glutamate receptor but also the composition, our results strongly support a role of DLG proteins in the efficiency of the synaptic vesicles release. More exactly in the localization and abundance of voltage dependent calcium channels.



EYE MOVEMENT DURING FREE VIEWING OF NATURAL IMAGES AS MARKERS OF SCHIZOPHRENIA.

Maldonado, P[1]., Mayol, Rocio[2].,Egaña, Jose[2].,Gaspar, Pablo[3].,Silva, Hernan[3].,[1]PDFB, BNI , Facultad de Medicina , Universidad De Chile.[2]Biomedical Neuroscience Institute, Medicina, Universidad De Chile.[3]Clinica Psiquiatrica, Medicina, Universidad De Chile.
(Sponsored by Supported By Iniciativa Cientifica Milenio BNI ICM P09-015-F)

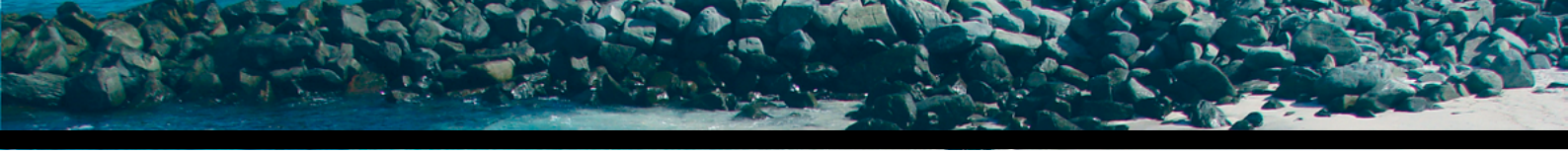
In schizophrenia, patients display dysfunctions during the execution of simple visual tasks such as antisaccade or smooth pursuit. In more ecological scenarios, such as free viewing of natural images, patients appear to make fewer and longer visual fixations and display shorter scanpaths. It is not clear whether these measurements reflect alterations in their proficiency to perform basic eye movements, such as saccades and fixations, or are related to high-level mechanisms, such as exploration or attention. We utilized free exploration of natural images of different complexities as a model of an ecological context where normally operative mechanisms of visual control can be accurately measured. We have found that patients exhibit similar scanpaths as well as saccades frequency and fixations duration. New we are exploring differences in saliency of stimuli to explore potential impairment in the magno-cellular and parvo-cellular pathways. These results will help elucidate the mechanisms of visual motor control that are affected in schizophrenia and contribute to the finding of adequate markers for diagnosis and treatment for this condition.

SYMPOSIUM
YOUNG NEUROSCIENTISTS SYMPOSIUM
Chair: Adrián Palacios

INNERVATION TARGET SPECIFICITY AND CELL-CELL INTERACTIONS DURING PERIPHERAL NERVE REGENERATION

CECI, M[1]., MARDONES-KRSULOVIC, C[1]., SANCHEZ, M[1]., VALDIVIA, L[1]., GUTIERREZ, D[1]., ALLENDE, M[1]., [1]DEVELOPMENTAL BIOLOGY LABORATORY, FACULTAD DE CIENCIAS , FONDAP CENTER FOR GENOME REGULATION - UNIVERSIDAD DE CHILE. (Sponsored by Funding: FONDECYT 3120073 (MLC), 1110275 And FONDAP 15090007 (MLA).

Introduction: Damage to peripheral axons usually leads to a better outcome compared to injuries to central nervous system; however it is currently unknown how neurons re-establish their target innervations to recover function after injury, and how accessory cells contribute to this task. To answer this, we used the zebrafish posterior lateral line (pLL) system. The sensory organs are located in the body surface and are innervated by afferent neurons, whose somas are located in the pLL ganglion. The peripheral projections of these neurons are enterely covered by Schwann cells, whereas their central projections generate a somatotopic sensory map in the hindbrain. **Materials and methods:** By electroablation we sectioned the pLL nerve. Genetic labeling of single axons and the use of transgenic markers of Schwann cells allowed us to individualize different components in this system and to characterize their behaviors during the regenerative process. **Results**: After axonal damage in the lateral line nerve, the overall anteroposterior sensory map is reproduced after its regeneration. However, there is a degree of promiscuity and original target organs are not reacquired with absolute fidelity. Furthermore, Schwann cells are required for directional extension and fasciculation of the regenerating nerve. **Conclusion**: The accessibility of the pLL nerve and the availability of transgenic lines that label their synaptic targets provides an outstanding in vivo model to study the different events associated with axonal extension, target reinnervation, and the complex cellular interactions between glial cells and injured axons during nerve regeneration.



MODULATION OF BK CHANNEL VOLTAGE GATING BY DIFFERENT AUXILIARY B SUBUNITS

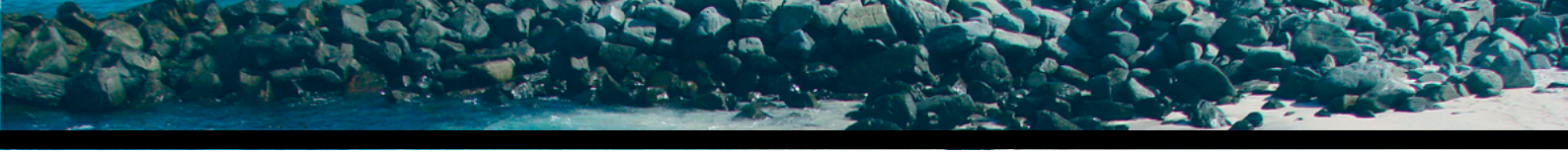
Castillo, Karen[1]., Contreras, Gustavo[1]., Lorenzo, Yenisleidy[1,2]., Pupo, A[2,1]., Neely, Alan[1]., Gonzalez, Carlos[1]., Latorre, Ramon[1]., [1]Centro Interdisciplinario de Neuriociencia, Ciencias, Universidad De Valparaíso. [2]Programa de Doctorado en Neurociencia Universidad De Valparaíso. (Sponsored by This Work Is Supported By FONDECYT Grants 1110430 (R.L.), 1120802 (C.G); ANILLO Grant ACT1104 (C.G.); FONDECYT Postdoctoral Fellowship 3140590 (G.F.C.); CONICYT Graduate Fellowship (YL And AP). The CINV Is A Millennium Institute)

BKα channels are key components for the physiology in most mammalian tissues and are modulated by ß subunits (ß1-ß4), which confer particular functional properties to the channel, for specific cellular needs. Minimal alterations in BK channel function may contribute to the pathophysiology of several diseases including hypertension, asthma, cancer, epilepsy and diabetes. Auxiliary ß1 and ß2 subunits are able to stabilize the BK voltage sensor equilibrium in the active conformation, whilst ß3 has no effect on voltage sensor balance. Despite the fact that the BK phenotype produced by each of the ß subunits has been well characterized, controversies exist regarding the molecular mechanisms by means of which these auxiliary subunits can modify the gating properties of BK channel. By constructing chimeric ß proteins between ß1 and ß3 subunits and by measuring gating currents, we were able to identify that the cytoplasmic elements of ß1 are responsible for the modulation of the voltage sensor of BK channel in absence of Ca^{2+}. Particularly, lysine residues K3 and K4 are necessary and sufficient to recover the gating charge movement features of α/ß1 channel phenotype. Moreover, we observe a left shift in more than 100 mV in the charge versus voltage relationships in BKα and BKα/ß1 channels in presence of Ca^{2+}. These results suggest that the voltage and calcium sensors in BK channel are strongly coupled, allowing fast responses to both physiological stimulus.

RYANODINE RECEPTOR AND ATLASTIN-2 SHAPE CALCIUM SIGNALS IN SINGLE RAT HIPPOCAMPAL NEURON DENDRITES

Cordova, John [1]., Ramirez, Omar[1]., Cerda, Mauricio[1,2]., Lobos, Pedro[1]., Paula-Lima, Andrea[1,3]., Couve, Andres[1,5]., Härtel, Steffen[1,4]., Hidalgo, C[5,1,6,7]., [1]Biomedical Neuroscience Institute, Faculty of Medicine, University of Chile.[2] Anatomy and developmental Biology Program, Faculty of Medicine, Univeristy of Chile. [3]Institute for research in Dental sciences, Faculty of Dentistry, University of Chile. [4]Anatomy and developmental Biology Program, Faculty of Medicine, University of Chile. [5]Physiology and Biophysics Program, Faculty of Medicine, University of Chile. [6]Centro de estudios moleculares de la célula, Faculty of Medicine, University of Chile. [7]Physiology and Biophysics Program, Faculty of Medicine, University of Chle. (Sponsored by Funding, CONICYT 21120689 (JAC), Fondecyt 3110157 (OR), 1150756 (APL), 1151029 (SH), 1140545 (CH), ICM P09-015-F (JAC, OR, APL, AC, SH, CH).))

Voltage gated potassium channels appear to be machines precisely build to transport potassium ions with remarkable efficiency. Both selectivity and voltage sensitivity appear to be highly optimized. To study ion conduction and the extent of the conformational changes leading to activation by voltage, we used sucrose as a tool, both viscous and osmotic effects, to estimate the pore dimensions and the volumetric changes in the voltage sensor during activation. Sucrose increased viscosity, allowing for an experimental condition in which the potassium current are limited by the diffusional access of potassium ions to the pore. Thus, making use of the theory of diffusion controlled reactions is possible to estimate the channel pore opening dimensions and explain how this dimension specify low or high conductance in K-channels. Sucrose increased osmolarity favor or disfavor the voltage dependent activation. Thus, by adding sucrose to either the external or the internal side of the channel we measured the osmotic work developed by the voltage sensor during activation. We propose that the overall osmotic work of the voltage sensor is small, but it is composed by very dissimilar works by the different components of the voltage sensor. Thus, we concluded that the energy route for each moving sensing charge must be different.



SWEET EXPERIMENTS: WHAT SUCROSE HAS TAUGHT US ABOUT POTASSIUM CHANNELS

Diaz, Ignacio[1]., Naranjo, David[2].,[1]Centro Interdisciplinario de Neurociencias, Facultad de Ciencias, Universidad de Valparaíso, CINV.[2]Centro Interdisciplinario de Neurociencias, Facultad de Ciencias, Universidad De Valparaíso. (Sponsored by This Work Was Supported By Fondecyt Grants 1120819 And 1131003)

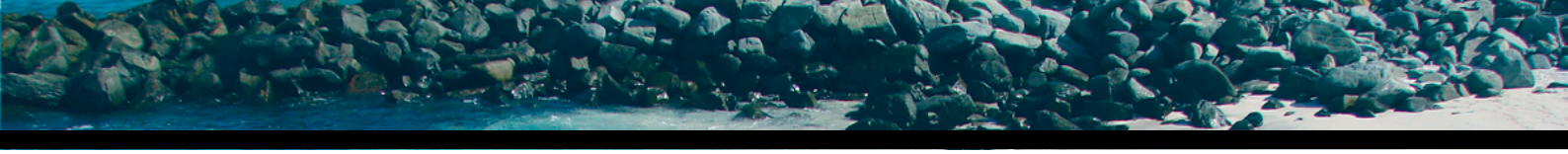
Voltage gated potassium channels appear to be machines precisely build to transport potassium ions with remarkable efficiency. Both selectivity and voltage sensitivity appear to be highly optimized. To study ion conduction and the extent of the conformational changes leading to activation by voltage, we used sucrose as a tool, both viscous and osmotic effects, to estimate the pore dimensions and the volumetric changes in the voltage sensor during activation. Sucrose increased viscosity, allowing for an experimental condition in which the potassium current are limited by the diffusional access of potassium ions to the pore. Thus, making use of the theory of diffusion controlled reactions is possible to estimate the channel pore opening dimensions and explain how this dimension specify low or high conductance in K-channels. Sucrose increased osmolarity favor or disfavor the voltage dependent activation. Thus, by adding sucrose to either the external or the internal side of the channel we measured the osmotic work developed by the voltage sensor during activation. We propose that the overall osmotic work of the voltage sensor is small, but it is composed by very dissimilar works by the different components of the voltage sensor. Thus, we concluded that the energy route for each moving sensing charge must be different.

SYMPOSIUM
EXTRINSIC AND INTRINSIC SIGNALS THAT MODULATE BRAIN FETAL/NEONATAL PROGRAMMING
Chair: Paola Haeger

NEUROIMMUNE AND NEUROVASCULAR EFFECTS OF 3RD TRIMESTER ETHANOL EXPOSURE

Valenzuela, Carlos[1]., Topper, Lauren[1]., Welch, Jason[1]., Mayfield, Jacob[1]., [1]Neurosciences, Medicine, University of New Mexico Health Sciences Center. (Sponsored by National Institutes Of Health Grants: R37-AA015614, P50-AA022534 And T32-AA014127)

Fetal alcohol exposure is a leading cause of preventable birth defects. The range of effects that can be produced by fetal alcohol exposure is denoted as Fetal Alcohol Spectrum Disorder (FASD). In this study, we investigated the role of the neuroimmune and vascular systems in the pathophysiology of FASD. To model binge-like alcohol exposure during a portion of the 3rd trimester of human pregnancy, we exposed rats to alcohol vapor inhalation. Animal procedures were approved by the UNM-HSC Institutional Animal Care and Use Committee. Pregnant Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN) and arrived at gestational day 14-16. Subsequently, we exposed dams with their pups to air or ethanol on postnatal days 3, 4, and 5 for 3-4 hours daily in the vapor chambers. We found that exposure to high levels of ethanol (blood ethanol concentration (BEC) = 0.4-0.5 g/dl) causes Purkinje cell degeneration in the cerebellar vermis at P6 and P45; however, this effect was not observed in the hippocampal formation. Significant increases in mRNA levels for pro-inflammatory cytokines (interleukin-1β and tumor necrosis factor-α) were observed in both the cerebellum and hippocampus during alcohol withdrawal periods. Importantly, marked astrocyte activation was observed in both the hippocampus and cerebellar vermis, whereas microglial activation was observed only in the cerebellar vermis. In the cerebral cortex of P6-7 rats, we detected some spontaneous micro-bleeds in the surface of the cerebral cortex of air exposed control pups. In brains from ethanol exposed pups, the number and size of bleeds per brain were significantly increased. Coronal brain sections showed that most of the micro-bleeds were located in the motor and somatosensory regions of the cerebral cortex. Micro-bleeds were also observed in rats exposed to lower levels of ethanol vapor (BEC = 0.08 g/dl). Activation of microglia and astrocytes was evident in the bleed area. At the behavioral level, rats exhibited alterations in gait (assessed with using the Catwalk test) and spatial memory (assessed using context pre-exposure contextual fear conditioning) at P45-50. These findings suggest that 3rd trimester alcohol exposure has time- and brain region-dependent, and that not only microglia but also astrocytes and vascular alterations play an important role in the mechanism of action of ethanol. Collectively, these effects could contribute to the behavioral deficits associated with FASD.



ROLE OF REACTIVE OXYGEN SPECIES IN THE ALCOHOL-DEPENDENT COGNITIVE DEFICIT IN RATS EXPOSED TO ETHANOL IN UTERO.

Haeger, Paola[1]., Contreras, Marcela[1]., Cortés, Paulina[1]., Muñoz, Daniela[1]., De La Fuente, Erwin[1]., [1]DEPARTAMENTO CIENCIAS BIOMEDICAS, FACULTAD DE MEDICINA, UNIVERSIDAD CATOLICA DEL NORTE. (Sponsored by FONDECYT 1140855)

Alcohol intake during pregnancy may generate severe effects in the cognitive development of offspring. Children with prenatal alcohol exposure may exhibit morphological and functional alterations, which are called by the generic term Fetal alcohol spectrum disorders (FASD). Particularly, prenatal alcohol exposure in both human and animal models produces learning and memory alterations, and increases vulnerability to alcohol and other substance abuse.

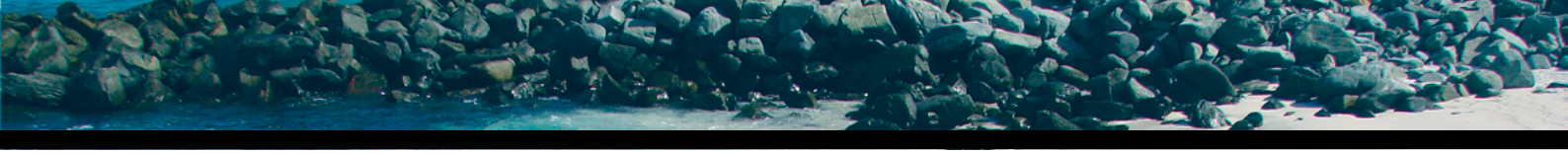
Ethanol exposure increases oxidative stress in developing organs, including the brain. Indeed, even a brief exposure to ethanol during gestation can produce perdurable imbalance between the levels of intracellular reactive oxygen species (ROS) and brain antioxidants, which can be correlated with cognitive deficits. Interestingly, antioxidant treatment, during maternal ethanol ingestion, has been repeatedly shown to improve behavioral deficits in rodent models of FASD. However, the impact of the general antioxidant treatment in the adult age of the offspring, and the specific ROS-dependent mechanism, has not been fully studied.

We developed a rat model of FASD, which has been evaluated at adult age (p70). These animals consumed around 25% more ethanol than control rats, during one day of a freechoice alcohol test; they spent significantly more time in the alcoholpaired compartment in the place preference conditioning test; finally, they showed a significant delay in spatial memory acquisition compared with control rats. The neurobehavioral status found in this model resembles FASD characteristics, previously described. We are currently performing studies to identify the molecular sources of ROS generation as well as the mechanism of their involvement in the deficit of memory acquisition, and increased vulnerability to alcohol dependence. Funded by Fondecyt grant N° 1140855

GESTATIONAL CHRONODISRUPTION IMPAIRS HIPPOCAMPAL EXPRESSION OF NMDA RECEPTOR SUBUNITS AND SPATIAL MEMORY IN THE ADULT OFFSPRING

Torres-Farfan, C[1]., Vilches, Nelson[2]., Spichiger , Carlos[1]., Mendez, Natalia[1]., Richter, Hans[1]., [1]Instituto de Anatomía, Histología y Patologia, Medicina, Universidad Austral De Chile. [2]Fisiopatologia, Medicina, Universidad de Chile. (Sponsored by Proyecto Fondecyt 1120938 (CTF) ANILLO ACT 1116 (CTF-HGR))

Epidemiological and experimental evidence correlates adverse intrauterine conditions with the onset of disease later in life. For a fetus to achieve a successful transition to extrauterine life, a myriad of temporally integrated humoral/biophysical signals must be accurately provided by the mother. We and others have shown the existence of daily rhythms in the fetus, with peripheral clocks being entrained by maternal cues, such as transplacental melatonin signaling. Among developing tissues, the fetal hippocampus is a key structure for learning and memory processing that may be anticipated as a sensitive target of gestational chronodisruption. Here, we used pregnant rats exposed to constant light treated with or without melatonin as a model of gestational chronodisruption, to investigate effects on the putative fetal hippocampus clock, as well as on adult offspring's rhythms, endocrine and spatial memory outcomes. The hippocampus of fetuses gestated under light:dark photoperiod (12:12 LD) displayed daily oscillatory expression of the clock genes *Bmal1* and *Per2*, clock-controlled genes *Mtnr1b*, *Slc2a4*, *Nr3c1* and *NMDA* receptor subunits 1B-3A-3B. In contrast, in the hippocampus of fetuses gestated under constant light (LL), these oscillations were suppressed. In the adult LL offspring (reared in LD during postpartum), we observed complete lack of day/night differences in plasma melatonin and decreased day/night differences in plasma corticosterone. In the adult LL offspring, overall hippocampal day/night difference of gene expression was decreased, which was accompanied by a significant deficit of spatial memory. Notably, maternal melatonin replacement to dams subjected to gestational chronodisruption prevented the effects observed in both, LL fetuses and adult LL offspring. Collectively, the present data point to adverse effects of gestational chronodisruption on long-term cognitive function; raising challenging questions about the consequences of shift work during pregnancy. The present study also supports that developmental plasticity in response to photoperiodic cues may be modulated by maternal melatonin.



EFFECTS OF METABOLIC INSULT ON THE POSTNATAL NEUROGENESIS AND BEHAVIOR.

Morales, Paola[1,2]., Esmar, Daniela[1]., Tapia-Bustos, Andrea[1]., Espina-Marchant, Pablo[1]., Gutiérrez-Hernández, Manuel[1]., Rubio, Mariana[1]., Rojas-Mancilla , Edgardo[1]., Pérez-Lobos, Ronald[1]., Palacios, Esteban[1]., Serth, Marcos[1]., Muñoz, Valentina[1]., Lespay, Carolyne[1]., Bustamante, Diego[1]., Herrera-Marschitz, Mario[1]., [1]BNI, Programme of Clinical & Molecular Pharmacology, Medical Faculty, ICBM, University of Chile. [2]Programme of Clinical & Molecular Pharmacology, Medical Faculty, ICBM, University of Chile.
(Sponsored by Supported By Institute Millennium-BNI (BNI P09-015-F) And FONDECYT (No1120079; 1110263) Grants)

Perinatal asphyxia (PA) primes the development of the CNS. Severe asphyxia has been linked to cerebral palsy, mental retardation, and epilepsy, while mild-severe asphyxia has been associated with attention deficits and hyperactivity in children and adolescents, and increased risk for low intelligence quotient score. Obstetric complications are also central risk factors of psychiatric diseases including schizophrenia, bipolar disorders and vulnerability to drug abuse, in particular to cocaine addiction.

PA affects mainly neurocircuitries of mesencephalon and hippocampus generating deficits in neuro-behavioural domains, including early neurological reflexes, locomotor activities, spatial memory, non-spatial working memory, reactivity to stress and anxious behavior.

Several compensatory mechanisms, including neurogenesis, have been proposed as mediators of endogenously triggered protection against cell death induced by PA. Indeed, increased neurogenesis has been observed in DG, CA1 and SVZ after PA. Neurogenesis can be regulated by a large number of molecules, including growth factors, neurotransmitters, such as dopamine and serotonin and other factors still under characterisation. The expression of basic fibroblast growth factor (bFGF) is upregulated in DG and SVZ following PA. We have reported evidence suggesting that bFGF, through activation of the MAPK/ERK pathway could be involved in the neurogenesis induced by PA. Also, modulator proteins of bFGF pathway, Spry (Sprouty), FRLT3 (leucine-rich repeat transmembrane protein) and Sef (similar expression to FGF) are upregulated after PA suggesting a fine regulation of the neurogenic process since Spry and Sef provide inhibitory regulation, while FRLT3 stimulates the activation of bFGF transduction pathway with specific temporal and regional patterns.

Dopaminergic system is vulnerable to PA. Recently, using organotypic cultures from DG, was found that dopamine (DA) fibres originated in substantia nigra (SN)/ventral tegmental (VTA) area targeting the hippocampus and SVZ, establishing anatomical and functional contacts with neuronal progenitors and diferentially modulates the neurogenesis. Recent studies have suggested that stimulation of D2 receptor promotes neuronal proliferation, providing perhaps a target for neuroprotection. These receptors are also located on astrocytes, releasing neurotrophic factors like bFGF, driving neurogenesis.

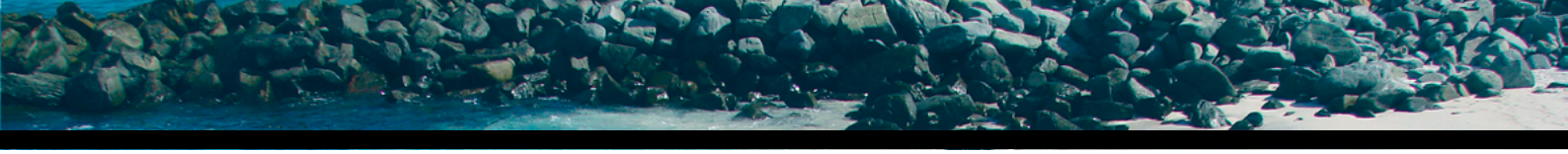
Therefore, further progress is needed in understanding the subjacent mechanisms involved in the modulation of neurogenesis after brain insults, in order to develop novel therapeutic strategies for restoring the damaged neurocircuitry.

SYMPOSIUM
PHYSIOLOGICAL AND STRUCTURAL INSIGHTS OF ION CHANNELS AND MEMBRANE RECEPTORS
Chair: Claudio Coddou

INTRACELLULAR ATP REGULATION OF THE EXTRACELLULAR ATP GATED P2X2 RECEPTOR CHANNEL

Coddou, C[1]., [1]Departamento de Ciencias Biomédicas, Facultad Medicina, Universidad Católica Del Norte. (Sponsored by FONDECYT 11121302)

Activation of P2X2 receptor channels (P2X2Rs) is characterized by a rapid current growth accompanied with a decay of current during sustained ATP application, a phenomenon known as receptor desensitization. Such facilitation of receptor desensitization, termed use-dependent desensitization (UDD), is observed in whole-cell recordings and is determined by intracellular domain calcium. Experiments with various receptor chimeras also indicate that the transmembrane and intracellular domains of P2X2R are required for development of calcium-dependent desensitization and that decrease in current amplitude slows receptor desensitization. Further experiments inhibiting several protein kinases and phosphatases demonstrate an allosteric nature of calcium action near the P2X2R pore. We further show that UDD is practically abolished in the perforated-cell and two-electrode voltage clamp configurations in HEK293 cells and *Xenopus* oocytes, respectively. Addition of ATP but not GTP in the pipette solution also abolishes UDD in whole-cell recordings, suggesting that a leak of ATP from cytosol to pipette permits the action of calcium. In contrast to calcium, intracellular ATP does not modulate P2X2R gating allosterically, as indicated in experiments with ATP-γ-S in the intracellular solution. Concordantly, intracellular injection of apyrase, an ectoATPase, or alkaline phosphatase in oocytes facilitated P2X2R desensitization. The addition of staurosporine and ATP to the pipette solution also abolishes UDD but the desensitization rates are faster. Mutagenesis studies of N- and C-terminal amino acids that are potential phosphorylation sites identified a critical role of the S363 residue in ATP intracellular action. These findings indicate that ATP not only activates P2X2R extracellularly but also determines the desensitization kinetics intracellularly through phosphorylation of P2X2R, which prevents development of intracellular allosteric calcium action. Therefore, the metabolic state of the cell expressing P2X2R could influence the gating properties of this receptor.



NEW INSIGHTS ON TRPV1 CHANNELS AS MODULATORS OF SYNAPTIC FUNCTION

Chávez, A,E. Instituto de Neurociencias, Facultad de Ciencias, Universidad de Valparaíso, Centro Interdisciplinario de Neurociencia de Valparaíso.

The equilibrium between excitation and inhibition is essential for proper brain circuit function. Changes in excitatory or inhibitory synaptic efficacy could have profound influence over this balance and are commonly linked to certain cognitive deficits, including autism and epilepsy. Increasing evidence suggests that endocannabinoid (eCBs) play an important role in regulating this balance, but the precise mechanisms of this regulation are not yet fully understood. In addition to binding to cannabinoid receptors (CB1Rs), certain eCBs such as anandamide (AEA) can also bind and activate the transient potential receptor of vanilloid type 1 (TRPV1). While TRPV1 receptors are mainly found in the peripheral nervous system, these receptors have also been found in the brain, where their localization and functional role is far less understood. Recently, we reported that the eCB anandamide acts on postsynaptic TRPV1 to suppress synaptic transmission presumably by reducing the number of AMPA-type glutamate receptors (AMPARs) at excitatory synapses as well as the number of $GABA_A$-type receptors at specific inhibitory synapses, strongly suggesting that eCB and TRPV1 might play an important role in regulating the excitatory/inhibitory balance require for proper brain function. Moreover, using selective pharmacology for serotonin (5-HT) receptor in acute rat hippocampal slices, we found that bath application of 5-HT depresses excitatory synaptic transmission in an input-specific manner in rat and mouse dentate gyrus via activation of 5-HT2a/cRs. Notably, this depression also requires activation of TRPV1 channels. This finding reveals a novel form of 5-HT-TRPV1 mediated regulation of excitatory synaptic transmission at central synapses. By elucidating how eCBs can modulate synaptic efficacy via TRPV1 channels within a neuronal circuit, the exact TRPV1 localization, the role of eCB-signaling in mediating TRPV1 activation, and the functional impact of such modulation is crucial not only for a more realistic representation of neural function, but also for the development of novel therapeutic approaches targeting the eCB system. In addition, unmasking the functional role of brain TRPV1 would allow for the development of novel analgesic strategies to control pain without affecting normal brain function.

PHARMACODYNAMIC INSIGHTS OF B-ADRENERGIC MECHANISMS: FROM CRYSTALS TO FUNCTION, A TALE FOR ALL CELLS/ RECEPTORS

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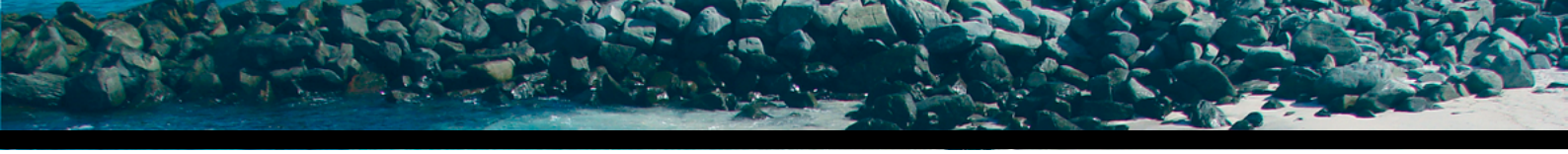
Recently, pharmacological experiments identified the expression and function of β1, β2 and β3-adrenergic receptors in endothelial cells from the rat arterial mesenteric bed and in-vivo protocols (Am J Physiol 297: H134, 2009). This study raised the novel concept that at least three β-adrenoceptors participate in the regulation of the vascular wall by acting on endothelial cells, since all these β-adrenoceptors are coupled to the net synthesis of endothelial NO. This finding, in addition to the well-known vasodilator role of the β-2-adrenoceptor in the smooth muscle, allowed proposing that adrenaline and related ligands play a role in the regulation of vascular resistance and in blood flow, particularly during exercise, via targets localized in both endothelial and vascular smooth muscle cells. The β-adrenergic receptor is a “first of a kind” transduction protein. During the fifties, the first adrenergic “pharmacophore” was designed; although, by then, no firm notion of the structural implications of this hypothesis was accessible. Latter, the coupling of the receptor to protein Gs was deciphered followed thereafter by its sequencing and the prompt identification of its main structural determinants in accordance with the putative pharmacophore unraveled 30 years before. The advent of its crystal structure laid the foundation to understanding its functioning at the atomic level and paved the road to scrutinize the workings of other G protein coupled receptors (GPCR). In fact, for about 7-8 years the rhodopsin and the β-adrenergic receptors were the routine paradigms for GPCR pharmacodynamics. The finding that the crystal structure of β1 and β2-adrenoceptors contain cholesterol molecules imbibed in the protein structure is compatible with the notion that allosteric modulation is inherent to the receptor pharmacodynamics. This finding recognized opportunities for the discovery of allosteric modulators in most GPCRs. The elucidation of the molecular structure of the β1 the β2-adrenoceptors has allowed the detailed understanding of their binding selectivity at the orthosteric site of each receptor subtype, accounting in addition for the differences between agonists and antagonists at each pharmacophore of each β-adrenoceptor subtype. This information will prove useful to discover novel “tailored ligands” for these receptors in direct benefit from patients requiring better drugs. One field of attention is of course, β2-agonists for treatment of asthma and related diseases as well as safer β-1 antagonists for cardiovascular drugs for the treatment of the many vascular diseases that currently use adrenergic drugs for therapy.



TRP CHANNELS IN NEURONAL PHYSIOLOGY AND PATHOLOGY

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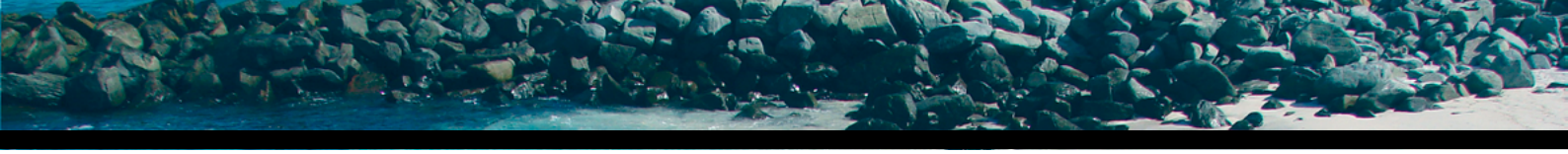
The *t*ransient *r*eceptor *p*otential (TRP) family of channels comprises more than 50 cation channels; they are present from algae to humans. These channels are composed of seven main subfamilies; TRPA, TRPC, TRPM, TRPML, TRPN, TRPP and TRPV; they are expressed in a variety of tissues participating in different cellular functions like intracellular calcium oscillation, immune response, cardiac pacemaking, cell migration, vascular tone, etc. New evidence showed that some of these channels are implicated in important neuronal functions like transduction of sensory stimulation, neuronal proliferation, differentiation, nerve growth, excitability control, synaptic transmission, neuronal damage and neuronal death. In this context, TRPC channels have been involved in the regulation of burst firing and intrinsic neuronal excitability through its sensitivity to membrane lipids. Additionally, TRPV channels regulate synaptic strength through endocannabinoid in central synapses. Moreover, TRPM channels has been implicated in physiological and pathological conditions, for example, TRPM7 is physiologically described as an entry way of trace metals like Mg^{2+}, however during ischemic episodes it drives neuronal Ca^{2+} overload. Furthermore, TRPM4 channels modulate the excitability by driving the afterdepolarization current, which increases the neuronal ability to fire continuous action potentials; also during ischemia-reperfusion injury, their non-regulated activation produces Na^{+} overload causing neuronal death. Additionally TRPM4 impaired function has been associated with axonal damage in animal models of multiple sclerosis. Importantly, TRPC3, TRPM2, TRPM4 and TRPM7 show different degrees of redox sensitivity suggesting that its redox modification could drive some of their functions. In this context, TRP channels are emerging as essential cellular switches allowing to understand the control of the neuronal homeostasis and how their impaired activity play a key role in pathological conditions and potentially bringing new drug target against neuronal disorders.

SYMPOSIUM
Aging and Neurodegeneration
Chair: Cecilia Hidalgo

CALCIUM DYSREGULATION IN A RODENT MODEL OF ALZHEIMER´S DISEAS

Paula-Lima, A[1,2]., More, J[2]., Barattini, P[2]., Adasme, T[2,3]., Hidalgo, C[2,3]., Valdes, JL[4]., [1]Institute for Research in Dental Sciences, Faculty of Dentistry, Universidad De Chile.[2]Biomedical Neuroscience Institute , Faculty of Medicine, Universidad De Chile.[3]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, Faculty of Medicine, Universidad De Chile.[4]Center for Neuroscience of Memory, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile. (Sponsored by Support: FONDECYT (1150756, 1100052, 11140580); BNI (P-09-015F).)

Introduction: Current evidence indicates that soluble beta-amyloid oligomers (AβOs) are likely causative agents of memory loss in age-related Alzheimer´s disease. Recently, we reported in rat primary hippocampal neurons that AβOs generate long lasting Ca^{2+}-signals. These signals, which arise from Ca^{2+}-induced Ca^{2+}-release from the endoplasmic reticulum (ER), are mediated by ryanodine receptors (RyR) and are sensitive to cellular redox state. The ER is in physical contact with mitochondria; thus, prolonged Ca^{2+}release from the ER promotes sustained mitochondrial Ca^{2+}-uptake, leading to excessive reactive oxygen/nitrogen species (ROS/RNS) generation and oxidative stress. We have reported that persistent RyR-mediated Ca^{2+}-signals invoked by soluble Aβ oligomers (AβOs) prevent the spine remodeling invoked by brain-derived neurotrophic factor, decrease RyR2 mRNA and protein contents and provoke mitochondrial fragmentation (Paula-Lima et al, 2011). Moreover, pre-incubation with N-acetylcysteine, an effective antioxidant glutathione precursor, abolishes the cytoplasmic Ca^{2+} increases and the mitochondrial fragmentation induced by AβOs (SanMartin et al, 2012). **Objectives:** Here, we investigated whether mitochondrial ROS/RNS generation modulates the RyR2 expression changes induced by AβOs, and if decreased hippocampal RyR2 expression affects hippocampal-dependent spatial learning and memory. **Materials and Methods:** Primary hippocampal neurons were transfected with mito-Pericam or HyPerMito to detect mitochondrial Ca^{2+} and hydrogen peroxide production, respectively, or were incubated with MitoSox to sense mitochondrial superoxide generation. Mitochondrial fragmentation was detected in neurons loaded with MitoTracker. Male rats, bilaterally injected intra-hippocampus with AβOs or antisense oligonucleotide anti-RyR2 (O-RyR2), were trained in the Oasis Maze task to evaluate hippocampal-dependent spatial learning and memory. Fluorescence images acquired by confocal or spinning disk microscopy were analyzed with ImageJ software; RyR2 mRNA expression was evaluated by q-PCR and protein content by immunofluorescence. **Results:** Intra-hippocampal injections of AβOs or O-RyR2 significantly decreased RyR2 protein content, without alterations in RyR3 protein levels, and impaired spatial learning. The decrease in RyR2 mRNA levels induced by AβOs required mitochondrial ROS/RNS generation. **Conclusions:** Our results suggest that redox-sensitive RyR2-mediated Ca^{2+}-release is crucial for spatial memory processes, and suggest that deficient RyR2-mediated Ca^{2+} signaling contributes to AβOs-induced learning and memory deficits. **Support:** FONDECYT (1150756, 1100052, 11140580); BNI (P-09-015F).



WNT SIGNALING STIMULATES NEURONAL GLUCOSE METABOLISM AND ENHANCES NEUROPROTECTION AGAINST AB OLIGOMERS

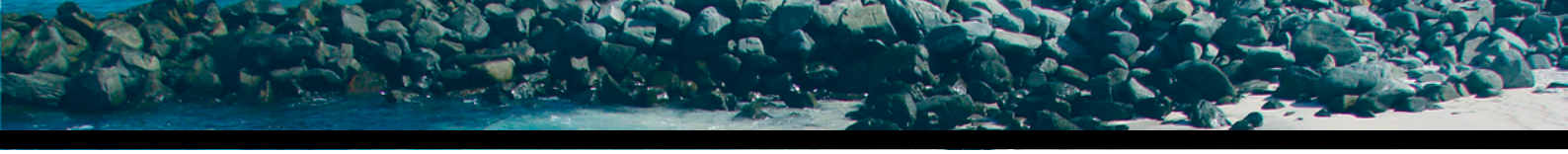
Inestrosa, N[1]., [1]Cell and Molecular Biology, Biological Sciences, Pontificia Universidad Católica De Chile.

The Wnt signaling pathway is critical for a number of functions in the central nervous system, including the regulation of the synaptic structure and function and the neuroprotection against several insults, including the amyloid-b peptide (Ab_{1-42}) oligomers of Alzheimer´s disease. The loss of Wnt signaling has been associated with several brain pathologies, including Alzheimer`s and Parkinson`s disease. In recent years, a new role has been suggested for the Wnt pathway as a central integrator of metabolic signals from peripheral organs to the brain. Most neurological disorders have been linked with a decrease in the brain's capacity to utilize glucose; however, despite the importance of this issue, little is known about the relationship between Wnt signaling and brain glucose metabolism and how this interaction might play a role in neuroprotection. Here we will present a study, where the activation of the canonical and the non-canonical Wnt signaling pathways induces a strong increase in glucose uptake and the glycolytic rate in cortical neurons. The effect of the canonical Wnt signaling was independent of the transcription of Wnt target genes. However, the effect of the non-canonical Wnt signaling required the intracellular generation of nitric oxide. Finally, we demonstrate that the protective effect of Wnt signaling against neurotoxicity induced by the Ab_{1-42} oligomers is partially dependent on the stimulation of glucose metabolism. Together, our data suggest that Wnt signaling stimulates energy metabolism in neurons and that this effect could be a key factor to prevent neuronal cell death.

THE WNT EFFECTOR ß-CATENIN IN MODELS OF AMYOTROPHIC LATERAL SCLEROSIS: ALLY OR FOE?

Pinto, Cristina[1]., **Henriquez, Juan Pablo[1]**., [1]Cell Biology, Biological Sciences, Universidad de Concepcion. (Sponsored by FONDECYT 1130321 And MINREB RC120003)

Introduction: Wnt pathways are associated to neurodegeneration. In mice models of amyotrophic lateral sclerosis (ALS), the expression of Wnt ligands is altered in motoneurons. Our previous findings showed that cultured ALS-like motoneurons display reduced Wnt activity. Here, we analyzed the localization of ß-catenin in motoneuron-like NSC34 cells expressing wild-type (NSC-hSOD1WT) or mutated (NSC-hSOD1G93A) superoxide dismutase-1 (hSOD1), as well as in spinal cord and neuromuscular junctions (NMJs) of mutant hSOD1 mutant mice. **Materials and Methods:** hSOD1-expressing NSC34 cells were double stained for ß-catenin along with MAP1B, vimentin, ubiquitin, LC3 and γ-tubulin. ß-catenin staining was also performed in vibrosections of hSOD1G93A mice spinal cords from P21 to P130 and cryosections of skeletal muscles. Western blots were performed in cytosolic and membrane fractions of control and ALS-like NSC34 cells. **Results:** NSC-hSOD1G93A cells display large accumulations of ß-catenin in the cell cortex. These structures are not amyloid, and are neither associated with aggresomes, nor with hSOD1 aggregates. Y489 and Y654 phosphorylated ß-catenin are not incorporated into these structures. In control NSC-hSOD1WT cells, lithium treatment translocates ß-catenin to the nucleus. In turn, in NSC-hSOD1G93A cells lithium decreased ß-catenin accumulations, in the absence of nuclear import. Western blot analyses showed that ß-catenin is accumulated in membrane fractions of NSC-hSOD1G93A cells. Motoneurons of presymptomatic ALS mice display ß-catenin structures, whereas control mice show strong nuclear ß-catenin staining. Even though ß-catenin labels the NMJ of control mice, this staining is lost in symptomatic stages of ALS mice. **Discussion**: ß-catenin is accumulated in ALS motoneurons as in other neurodegenerative conditions; but, different to them, it does not aggregate in amyloid structures. These structures could be related to the observed decrease in Wnt pathway activation. Our novel findings also reveal a potential role for ß-catenin in the maintenance of the NMJ in the context of ALS.



AGING AND NEURODEGENERATION: THE MITOCHONDRIAL CONNECTION

Nuñez, Marco T.[1]., Urrutia, Pamela J.[1]., Aguirre, Pabla[1]., Garcia-Beltran, Olimpo[2]., Cassels, Bruce K.[2]., Tapia, Victoria[1]., Mena, Natalia P.[1]., [1]Biology, Sciences, Universidad de Chile. [2]Chemistry, Sciences, Universidad de Chile. (Sponsored by This Work Was Supported By Grants Fondecyt 1130068 And ACT-1114 From PIA, CONICYT.)

Mitochondria are intrinsic sources of ROS that play a vital role in the regulation of intracellular Ca^{2+} and apoptotic processes. In recent years, increasing attention has been given to the role of mitochondrial dysfunction in the pathogenic development of various neurodegenerative disorders. Neurons are particularly sensitive to ROS and ATP imbalances derived from mitochondrial dysfunction because of their unique elongated morphology and their dependence on ATP to propagate electrical signals, maintain ionic gradients, and facilitate anterograde and retrograde axonal transport. Iron accumulates with age in the healthy human brain. Several recent reports have described brain mitochondrial dysfunction with iron accumulation in neurodegenerative disorders such as PD, AD, HD and a less investigated group of disorders known as NBIA (Neurodegeneration with Brain Iron Accumulation), characterized by the presence of high brain iron, particularly within the basal ganglia. Here we will review the most prominent evidence that links mitochondrial dysfunction and iron accumulation, and present recent evidence on the intervention of a vicious cycle mitochondrial dysfunction–iron accumulation by the use of novel iron chelators with mitochondria targeting and BBB permeability, and by silencing of the cellular iron homeostasis regulator IRP1, which is spuriously activated during mitochondrial dysfunction.

SYMPOSIUM
NEUROPHARMACOLOGY OF STRESS, ANXIETY AND DEPRESSION
Chair: Javier Bravo

INFLUENCE OF MATERNAL EXPERIENCE ON BEHAVIORAL RESPONSE TO THE MATERNAL SEPARATION STRESS IN MOTHER RATS

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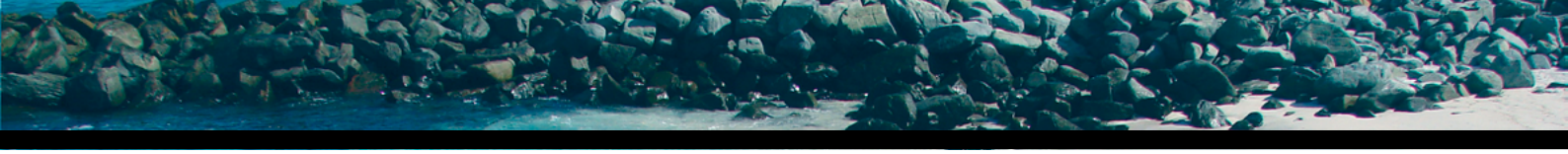
Transition to motherhood induces a number of extraordinary physiological, neuroendocrine and behavioral modifications. Therefore pregnancy and postpartum are periods of maximum neuronal and behavioral plasticity in a female life. In laboratory rodents, the chronic effects of postnatal manipulation of the infant-mother relationship have been studied experimentally for more than 50 years. While most of the studies in which the paradigm of maternal separation is used have focused their attention on the effects on offspring, few have focused on the effects on maternal behavior and physiology. Although limited, recent work has begun to document the effect of maternal separation stress on dams. We hypothesized that lengthy separation from their pups would affect emotion, maternal behavior and neuroendocrine parameters. Our work demonstrated that the stress of maternal separation during the postpartum induce alterations in the dam\'s pup-retrieval behavior, increases anxiety-like behavior, and has a detrimental effect on cognitive processes which is prevented by the rewarding aspects of physical contact with pups. Additionally we investigated whether reproductive experience (number of pregnancies and parturition) affects the behavioral response to an environmental stress as the early mother-pup separation by studying parameters known to be affected by long-term stress. Primiparous (1 reproductive experience) and multiparous (MP) (2 reproductive experiences) age-matched female Wistar rats were subjected to either animal facility conditions or daily 4,5h of separation from pups (MS) from postpartum day (PPD) 1-21. Maternal behavior was evaluated during early postpartum. After weaning, anxiety and spatial memory were assessed. The preliminary results suggest that MP females show a greater efficiency in maternal care. During early postpartum, multiparity and separation from pups induces an increase of active maternal behaviors. MP rats show a trend towards better performance in spatial memory. Contrary to expected, MP females showed increased anxiety-like behaviors. Although preliminary, the present results support the conclusion that reproductive experience influences the maternal response to stress. Understanding how a disturbed mother-infant relationship affect the neurochemical, physiological and behavioral profiles in dams will contribute to a better knowledge of postpartum psychiatric disorders and the detrimental outcome of separations for both the mother and child.



CROSS-TALKS BETWEEN STRESS AND POLYUNSATURATED FATTY ACIDS: ROLE ON DEPRESSIVE DISORDERS

Dagnino-Subiabre, Alexies[1]., Pérez, Miguel[2]., Peñaloza-Sancho, Valentín[1]., Fuenzalida, Marco[2]., [1]Laboratory of Behavioral Neurobiology, Center for Neurobiology and Brain Plasticity, Institute of Physiology, Faculty of Sciences, Universidad de Valparaíso. [2]Laboratory of Neural Plasticity, Center for Neurobiology and Brain Plasticity, Institute of Physiology, Faculty of Sciences, Universidad de Valparaíso. (Sponsored by This Work Was Supported By FONDECYT 1141276 Grant (Alexies Dagnino). Labsite: www.stress.cl.)

While chronic stress induces dendritic atrophy in the hippocampus and impairs learning and memory, supplementation with n-3 polyunsaturated fatty acid (PUFA) is known to improves memory of unstressed rats. Whether n-3 PUFA supplementation could improve dendritic morphology and memory of stressed rats remain unknow. Male Sprague-Dawley rats were randomly assigned to unstressed and stressed (chronic restraint stress) experimental groups. Afterward, animals were supplemented with n-3 PUFAs (DHA and EPA mix) or vehicle. Dendritic morphology and synaptic transmission in the hippocampus were evaluated by Golgi stain and patch-clamp tools, respectively. The Y-maze and Morris water maze were used to analyze the effects of chronic stress on memory consolidation. Supplementation with n-3 improved dendritic architecture and restored the frequency of inhibitory postsynaptic currents of hippocampal pyramidal neurons of stressed rats. In addition, n-3 supplementation improved spatial memory. Our results demonstrate that n-3 supplementation had three beneficial effects on stressed rats: prevents dendritic atrophy in CA3, restores the GABA release probability in CA1 and improves spatial memory. We speculate that n-3 supplementation could be used in the treatment of stress-related psychiatric disorders such as depressive disorders.

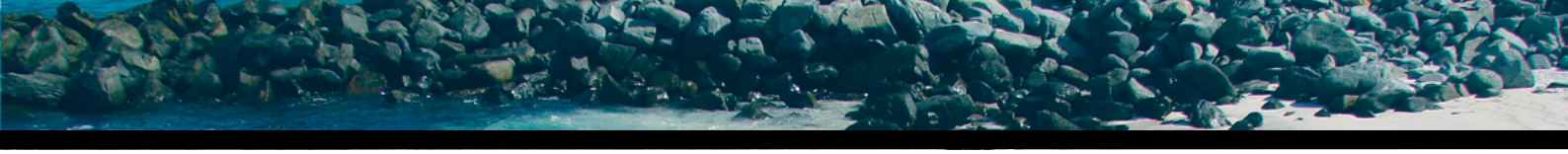
EARLY-LIFE INTESTINAL DYSBIOSIS AND ITS IMPACT ON STRESS-RELATED BEHAVIOURS IN YOUNG RATS

Bravo, Javier[1]., [1]GRUPO DE NEUROGASTROBIOQUÍMICA, LABORATORIO DE QUÍMICA BIOLÓGICA, INSTITUTO DE QUÍMICA, FACULTAD DE CIENCIAS, PONTIFICIA UNIVERSIDAD CATÓLICA DE VALPARAÍSO. (SPONSORED BY FUNDED BY: VRIEA PUCV # DI 037.386/2014, FONDECYT #1130213 & FONDECYT #1140776.)

Early-life exposure to microorganisms is key to the development of the stress response in animals. For example, germ-free mice have reduced anxiety-like behaviours despite producing an exaggerated release of corticosterone upon stressful stimuli. This suggests that the presence of microbes during early-life is necessary to adequate couple behavioural and physiological stress responses.

The communication between the gastrointestinal tract (GIT) and the central nervous system (CNS) is the basis to explain why interventions in gut bacteria affect stress-related behaviours, corticosterone levels and brain gene expression. Moreover, alterations in this conversation between the intestinal microbiome, GIT and CNS during early-life could be the biological substrate to gastrointestinal dysfunction and stress-related psychiatric disorders that might appear throughout life.

The infant gut begins to get colonized at birth by cross-contamination with the mother's vaginal, faecal and skin microbiota. In humans, by the age of 2 years, the gut microbiota resembles that of an adult. However, alterations in the balance between putative species of "protective" bacteria versus "harmful" ones in early-life can occur, affecting the microbiome-gut-brain axis in a way that affects how the individual copes with an ever changing environment later in life. These episodes of dysbiosis can occur in many forms, from nutritional alterations, to exposure to wide spectrum antibiotics. Interestingly, the effects of early-life dysbiosis on CNS function and physiological responses to stress are evident in infantile rats. This presentation will review empirical evidence on two animal models of early-life dysbiosis: 1) dietary supplementation of infantile rats with a Lactobacillus (a probiotic), inulin (a prebiotic) and the mixture of both and 2) perinatal exposure to broad-spectrum antibiotics. In both models, the effect of dysbiosis on stress-related behaviours and relevant molecular markers associated with the stress response and/or relevant to the neuropharmacology of stress was evaluated at a young age. All of these findings are observable during infancy, suggesting that further interventions can be made in order to prevent the occurrence of stress-related psychiatric disorders later in life.



NEUROPHARMACOLOGY OF THE BRAIN-GUT-MICROBIOME AXIS: FOCUS ON SEROTONIN AND TRYPTOPHAN METABOLISM

Clarke, G[1]., [1]Psychiatry/APC Microbiome Institute University College Cork.

The brain-gut axis is a bidirectional communication system between the central nervous system and the gastrointestinal tract. Accumulating evidence points to a critical role for the gut microbiome in regulating the normal functioning of this axis. Dysregulation of this reciprocal communication network impacts substantially on physiology, brain and behaviour across the lifespan. In particular, early life perturbations of the developing gut microbiota can impact on brain development and potentially lead to adverse mental health outcomes later in life. This ability of the gut microbiota to influence brain function is supported by both clinical and preclinical studies and extends to complex behaviours relevant to anxiety, depression, pain and cognition. Serotonin functions as a key neurotransmitter at both terminals of the brain-gut axis and it is becoming clear that the microbial influence on the serotonergic system may be an important neurobiological consequence of perturbations to the gut microbiome. There is also substantial overlap between behaviours influenced by the gut microbiota, those which rely on intact serotonergic neurotransmission and the prominent features of stress-related microbiome-gut-brain axis disorders such as irritable bowel syndrome. The mechanisms underpinning this crosstalk require further elaboration but may be related to the ability of the gut microbiota to control host tryptophan metabolism along the kynurenine pathway, thereby simultaneously reducing the fraction available for serotonin synthesis and increasing the production of neuroactive metabolites. This dual impact in addition to the ability to regulate relevant CNS receptor expression gives the gut microbiome a broad neuropharmacological profile. In addition, there are neural processes in the gastrointestinal tract which can be influenced by local alterations in serotonin concentrations with subsequent relay of signals along the scaffolding of the brain-gut axis to further influence CNS neurotransmission. Taken together, these findings firmly establish the gut microbiota as a critical node in the brain-gut axis and one which may be amenable to therapeutic interventions. An improved appreciation of these dynamic interactions can bring benefits across diagnostic, preventative and treatment domains.

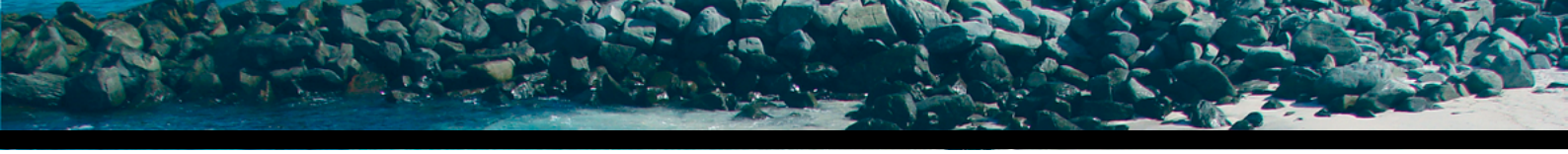
SYMPOSIUM
PHARMACOLOGICAL APPROACHES FOR PATHOPHYSIOLOGICAL CONDITIONS ASSOCIATED WITH HYPOXIA AND OXIDATIVE STRESS
Chair: Rodrigo Castillo

CARDIAC HYPOXIC INJURY AND OXIDATIVE STRESS: PROTECTIVE STRATEGIES AND POTENTIAL CLINICAL APLICATIONS.

Castillo, R[1]., Farías, Jorge[2]., Herrera, Emilio[3]., Carrasco-Pozo, Catalina[4]., Sepúlveda, Nestor[5]., [1]Programa de Fisiopatología, Instituto de Cs Biomédicas, Facultad de Medicina, Universidad de chile. [2]Departamento de Ingeniería Química, Facultad de Ingeniería y Ciencias, Universidad de la Frontera, Universidad De La Frontera. [3]Programa de Fisiopatología Oriente, ICBM, Facultad de Medicina, Universidad de Chile, Universidad de chile. [4]Departamento de Nutrición, Facultad de Medicina, Universidad De Chile. [5]Laboratorio de Producción Animal, Facultad de Ciencias Agropecuarias y Forestales, Universidad De La Frontera. (Sponsored by Fondecyt 11110246 (RLC.); 1151119 (EHV.); 1130232 (CCP.))

Hypoxia is a pathophysiological condition associated with several responses at cardiovascular, pulmonary and vascular levels, which may derive in chronic diseases. This is relevant in human populations exposed to high altitude, in either chronic continuous (permanent inhabitants) or intermittent hypoxia (IH) (high altitude workers, tourists and mountaineers). In Chile, it is estimated that 1.000.000 people live at highlands and more than 55.000 work in high-altitude shifts. IH is associated with the development of systemic hypertension and left ventricular dysfunction. At present, however, our understanding of the basic mechanisms linking IH and cardiovascular dysfunction is limited by the pathophysiological heterogeneity of hypoxic patients and the presence of multiple confound and comorbid conditions, including obesity and previous cardiac impairments. Moreover, the great variety of responses ranges from no clinical effects to strong pulmonary hypertension and vital risk. Consequently, there is a serious need for the development of experimental models to study the mechanisms involved in the cardiovascular responses to IH and the potential deleterious effects.

Acute exposure to high altitude has been shown to induce oxidative stress in healthy human lowlanders, as indicated by an increase in free radical formation. However, IH may induce an ischemic preconditioning-like in some animal models. Therefore, pharmacological preconditioning strategies have acquired importance in the development of novel therapies. Compelling data show cardiovascular beneficial effects in consuming fatty acids highly present in fish, such as omega 3 (Ω3), docosahexanoic acid (DHA 22:6 Ω3), and eicosapentanoic acid (EPA 20:5 Ω3). These fatty acids regulate cell membrane physicochemical properties (i.e., fluidity, organization and permeability) that affect signaling pathways, with probable antioxidant and antiinflammatory effects on cardiac tissue. Also, dietary Ω3 would induce a form of preconditioning, nutritional preconditioning, limiting hypoxic cardiac injury, and myocardial infarction and endows cardioprotection as powerful as ischemic preconditioning. These mechanisms have been showed in some clinical trials in coronary and heart failure patients. Consequently, it is desirable explore the effects of Ω3 supplementation in human populations exposed to IH. Grant: Fondecyt 11110246 (RLC.); 1151119 (EHV.); 1130232 (CCP.)



PERINATAL HYPOXIA AND OXIDATIVE STRESS: MECHANISMS AND POTENTIAL NEW THERAPIES.

Herrera, Emilio A[1,2]., Ebensperger, German[1]., Reyes, Roberto[1]., Llanos, Anibal[1,2]., [1]Programa de Fisiopatología, ICBM, Facultad de Medicina, Universidad de Chile. [2]International Center for Andean Studies (INCAS) Universidad de Chile. (Sponsored by Fondecyt 1110595, 1120605, 1130424, 1140647 & 1151119.)

The etiology of several non-communicable diseases has been linked with adverse perinatal conditions, such as hypoxia and oxidative stress. Both conditions are seen in chronic hypobaric hypoxia at high-altitude (>2,500m). Gestation and birth at highlands induce intrauterine growth restriction and pulmonary hypertension of the neonate. Neonatal pulmonary hypertension may induce cardiopulmonary remodeling and right heart failure if sustained in time. This is a pathophysiologic condition with multifactorial etiologies, due to an imbalance between vasodilator and vasoconstrictor mechanisms and maladaptive vascular remodeling of pulmonary arteries. Several studies have defined the mechanisms involved in the elevated pulmonary vascular resistance and remodeling, deriving in novel therapeutic proposals tested in animal models and some of them have reached the clinical practice. These approaches include treatments aiming to enhance vasodilator pathways such as nitric oxide-guanylyl cyclase-GMP, prostaciclyn-adenylate cyclase-AMP; and to decrease vasoconstrictor pathways such as endothelin-1 and calcium channels, among others. Further, in the last years, antioxidants and antiproliferative agents has positioned as important therapeutic adjuvants to prevent and/or treat pulmonary hypertension of the neonate, such as melatonin and carbon monoxide. New treatments are badly needed since NO administration fails in 40% of the neonates with pulmonary hypertension. Therefore, we will review some of these novel treatments to attenuate high-altitude perinatal complications.

OCCUPATIONAL EXPOSURE TO CHRONIC INTERMITTENT HYPOXIA: BETWEEN ACCLIMATIZATION, INTOLERANCE AND PROTECTION.

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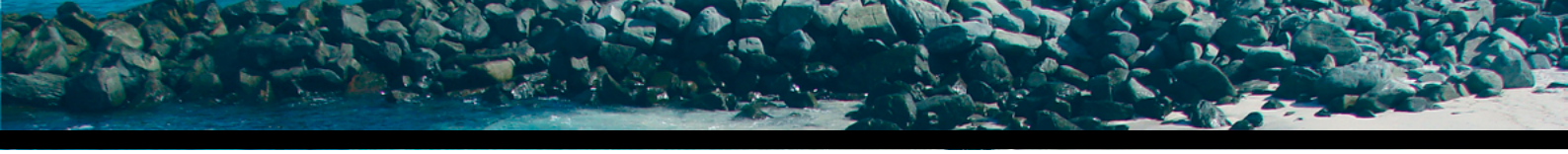
Native sea level workers, occupationally exposed to chronic intermittent hypobaric hypoxia, CIH, may be affected in their health and safety by environmental and ergonomic factors, atmospheric and O2 pressure, temperature, humidity, solar radiation, climate and unusual physical-mental exigencies. Hypobaric hypoxia is the most important factor. From 3000 meters, those exposed show signs in their ventilatory response, elevated EPO, Hb, pH, blood pressure, pulse, coronary and cerebral blood flow, and pulmonary pressure, while remaining stable cardiac output and stroke volume; but during sleep they have periodic breathing, drop SatO2 and rise central apnea/ hypopnea.

The type of exposure to altitude, acute, chronic or intermittent, determines the intensity and timing of these signs. In CIH that is more diverse and complex, because at cycling days in normobaria and hypobaria the subject goes through chronic, acute and deacclimation exposure. In homogeneous labor groups by age, health status, altitude and ergonomic needs, the answer to the CIH has been heterogeneous, since excellent tolerance to proper acclimatization even intolerance. At higher altitudes more heterogeneity.

In occupational health is strategic achieve better acclimatization and prevent the two types of intolerance, aggravation of pre-existing conditions, or occurrence of disabling altitude illnesses as acute mountain sickness, pulmonary edema, cerebral edema, pulmonary hypertension, sleep disturbances, reactive hypertension, etc. Also interested occupational physical-muscular work tolerance, due to physiological VO2max decline, with risk of fatigue facing intense effort.

Adequate tolerance and acclimatization is prioritized, with staff not carrying hypoxia incompatible illnesses, controlling the quality of sleep, screening and treatment of altitude illnesses, promoting healthy lifestyle, and design appropriate worksites. Besides mitigation measures, such weatherization dormitories, use of acetazolamide and supplemental oxygen in case of central apneas.

Recent biomedical information is confirming some cardioprotective effects of chronic hypoxia, which for decades had already been pointed out in inhabitants of the Peruvian altiplano. Evaluations in controlled doses of HIC indicate positive effects on cardiac hemodynamics, homeostasis of redox regulation and calcium, modulation of apoptoics signals and neovascularization. This opens new horizons for the epidemiology tolerance of workers to occupational exposure to CIH.



PHARMACOLOGICAL STRATEGIES FOR THE PREVENTION OF THE EFFECT OF INTERMITTENT HYPOBARIC HYPOXIA IN RAT TESTIS.

Farias, Jorge[1]., Castillo, Rodrigo[2]., Figueroa, Elias[1]., Short, Stefania[1]., Zepeda, Andrea[1]., Figueroa, Carolina[1]., [1]Departamento de Ingeniería Química, Facultad de Ingeniería y Ciencias, Universidad de La Frontera. [2]Programa de Fisiopatología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile. (Sponsored by Fondecyt 1151315 (JGF); 11110246 (RLC))

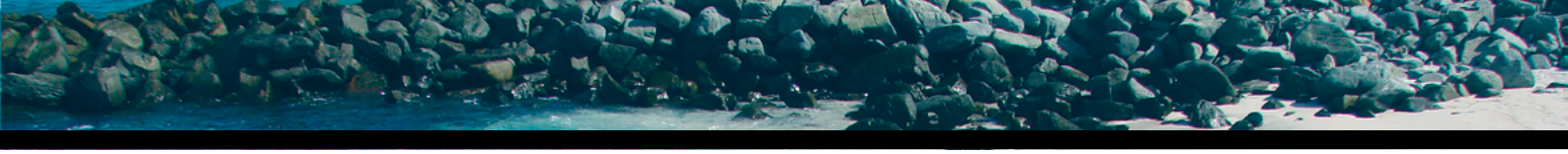
In Chile, due to the intensive activity developed in confining areas of the Andes Mountains ranging in altitude over 4000 asl, there has been an increasing intermittent migration of human resources to high altitude (HA) lands. This unusual condition, defined as hypobaric hypoxia, affects notoriously in any living organism and there shows a series of pathophysiological responses. Studies performed in rats under chronic hypobaric hypoxia and intermittent hypobaric hypoxia have registered changes in testicular morphology together with loss of spermatogenic cells in all stages of spermatogenic cycle. Furthermore, recent experimental evidences reinforced the existence of an oxidative metabolism in epididymis of rats subjected to hypobaric hypoxia due to the increase in the regulator enzyme expression of reactive oxygen species (ROS), This increase in the production of ROS induced a rise in apoptosis at germinal cell level, leading to a state of hypo-spermatogenesis that may jeopardise masculine fertility. Therefore, the eventual development of oxidative stress in spermatogenic cells and consequently the spermatozoids of workers subjected to HA, either chronic or intermittent, turns out to be critical when it poses as an imminent risk to the viability and quality of the reproductive cells of workers subjected to intermittent hypobaric hypoxia. For several years, the study of the responses to hypoxic insults and pharmacological targets has been the motivation of our group. In this case, the antioxidant supplementation with ascorbate, melatonin or blueberries have been used to attenuate this prooxidant imbalance and their derived functional and structural consequences in reproductive cells. This work describes some of the mechanisms underlying hypoxic responses and the novel potential therapeutic approaches in rat model of intermittent hypoxia.

SYMPOSIUM
MEMORY AND STRESS
Chair: Jimmy Stehberg

A GABAERGIC SIGNALING WITHIN THE BASOLATERAL AMYGDALA COMPLEX MODULATES THE INFLUENCE OF STRESS ON FEAR MEMORY

Molina, V[1]., Rodriguez Manzanares, P.[1]., Ortiz, V[1]., Espejo, P.[1]., Giachero, M.[1]., Calfa, G.[1]., Calfa, G.[1]., Isoardi , N.[1]., Carrer, H.[1]., Carrer, H.[1]., Martijena, I.[1]., Maldonado, N.[1]., [1]Departamento de Farmacologia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

There is consensus that stressful experiences enhance fear memory formation. This influence is highly adaptive since it is extremely relevant for the animal's survival to anticipate and avoid potential dangerous stimuli. The Amygdala Basolateral Complex (BLA) is crucially implicated in fear memory formation. It is well recognized that GABAergic interneurons within the BLA are responsible for controlling the activity of projecting glutamatergic cells through feedforward and feedback inhibition. Findings from our laboratory have revealed that a single restraint experience elicited BLA neuron hyperexcitability, which resulted from the reduction of recurrent GABAergic inhibition. This view was further supported by pharmacological evidence, since intra-BLA infusion of midazolam (MDZ), a positive modulator of GABAa sites, prior to stress prevented the enhanced fear memory of stressed animals. In contrast, blockade of GABAa receptors in the BLA, but not in the adjacent CeA, facilitated fear memory, similar to the ameliorating influence induced by stress. Moreover, the stress-induced promoting effect on the emergence of associative fear memory is coincident with the facilitating influence of stress on LTP generation in BLA, a neuroplasticity process linked to fear memory formation. Accumulating evidence suggests that the dorsal hippocampus (DH) is a downstream target of BLA neurons in contextual fear memory and hippocampal structural plasticity is proposed to provide a substrate for the storage of long-term memories. Recent data showed that prior stressful experience promoted contextual fear memory and enhanced dendritic spine density in the DH. Intra-BLA infusion of MDZ prevented the facilitating influence of stress on structural remodeling in DH. Similarly to the stress-induced effects, the blockade of GABAa sites within the BLA ameliorated fear memory and induced structural remodeling in the DH. Overall, these findings suggest that stress, by reducing this inhibitory GABAergic control, would result in an unmasked activation of projecting neurons of the BLA, facilitating fear memory and promoting structural changes in the DH associated to contextual fear memory.



LONG TERM MEMORIES THAT ARE REACTIVATED WITHOUT BEING BEHAVIORALLY EXPRESSED

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During the consolidation process the memory traces are stabilized and, consequently, memories were conceived as fixed after being consolidated. This fixed memory trace paradigm was withdrawn; long-term memories are not invariable. Activated by reminder cues, the memory trace can be unstabilized again during the reconsolidation process. The withdrawal of the aforementioned paradigm has led to new perspectives about several mnemonic processes. In this context, we will discuss our studies on the modulation of memory expression during reconsolidation as a biological mechanism -conserved throughout evolution- that enables continuous updating of memory. We propose that during both memory consolidation and reconsolidation, neuromodulators can determine the probability of the memory trace to guide behavior, i.e. they can either increase or decrease its behavioral expressibility without affecting the potential of persistent memories to be activated and become labile. Moreover, the positive modulation of memory expression during reconsolidation can occur even if memory is unexpressed. This view offers a new view regarding both weak trainings, which do not generate long term memories, and experimental amnesia: memory retrieval cannot be conceived as thesaurus of memory expression during the testing sessions. This new view show that even in human declarative memory the periods in which long-term memory can be activated and become labile during reconsolidation exceeds the periods in which that memory is expressed, providing direct evidence that conscious access to memory is not needed for reconsolidation. In the hypothesis presented, memory expressibility - the outcome of experience-dependent changes in the potential to behave- is considered as a flexible and modulable attribute of long-term memories. Expression seems to be just one of the possible fates of re-activated memories.

A ROLE FOR THE INSULAR CORTEX IN FEAR OF THE NEW AND ANXIETY

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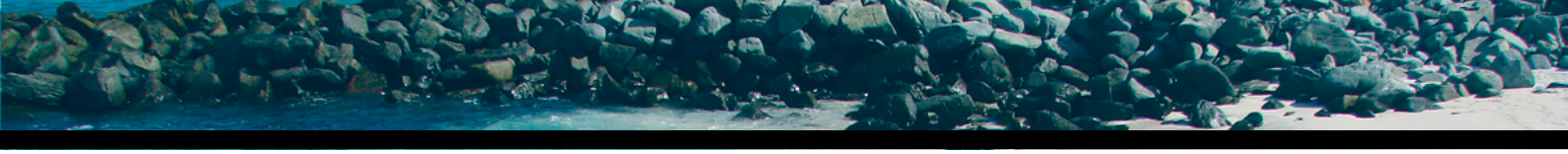
The Insular cortex is buried deep within the temporal lobe and is a complex structure that receives visceral, gustatory, somatosensory, pain, visual and auditory information. The Insula has also been associated to emotions and its augmented activity is common to all anxiety-related disorders. Here we show that the Insula regulates general anxiety, via mediating within the brain, the systemic effects of stress hormones adrenaline and glucocorticoids. Moreover, glucocorticoids acting at the Insula show paradoxical effects, giving new clues into the complexities of orchestrating behavior in response to stress and arousal. This work was funded by FONDECYT N°1130724.



THE ROLE OF ASTROCYTE-DERIVED EXOSOMES IN THE STRESS RESPONSE

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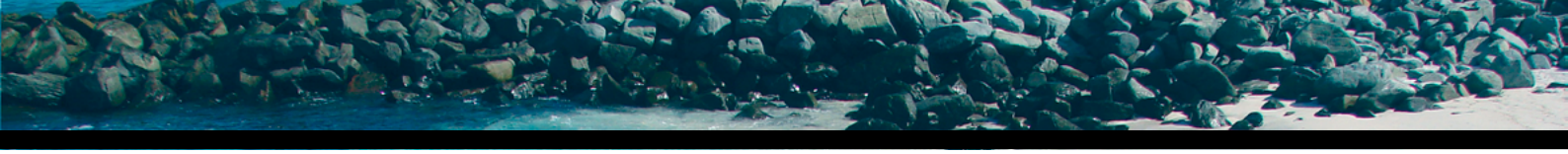
The search for animal models of mood disorders and of their subtypes, supported by the presence of biomarkers, is a yet unmet goal that would be useful for their diagnosis, treatment and the monitoring of their progression and treatment responsiveness. Recently, exosomes, i.e. extracellular vesicles of 30-100 nm that originate in the endosomal pathway, have emerged as potent mediators of diseases and as such, as markers of disease processes. Exosomes contain proteins and RNA species (both mRNA and miRNAs), affecting the function of target cells by regulating their gene expression.

We induced depressive-like behaviors in rats by exposure to chronic stress, attained by procedures that restrict movement of animals either by restriction in small cages or by immobilization in bags. Depressive-like behaviors in rats were reverted selectively by antidepressant drugs acting either on serotonin or noradrenalin neurotransmission. Moreover, when searching for a biomarker we found that the glycolytic enzyme aldolase C was differentially present in exosomes derived from the cerebrospinal fluid (CSF) or from the blood plasma of stressed animals. The central origin of aldolase C in plasma exosomes was demonstrated by *in utero* electroporation of forebrain astrocytes with GFP-aldolase C. In addition, exosomes in stressed animals contain a distinct miRNA signature. The effect of stress-derived exosomes of astrocyte origin and of stress-regulated exosomal miRNAs on neuronal function and morphology, as well as of their target genes in recipient cells, is currently under investigation.

We have found a novel form by which stress may affect neuronal function by volume transmission and possibly, may affect peripheral tissues as well.

ORAL PRESENTATIONS I



ACUTE AND CHRONIC AMPHETAMINE TREATMENTS MODULATES DIFFERENTIALLY NURR1 AND NF-KB P65 EXPRESSION IN THE RAT VENTRAL TEGMENTAL AREA

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Amphetamine is a powerful psychoactive drug. The rewarding and reinforcing properties are due to its capacity to elevate extracellular dopamine levels in the ventral midbrain area and its target nuclei through the reversal of the dopamine transporter (DAT) cycle Nurr1 is a nuclear receptor essential for the development and survive of midbrain dopaminergic neurons. This transcription factor regulates the expression of key genes of the dopaminergic phenotype as DAT and tyrosine hydroxylase (TH), the rate-limiting enzyme in the dopamine synthesis.

Moreover, drugs of abuse are known as triggers of inflammatory processes and damage in the brain. Exposure to cocaine and methamphetamine alters the transcription factor NF-kB levels in PC12 cells. Interestingly, Nurr1 and NF-kB p65 interact to regulate inflammatory genes in response to lipopolysaccharide (LPS) in microglia and astrocytes.
It is well known that recurrent drugs consumption causes long-lasting changes in dopaminergic neurons. So, we are interested in revealing the molecular mechanism underlying drug abuse. In this work we study the effects of acute and chronic amphetamine exposure in the expression of Nurr1, NF-kB p65 and TH in the rat midbrain region.
Male Sprague-Dawley rats weighing about 280g were injected with amphetamine (1.5 mg/kg) acutely or every day during fourteen days (chronic). Our data show that Nurr1 and NFkB-p65 co-localize in TH-positive cells in the rat VTA. Nurr1, TH and NFkB-p65 expression was assessed by Western blots of ventral midbrain total protein extracts. Acute amphetamine treatment induced an increase in Nurr1, TH and NFkB-p65 protein levels. Chronic amphetamine treatment decreased Nurr1 and NFkB-p65 protein levels, but TH was unchanged compared to saline-treated rats. Our results show that Nurr1 in midbrain dopaminergic neurons responds in a different way to acute or chronic amphetamine treatment and suggest that Nurr1 and NFkB-p65 could mediate a common response to amphetamine.

KOR-DEPENDENT POTENTIATION OF QUINPIROLE-INDUCED SENSITIZATION: NEUROCHEMICAL INTERACTION AND COHABITATION WITH D2 RECEPTORS.

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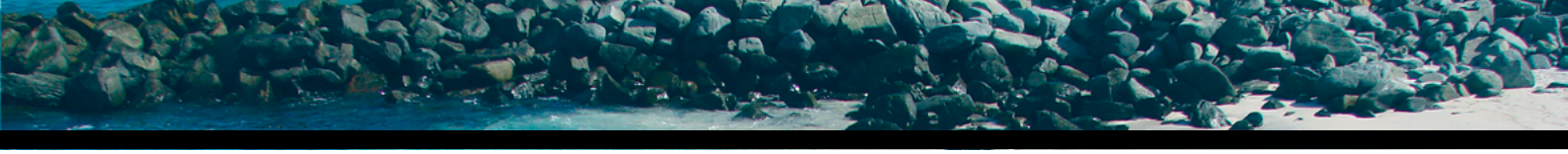
Kappa opioid receptors (KOR) are located presinaptically on dopaminergic terminals of the nucleus Accumbens (NAc). Similar to D2R autoreceptors, acute activation of KOR decreases dopamine extracellular levels and locomotor activity. Interestingly, co-activation of KOR potentiates the locomotor sensitization induced by repeated administration of the D2R agonist, quinpirole. Our previous microdialysis studies showed that repeated treatment with U69593 (U69), a KOR agonist, decreases the inhibitory D2R autoreceptor control on tonic dopamine release. Therefore, we have hypothesized that KOR-dependent potentiation of QNP-induced locomotor sensitization is due to a reduced inhibitory D2R autoreceptor function. Here, we show the neurochemical and molecular interactions between D2R and KOR in the NAc of rodents. Male *Sprague-Dawley* rats were administered with a mix of U69 and QNP every 2-3 days until 8 injections were completed. Horizontal locomotor activity was measured immediately after each injection. Forty-eight hours after the last injection, rats were anesthetized for Microdialyisis or fast scan cyclic voltammetry (FSCV) in the NAc. After baseline collections, rats were injected with U69 and thirty minutes later with QNP. Microdialysis data shows that KOR dependent potentiation of locomotor sensitization is accompanied by decreased dopamine and a conserved D2 autoreceptor function. FSCV data show a similar rapid decrease of phasic dopamine release in both control and drug-treated rats, when injected with QNP and U69. To evaluate whether KOR and D2R colocalize in any neuronal sub-structure, immunofluorescence assays were carried out in synaptosomes free of post-synaptic elements, mice brain slices and cultured dopamine neurons. Immunofluorescent assays in NAc synaptosomes showed that 20% of KOR positive label co-localize with D2R. In addition, mesencephalic primary cell culture showed that D2R-like labelling is mainly located on somato-dendritic profiles of TH-positive neurons, while KOR is located mainly on axon profiles. Immunofluorescence in mice NAc tissue slices showed that D2R-like labelling is mainly postsynaptic and partially colocalizes with KOR-like labelling, probably in GABA projecting neurons. Together, our data indicate that presynaptic inhibitory control over dopamine is maintained after repeated KOR and D2R activation and that U69 potentiation of QNP-induced locomotor sensitization is not linked to an increase of phasic dopamine release. In addition, histological data support the possibility of a postsynaptic molecular interaction between KOR and D2R.



Hypothalamic synaptic plasticity disturbance caused by a chronic high fat diet feeding

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(Sponsored by Fondecyt 1140162, PFB 01/2007.)

The hypothalamus is the main structure of the body in charge of energy balance homeostasis, since the hypothalamus senses the nutritional state of the body and orchestrates feeding behavior and energy expenditure in order to maintain energy homeostasis. This system is primarily controlled in the arcuate nucleus of the hypothalamus (ARC), which holds two major neuronal groups involved in sensing the peripheral signals, nutrient availability and regulate caloric intake. These neurons are the Pomc neurons which are activated by anorexigenic peripheral signals and release α-MSH to the various target nuclei. On the other hand are the Agrp neurons, which are activated by orexigenic peripheral signals, releasing AgRP and GABA. These two groups of neurons have opposing functions, decreasing caloric intake/increasing energy expenditure and increasing caloric intake/decreasing energy expenditure, respectively. As a cause of the pandemic health issue caused by obesity, many studies have focused on the effect of diet induced obesity, as an experimental model of the present globalized “western diets”, showing alterations in the brain, in cognition and also metabolic disturbances. Diet induced obesity causes alterations in energy balance control by altering the capability of neurons located in the hypothalamus to interconnect and integrate peripheral signals into this negative feedback loop. This cause altered health conditions such as leptin resistance, insulin resistance, metabolic disease, chronic inflammation among other alterations. In this work, mice where fed with a high fat/low carbohydrate diet to evaluate the effect of a ketogenic/non-overweighting diet in the hypothalamic synaptic plasticity and to comprehend the molecular events that conform the metabolic and neuronal alterations caused by high fat diets in different groups of neurons. For this purpose various assays were performed including the analysis of mRNA expression levels, western blot analysis, immunofluorescence and also food intake experiments. Mice fed with a high fat diet showed alterations in the expression of genes and proteins involved in energy balance and synaptic plasticity as well as changes in hypothalamic morphology. In addition, mice fed with a high fat diet had similar food intake and body weight in relation to control mice. This study provides an insight of the molecular changes caused by chronic high fat diet consumption in murine hypothalamus and better understanding of the molecular mechanisms by which the metabolic disturbances occur during high fat diet feeding.

EXPOSURE TO AN ENRICHED ENVIRONMENT DURING PREGNANCY AND LACTATION MODULATES FEEDING BEHAVIOR IN ADULT OFFSPRING

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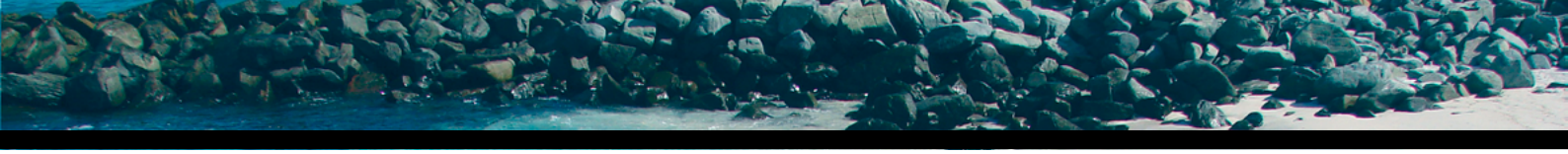
A growing body of evidence suggests that environmental factors during early stages of development are capable of modifying the metabolic phenotype observed during adulthood. In addition, a previous study demonstrated that the postnatal exposure of mice to an enriched environment, a widely used paradigm to induce synaptic plasticity, increases hypothalamic sensitivity to metabolic signals that control feeding behavior. Here, we evaluated the effect of exposing mice to an enriched environment during pregnancy and lactation on adult offspring feeding behavior and body weight. In order to address the phenotypic and behavioral effects of this early stage- environmental intervention, C57/B6 breeding pairs of mice were exposed to either an enriched environment or standard conditions. Male offspring from both conditions were housed in standard cages from weaning to adulthood. Body weight was recorded weekly and both food intake and locomotor activity was tested in metabolic cages at three, seven and twelve weeks of age.

Our results show that mice from breeding pairs exposed to enriched environment exhibited an increase in body weight between 3 and 6 weeks of age. After this period of time, we did not observe any difference in the body weight of mice from breeding pairs exposed to each of the environmental conditions. The reduction in body weight gain exhibited by mice from breeding pairs exposed to an enriched environment after 6 weeks of age was not associated to an increased locomotor activity, but rather with a reduction in food intake that remained until later ages. To evaluate whether this difference in feeding behavior was associated to an epigenetic modification during exposure to an enriched environment, impacting the post-weaning gene expression pattern, we first evaluated the expression of hypothalamic genes associated to energy homeostasis. Our results show a decreased expression of the anorexigenic gene *Pomc*, which may explain the decreased food intake observed in adult mice from breeding pairs exposed to an enriched environment. Currently, we are assessing methylation analysis of a CpG island located at the *Pomc* promoter, in order to associate the phenotype observed in adult mice with epigenetic modifications which occurr during the fetal development/lactation period. In summary, our results show that exposure to an enriched environment during pregnancy and lactation impacts on feeding behavior exhibited by offspring during adulthood as a consequence of changes in the expression of the anorexigenic gene *Pomc*

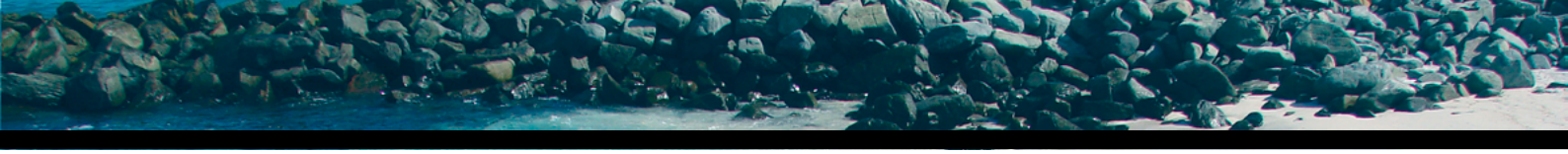


ASSESSING EXPOSURE TO ORGANOPHOSPHATE PESTICIDES, BIOMARKERS AND NEUROPSYCHOLOGICAL OUTCOMES IN RURAL POPULATIONS OF CHILE

Ramirez, M[1]., Zuñiga-Venegas, Liliana[2].,Corral-Zavala, Sebastian[3].,Pancetti Vaccari, Floria[2].,Sandoval-Guzman, Rodrigo[2].,[1]SALUD PUBLICA, MEDICINA, Universidad Católica Del Norte.[2]Biomedical Sciences, Medicine, Universidad Católica Del Norte.[3]Psychology, FACSO, Universidad de Chile. (Sponsored by This Research Was Supported By: FONDEF: Fondo De Fomento Al Desarrollo Científico Y Tecnológico, CONICYT Chile. Grant Number D09I1057)

The increased agricultural activity that Chile has experienced in recent years has resulted in increased use of pesticides. According to the records of the monitoring system of the Ministry of Health, the pesticide family mainly involved in episodes of acute poisoning corresponds to organophosphates, causing a 39% of acute intoxications. While acute poisonings are easily diagnosed, chronic exposure often goes unnoticed. The main problem for monitoring chronic exposure is the lack of highly sensitive biochemical biomarker capable of being monitored in a body fluid. Biomarkers available today only serve to diagnose acute poisoning. The project sought to determine whether the activity of the erythrocyte enzyme called **acylpeptide hydrolase (APEH)** serves as a biomarker for biomonitoring of human populations exposed to different levels of organophosphate pesticides; correlating its catalytic activity with cognitive performance. The project recruited a total of 268 volunteers of which **87 were occupationally exposed (OE), 81 were environmentally exposed (EE) and 100 as a reference group (RG).** The population was homogeneous in age range, alcohol intake, drugs consumption and smoking habits; however there were differences in gender and educational level, being the indirectly exposed group those presenting a higher number of women and educational level than the other two groups. The interview consisted in a neuropsychological evaluation and a blood sampling for **measuring erythrocyte acetylcholinesterase (AChE), plasma cholinesterase (BChE) and erythrocyte APEH** activities. Neuropsychological assessment included**: general mental state, memory, language, attention, praxis, executive function, motor coordination and mood**. The results of the blood tests indicated that the enzyme activities found to be inhibited by high environmental burden of organophosphates (during fumigation) were APEH in the environmentally exposed (EE) group and plasma cholinesterase in the occupational exposed (OE) group. Regarding to cognitive performance, both exposed groups showed abnormal results in most of the areas evaluated; **being most affected memory, executive function and fine motor coordination**. Six predictive models were generated to relate enzymes activity with cognitive outcomes during fumigation period using random forest analysis. When analyzing **delta (Δ) of enzymatic activity**, **APHE showed to be a good predictive variable for memory.** BChE and AChE showed significant power to predict executive function and motor control respectively.Other variables with significant strength were the number of study years and alcohol consumption.

ORAL PRESENTATIONS II



THE NEUROPROTECTIVE EFFECTS OF AN ERYTHROPOIETIN ISOFORM WITH LOW GLYCOSYLATION AGAINST THE ß AMYLOID STRESS.

Castillo, Carolina[1,2]., Hidalgo, Angela[1].,Silva-Grecchi, Tiare[2].,Fuentealba, Jorge[2].,Toledo, Jorge[1].,[1]Biotechnology and Biopharmaceuticals Laboratory, Departament of Pathophysiology., School of Biological Sciences, University of Concepcion.[2]Screening of neuroactive compounds Laboratory. Departament of Physiology., School of Biological Sciences., University of Concepción.

Erythropoietin is a glycoproteic hormone that weighs 34 KDa. Its principal function is to regulate the erythropoiesis; however since the discovery of its receptor (EpoR) in other non-erythroid tissues, like central nervous system (SNC) their function has been related with other pleiotropic effects. In the SNC has been observed a neuroprotective effect of this hormone that's related with the upregulation of antiapoptotic pathways and directly related with activation of its receptor. In patients with Alzheimer's disease exists an increase of EpoR expression *versus* control patients. The objective of this proyect is to evaluate the neuroprotective effect *in vitro* against the beta amyloid stress (Alzheimer's related protein) of an erythropoietin isoform with low glycosylation and without hematopoietic activity produced trough adenoviral transduction of goat's mammary gland (EpoL) and their dependence of the activation of EpoR. For this purpose we preincubated cortical neurons or PC12 cells with EpoL for an hour and then we incubated these cells with β-amyloid peptide or with an uncoupling of respiratory chain of electrons in mitochondria (FCCP), like a model of oxidative stress. The cells preincubated with EpoL shown an increase of the cellular viability of 32% more than the control cells, and an increase of 26% more than cells incubated with an inhibitor of activation of EpoR. Preincubated cells with EpoL prevent the increase of calcium transients induced by beta amyloid peptide. These results agree with different expression of EpoR and with the 50% of upregulation of BCL2 gene observed in the same treatments by qRT PCR experiments and also agree with an increase of immunoreactivity of EpoR in immunofluorescence data. With these results we have shown that EpoL has a neuroprotective effect against the stress induced by Aβ and also against oxidative stress. Further this effect is mediated by EpoR activation and also the anti-apoptotic pathways activation. Therefore these results suggested that EpoL can be used like a neuroprotective agent against neurodegenerative diseases.

BASAL CILIARY ACTIVITY DEPENDS ON ATP RELEASE IN MOUSE TRACHEAL EPITHELIAL CELLS *IN VITRO*.

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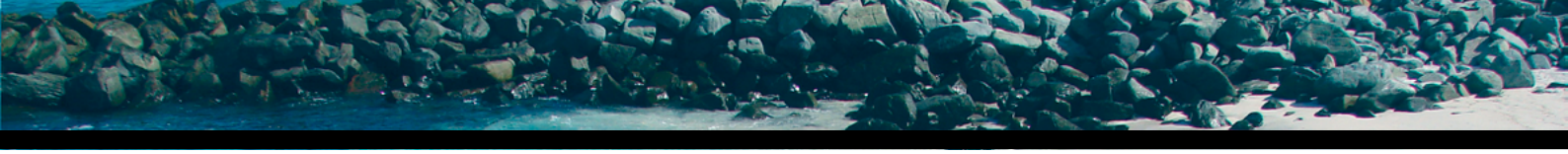
In mucociliary epithelia from respiratory tract, ciliary beating is determinant in the velocity and effectiveness of mucociliary clearance (MCC). ATP is known to increases ciliary beat frequency (CBF) in ciliated cells, effect that is mediated by purinergic receptor activation and the subsequent increase in intracellular Ca^{2+}. In respiratory epithelial cells, ATP can be released constitutively or following mechanical stimulation (MS) through a possible mechanism mediated by pannexin 1 (Panx1) or connexin 43 (Cx43) hemichannels (HCs), however, it unclear if ATP release is a regulated mechanism that contribute to basal ciliary activity. The aim of this study was to determine whether extracellular ATP (eATP) release contributes to regulate basal ciliary frequency. We used primary cultures from mouse trachea epithelial ciliated cells (MTEC). CBF was recorded using videomicroscopy (Sisson Ammons Video Analysis) and Atomic Force Microscopy (AFM) was used to caractherize ciliary function. eATP was measured by luminometric assay using luciferin/luciferase and $[Ca^{2+}]_i$ were measured using FURA 2AM. Apyrase (50 U/mL), an ectonucleotidase that hydrolyzes ATP, significantly lowered eATP levels compared to vehicle (3.8 ± 1.4 versus 8.1 ± 0.8 pmol/cm^2 after 1 min. treatment, *p<0.05). Apyrase also reduced CBF in a 45.5 ± 2.3 % (n=4), effect that correlates with a $[Ca^{2+}]_i$ reduction. Simultaneous treatment with carbenoxolone (CBX) (100 µM), a HCs inhibitor, and oxidized ATP (oATP) (100 µM), a P2X7-R antagonist, produced a reduction of CBF compared to basal activity of 57.5 ± 3.0 % after 5 min of incubation, returning to baseline after 20 min. This reduction was prominent compared with oATP alone (6.8 ± 1.7 %, *p<0.05) and CBX alone (24.9 ± 6.8 %, n=12). In addition, concomitant treatment with CBX, oATP and apyrase reduced the basal CBF in 85.2 ± 4.8 % (n=10), in concordance with a reduction on eATP levels (19.9 ± 9.5 pmol/cm^2 for vehicle, n=11, versus 2.6 ± 0.1 pmol/cm^2 for treatment, n=4, * p<0.05). Furthermore, using AFM we topologically detected deflection, amplitude, phase and height of MTCE. Using AFM-single force mode, we measured areas with oscillatory deflection patterns whose dominant frequency was between 4 to 14 Hz, a similar frequency range detected with videomicroscopy. These results suggest that ATP release from epithelial ciliated cells is required to maintain basal ciliary activity associated to $[Ca^{2+}]_i$ homeostasis. The underlying molecular mechanism might involve HCs and P2X7-R, through an autocrine mechanism that regulates basal ciliary activity in respiratory epithelium. Fondecyt Postdoctorado 3150652. Fondecyt 1120169.



MODELING THE SENSITIVITY OF COLD THERMORECEPTOR NEURONS AND COLD NOCICEPTORS IN TERMS OF I_{TRPM8} AND I_{KD} CURRENT EXPRESSION.

Herrera Pacheco, Gaspar[1]., Olivares, Erick[1].,Madrid, Rodolfo[2].,Orio, Patricio[1].,[1]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad De Valparaíso.[2]Departamento de Biología, Facultad de Química y Biología, Universidad De Santiago De Chile.

Cold thermoreceptor neurons (CTs) and cold nociceptors (CNs), active in the innocuous and noxious cold range respectively, are responsible for the detection of environmental cold temperatures in the somatosensory system. Molecular, cellular and behavioral evidences are consistent with the view that TRPM8, a cold- and menthol-gated cation channel, is the main molecular determinant of cold-sensitivity, and suggest that the broad detection range and distribution of thermal thresholds mainly arises from the counterbalance in the functional expression of TRPM8 (I_{TRPM8}) and Kv1.1-1.2 channels (I_{KD}). The small diameter and low density of peripheral receptors precludes the optical and electrophysiological approaches, so it is not clear to which extent observations obtained in cultured primary sensory neurons can be extrapolated to peripheral thermotransduction occurring at the nerve endings. Here we use a conductance-based mathematical model of a generic CT, and computer simulations under different temperature regimes, to understand the contributions of different conductances to cold responses. In this model, the fine sensitivity to fast temperature drops is due to a depolarizing TRPM8-dependent current, which generates large changes in the firing rate of the cold receptor. A calcium-dependent desensitization causes this response to be transient. Exploration of the parameters space was performed to study how the TRPM8 and K_D conductances determine the detection thresholds and maximum firing rates. Thermal thresholds of the models are correlated to TRPM8 and K_D conductance densities, in agreement with functional studies in cultured cold-sensitive neurons. Models with low threshold and the characteristic features of the dynamic and static response of CTs have high TRPM8 and low K_D conductance levels. Conversely, high detection thresholds and firing properties of CNs are associated to high K_D and low TRPM8 conductance densities. We provide the first comprehensive mathematical model of both CTs and CNs including the excitability brak potassium current I_{KD}. Making use of it, we also show that neuropathic painful hypersensitivity to cold can be explained as a consequence of an impairment in this brake potassium current. Supported by Grants Fondecyt 1130862, 1131064 and ACT1104, ACT1113. CINV is supported by the Millennium Scientific Initiative of the Ministerio de Economía (Chile).

STUDIES ON THE ROLE OF RHOA-RHO KINASE ACTIVATION IN AN ANIMAL MODEL OF METABOLIC SYNDROME

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(Sponsored by Fondecyt 1141051, Beca Doctoral Conicyt 21140004, Puente VRI-UC 20/2013)

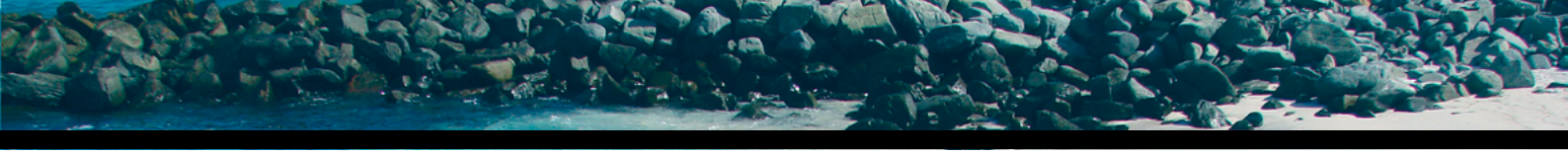
Metabolic Syndrome (MetS) is a collection of cardio-metabolic risk factors. Endothelial dysfunction (ED) and atherosclerosis are chronic conditions that are the foundation behind cardio-ischemic complications. RhoA/Rho-kinase (ROCK), which regulates the cellular actin dynamics and other functions, has been associated to the pathogenesis of cardiovascular diseases and a RhoA/ROCK inhibitor, Fasudil, is currently used to treat pulmonary hypertension. However, RhoA/ROCK over-activation in relation to MetS has not been completely addressed.

Aim: To study in a MetS animal model the participation of RhoA/ROCK activity associated to MetS phenotype and ED signs. **Methods:** Groups of 12 male Sprague Dawley rats (100-125 gr) werefed with high fat diet or chow diet ad libidum for 14 weeks. At week 12 the groups were subdivided for Fasudil 100mg/kg/day or vehicle oral treatment. Weight, food consumption, triglycerides, glucose and blood pressure were monitored throughout the protocol.

Oxidative stress markers (AOPP and TBARS), Nitric Oxide (NO) (Griess method), IL-6, sCD40L, siCAM-1 (ELISA) and insulin were measured in serum. Aortas lysates were used to determine RhoA/Rho-kinase activity, pAkt/Akt, Nrf2/ERK and peNOS/eNOS by Western Blot. Triglycerides content was studied by Sudan IV and lipid oil red staining and MMPs activity by zymography.

Results: Rats fed withhigh fat diet gained significant weight starting from the second week, without modifying the food rate consumption. They showed higher glucose and insulin levels, systolic blood pressure, triglycerides, cholesterol and larger abdominal circumference than animals treated with chow diet. These MetS characteristics were associated to higher levels of TBARS and AOPP, uric acid, IL-6, sCD40L and ED signs (siCAM-1 and lower levels of NO) compared to controls. Aortas from MetS animals showed over-activation of RhoA/ROCK and reduced levels of pAkt, peNOS and Nrf2 as well. Animals resembling a MetS condition also showed elevated levels of E-Selectin, MMP2 activity, engrossed media intimae and lipid infiltration than chow diet fed rats. Two weeks of Fasudil treatment reverted significantly the alterations due to high fat diet.

Conclusions: Our results show that rats fed with high fat diet showed biochemical and pathological characteristics of MetS. ROCK inhibition was associated with a significant improvement of these abnormalities. These findings suggest that activation of RhoA-ROCK pathway may play an important role in the pathogenesis of MS and its inhibition might be considered as a potential therapeutic target for this condition.



K_{CA}3.1-DEPENDENT HYPERPOLARIZATION ENHANCES INTRACELLULAR CA^{2+} SIGNALING INDUCED BY FMLF IN DIFFERENTIATED U937 CELLS

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Background: Formylated peptides are chemotactic agents generated by pathogens. The most relevant peptide is fMLF (formyl-Met-Leu-Phe) which participates in several immune functions, such as chemotaxis, phagocytosis, cytokine release and generation of reactive oxygen species. In macrophages fMLF-dependent responses are dependent on both, an increase in intracellular calcium concentration and on membrane potential hyperpolarization. However, the molecular entity underlying this hyperpolarization remains unknown and it is not clear whether changes in membrane potential are linked to the increase in the $[Ca^{2+}]_i$. **Methods:** U937 cells were differentiated using 1mM dibutyryl cAMP for 48 h to obtain a macrophage-like cell model. fMLF-dependent responses were studied using patch clamp and Ca^{2+} imaging techniques in differentiated U937 cells. Pharmacological and molecular approaches were used to study the molecular entity of the ion channel underlying fMLF-dependent response. **Results:** Differentiated U937 cells exposed to 100 nM fMLF responded with a rapid increase in the $[Ca^{2+}]_i$ and hyperpolarization reaching ~ -70 mV. Both, the increase in the $[Ca^{2+}]_i$ was diminished and the hyperpolarization was significantly more shorter in the absence of external Ca^{2+}. As a Ca^{2+}-dependent K^+ channel underlay the hyperpolarization triggered by fMLF, pharmacological and knock down techniques were used to determine that K_{Ca}3.1was the molecular entity of the K^+ channel. Finally, the activity of K_{Ca}3.1 enhanced fMLF-dependent increase in the $[Ca^{2+}]_i$ in the differentiated U937 cells. **Discussion:** We demonstrate by means of pharmacological and molecular biology tools that fMLF induces a Ca^{2+}-dependent hyperpolarization via activation of the K^+ channel K_{Ca}3.1 and thus, enhancing fMLF-induced intracellular Ca^{2+} increase through an amplification of the driving force for Ca^{2+} entry. Consequently, enhanced Ca^{2+} influx would in turn lengthen the hyperpolarization, operating as a positive feedback mechanism for fMLF-induced Ca^{2+} signaling.

SOFARCHI Members Incorporation

ENDOTHELIAL CELLS DIFFERENTIATED FROM MESENCHYMAL CELLS ISOLATED FROM WHARTON'S JELLY PROMOTE TISSUE REGENERATION IN HYPERGLYCEMIC MOUSE.

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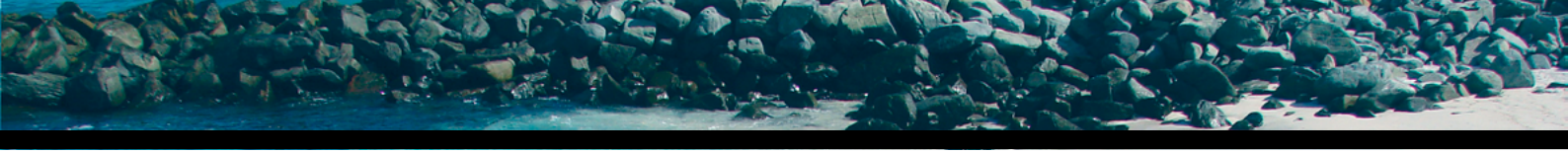
Mesenchymal stem cells (MSCs) have a high potential for differentiation, proliferation and plasticity and low immunogenicity, making them an excellent cell source for tissue repair. However, one of the main sources of MSCs is bone marrow, which reduces MSC isolation, it is poorly efficient and unpleasant for patients.The aim of this study was to determine whether MSCs isolated from Wharton\'s jelly of human umbilical cords can favor tissue repairing in vivo in hyperglycemic condition. MSCs were isolated from human Wharton\'s jelly by digestion with collagenase type I. Endothelial trans differentiation was induced for 14 (hWMSC-End14d). Immunophenotyping was performed using mesenchymal (CD90, CD73, CD105) and endothelial (Tie-2, KDR, eNOS, ICAM-1) markers. Endothelial trans-differentiation was demonstrated by the expression of endothelial markers and their ability to synthesizes nitric oxide (NO). hWMSCs can be differentiated into adipocytes, osteocytes, chondrocytes and endothelial cells. Moreover, these cells have a high expression of CD73, CD90 and CD105 but low expression of endothelial markers. hWMSCs-End express high levels of endothelial markers at 14 and 30 days of culture, and they can synthesize NO. After two months of treatment, the mouse exhibits high blood glucose levels (2-fold) and insulin (5 fold). Injection of conditioned media from hWMSC-End30d cultures in a mouse model of skin injury accelerated wound healing compared with animals injected with hWMSC non-differentiated or control injected with vehicle. These results demonstrate that differentiated hWMSC-End promotes neovascularization in hyperglycemic animal models probably through the secretion of pro-angiogenic soluble factors.



INHIBITION OF ETHANOL POTENTIATION OF GLYCINE RECEPTOR BY SMALL MOLECULES. *IN VITRO* AND *IN VIVO* STUDIES

Guzman, Leonardo[1]., San Martín, Loreto[1].,Aguayo, Luis[1].,Jin, Chunyang[2].,Jimenez, Verónica[3].,Cerda, Fabián[1].,[1]Fisiología, Ciencias Biológicas, University of Concepcion.[2]Center for Drug Discovery, Research Triangle Park, Research Triangle Institue.[3]Departamento de Ciencias Químicas Universidad Andres Bello. (Sponsored by FONDECYT 1131004, FONDEF CA12i10280.)

Introduction. Glycine receptor (GlyR), an inhibitory ligand gated ion channel is potentiated by ethanol by mean of the interaction of Gβγ with the intracellular domain of this channel. Previous studies have determined the Gβγ interacting region and a peptide (C7) that inhibit the ethanol potentiation of GlyR. Based on these results a virtual screening was performed to identify small molecules that mimics the C7 effects. *Objectives*. To analyze the effectiveness of selected molecules *in vitro* and *in vivo* and to determine their specificity to the ethanol potentiation of GlyR. *Materials and methods*. HEK cells overexpressing GlyR were registered on patch clam experiments in whole cell configuration. Small molecules were intracellularly added in the register pipette solution. Spontaneous synaptic activity was registered in hippocampal neurons. HL60 cell activated with fMLP were assayed in terms of intracellular Ca^{2+} levels. GIRK channels were activated through a GABAB agonist. Mice intoxicated with ethanol were analyzed to determine the righting-reflex recovery time. *Results*. The IC_{50} of two small molecules for ethanol potentiation of GlyR was 25±5 and 26±4 µM. The decay time constant of synaptic events was reduced to 8 and 3%. PLCβ activity was not affected by the presence of molecules, and GIRK channel was inhibited to 30 and 22% respectively. Finally, to demonstrate the effectiveness of molecules in ethanol intoxication, loss of righting reflex (LORR) experiments were performed in mice intoxicated with ethanol. For one of the molecules recovery was 10 minutes faster than control. *Conclusions*. Small molecules were identified to have inhibitory effect on the ethanol potentiation of GlyR activity. That effect was confirmed in *in vivo* experiments. These results confirm that the Gβγ - GlyR interaction could be pharmacologically modulated in order to reduce the effects of ethanol intoxication. Miembro patrocinante: Dr. Jorge Fuentealba

CONFORMATION-SPECIFIC MODULATION OF SYNAPTIC A3-CONTAINING GLYCINE RECEPTORS OF THE SPINAL DORSAL HORN ALLEVIATES CHRONIC INFLAMMATORY PAIN.

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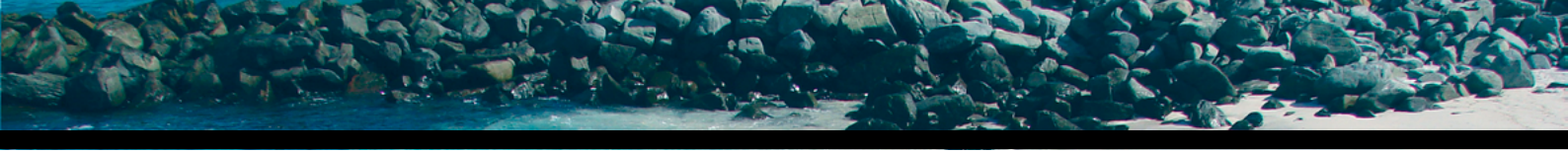
Diminished inhibitory neurotransmission in superficial spinal dorsal horn contributes to chronic pain. A PGE_2-mediated PKA-dependent phosphorylation of α3 glycine receptors (α3GlyR) at serine 346 appears to be particularly relevant for the generation of hyperalgesia in inflammatory pain states. Restoring the activity of spinal α3GlyR through positive allosteric modulators may thus constitute a rational approach against inflammatory pain. We have investigated the modulation of recombinant and native GlyRs by the non-anesthetic propofol analog 2,6-di-tert-butylphenol (2,6-DTBP) and its potential effects in behavioral pain models. We found that 2,6-DTBP is a positive allosteric modulator of homomeric α3GlyR. Heteromeric α3βGlyRs were much less sensitive than homomeric receptors, indicating that inclusion of GlyR β subunits in the receptor complex reduces allosteric modulation. Experiments performed in α3GlyR carrying point mutations mimicking either the phosphorylated or the non-phosphorylated state (S346E or S346A) revealed that phosphorylation at S346 did not influence the sensitivity of homomeric receptors 2,6-DTBP. However, heteromeric S346E point mutated α3βGlyRs displayed a higher sensitivity to 2,6-DTBP than S346A mutated receptors. We then went to investigate the effects of 2,6-DTBP on glycinergic IPSCs of superficial dorsal horn neurons using spinal cord slices. 2,6-DTBP caused only a minor potentiation of gly-IPSCs recorded in naïve slices. However, 2,6-DTBP significantly prolonged gly-IPSCs after pretreatment of the slices with PGE_2. These data suggest that inflammation-induced phosphorylation increases the susceptibility of synaptic α3GlyR to modulation by 2,6-DTBP. In behavioral studies, we found that 2,6-DTBP alleviated hyperalgesia in the zymosan A and CFA models of inflammatory pain in wild-type mice without altering muscle strength, motor coordination or locomotor activity. Noteworthy, the analgesic effect of 2,6-DTBP was reduced to about 30% in α3GlyR-deficient mice, suggesting a major role of α3GlyR in the mechanism of action of 2,6-DTBP *in vivo*. In summary, our data establish that potentiation of synaptic α3GlyRs is a promising strategy against chronic pain and that 2,6-DTBP has a unique pharmacological profile favoring an interaction with α3GlyR modified by phosphorylation induced by peripheral inflammation.



REDUCTION OF ACUTE ANTHRACYCLINE CARDIOTOXICITY THROUGH THE DECREASE OF THE OXIDATIVE INJURY IN PATIENTS WITH BREAST CANCER.

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Anthracyclines is a family of agents used in cancer chemotherapy with a limited use by the cardiotoxicity occurrence, which has not been adequately prevented yet. Cardiotoxicity can be presented in acute form, evidenced through different biomarkers. Pathophysiological cardiotoxicity mechanisms include generation of reactive oxygen species and are different to antineoplastic mechanisms. The aim of our study was to evaluate the effect in the acute anthracyclines cardiotoxicity of two pharmacological interventions based on strengthening of antioxidant defense system by administration of carvedilol and omega 3 fatty acids in breast cancer patients. A placebo-controlled, randomized, double-blind clinical trial was performed in 36 female patients with breast cancer (50.2±9 years) and indication of anthracyclines. Patients were assigned to 3 different groups to receive in the first cycle of chemotherapy since 7 days before until 7 days post treatment with anthracyclines: a) omega-3 1g every 12h plus carvedilol placebo every 12h; b) carvedilol 12.5 mg every 12h plus omega-3 placebo every 12h; c) carvedilol placebo every and omega-3 placebo every 12h. During the first cycle of chemotherapy, patients were controlled with 3 blood samples: pre-chemotherapy, +3 and +5 days after chemotherapy. Electrocardiographic controls were also performed: pre-chemotherapy, 6 hours and +3 day after chemotherapy. Primary endpoint of acute cardiotoxicity was NT-proBNP. Secondary endpoints were other markers of acute cardiotoxicity: QTc (interval and dispersion) and serum troponin T. In addition, effects on the redox status were evaluated: total plasma antioxidant capacity, erythrocyte thiol index, plasma F2-isoprostanes and activities of antioxidant enzymes in erythrocyte (SOD, CAT and GSH-Px). Patients showed a significant increase of NT-proBNP levels at +3 day, however there were no differences between intervention groups. Secondary endpoints of acute cardiotoxicity neither showed differences between groups. In oxidative stress biomarkers, placebo group showed higher levels of lipoperoxidation and a lower activity of the three erythrocyte antioxidants enzymes in +3 and +5 days, effects which were prevented by the two interventions. The decreased activity of antioxidant enzymes in the placebo group was associated with an increased in the thiol index. The interventions with carvedilol or omega-3 in patients with breast cancer did not achieve a reduction in acute cardiotoxicity biomarkers. However, the two interventions were able to reduce the systemic oxidative injury and prevent the decreased activity of erythrocytes antioxidant enzymes. Perhaps the reduction in systemic oxidative stress could contribute to a reduction of the effects of oxidative stress on the heart, thus should be studied the long term effects of these interventions.

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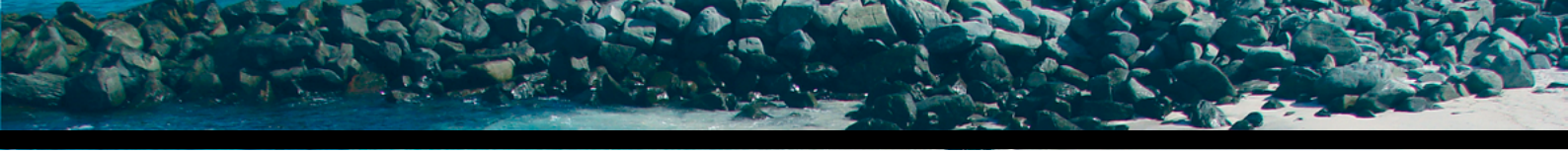


1) **Role of PKC in amphetamine- and cocaine-induced increase in dopamine and glutamate extracellular levels in rat ventral tegmental area**

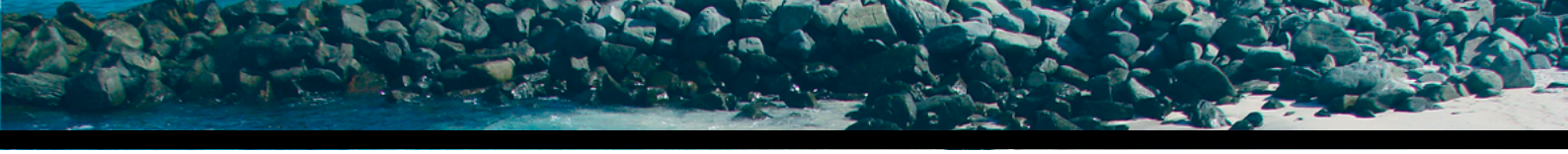
Abarca, Jorge[1]., Sotomayor-Zarate, Ramón[2].,Gysling, Katia[1].,[1]Departamento de Biología Celular y Molecular , Facultad de Ciencias Biológicas , Pontificia Universidad Católica De Chile.[2]Instituto de Fisiología, Facultad de Ciencias, Universidad de Valparaíso.
(Sponsored by Funded By FONDECYT Grant N° 1150244)

Dopaminergic transmission at synapses is terminated primarily by the removing of dopamine (DA) from the synaptic cleft by the DA transporter (DAT). Amphetamine and cocaine increase extracellular levels of DA by differential interactions with DAT. There is evidence that PKC activity is necessary for amphetamine-induced increase in DA leves in nucleus accumbens. In this work we have studied the effect of intra Ventral Tegmental Area (VTA) infusion of RO-31-8220 (PKC inhibitor) on extracellular levels of DA and glutamate (GLU) induced by intra VTA infusion of amphetamine or cocaine in control rats. Intra VTA infusion of 30 uM amphetamine or 100 uM cocaine significantly increased DA and GLU extracellular levels. Co-infusion of 10 uM RO-31-8220 inhibited amphetamine- but not cocaine-induced increase in VTA DA and GLU extracellular levels. Thus, our data show that PKC activity in the VTA is necessary for amphetamine- but not cocaine-induced increase in VTA DA and GLU extracellular levels.

2) **Potential neuroprotective effects of a series of indole derivatives in a model of Alzheimer´s Disease**

Perez, Nelson[1]., Peters, Christian[2].,Bascuñan, Denisse[2].,Aguayo, Luis[2].,Pessoa-Mahana, Hernan[1].,[1]Quimica Organica y Fisicoquimica, CQF, Universidad De Chile.[2]Physiology, Biological Sciences, Universidad De Concepción. (Sponsored by This Research Was Funded By Fondecyt 1140473 And 1130347)

Introduction. Alzheimer´s disease (AD) is one of the major neurodegenerative disorders affecting the elderly and is characterized by a progressive loss in cognitive functions, memory and learning. Current drug therapy for AD is designed to mitigate the disease without being able to stop or reverse it due to the many associated mechanisms of the disorder. The amyloid beta peptide (Aβ) is one of the most critical factors that contributes to the diverse mechanisms of toxicity, such as its capacity to bind to the membrane and form pores that increase the permeability to ions. Among the compounds that have been tested against Aβ toxicity are those having an indolic ring. Indole derivatives compounds are characteristic of a large number of alkaloids and molecules with therapeutic interest, such as Melatonin and Indomethacin, which have been shown to inhibit aggregation and toxicity of Aβ. In addition, these indole-derivatives have demonstrated good lipophilicity and can penetrate reasonably well the blood brain barrier. **Main Aim.** To obtain indole-derived molecules capable of reducing the neurotoxicity of Aβby interfering with its ability to bind to neuronal membranes. **Materials and Methods.** An initial screening was done to >100 molecules of indolic nature that were previously synthesized in the laboratory, and their capacity to counteract Aβneurotoxicity was determined in PC12 cells using the MTT assay. We also performed turbidity assays for aggregation and Dot Blot assays to evaluate the capacity of the molecules to inhibit the binding of Aβ to the membrane. In addition, docking analysis were done to assess the most probable interactions of the molecules with the Aβ peptide. **Results.** We found that a number of the molecules tested significantly antagonized the toxicity of Aβ. For example, 5 µM M88 was able to reduce the toxicity on cellular viability assays caused by application of 1 µM Aβ(65 vs. 85% of control, n=3). Studies of turbidity and Dot Blot revealed that some compounds inhibited the aggregation and Aβ association to membranes. For instance, M55 inhibited aggregation by 45±3% and membrane association by 60±4% (n=3). Interestingly, docking studies showed favorable π-stacking interactions with amino acids Phe19 and Phe20 of Aβ. **Conclusions**. Our data show that some indolic compounds have the ability to reduce the toxicity induced by Aβ, in addition to inhibiting the binding of Aβ to the membrane. Current experiments using immunocytochemistry and patch clamp techniques will further evaluate these molecules and their capacity to interfere with Aβ pore formation in neuronal membranes.



3) **Effects of amphetamine sensitization on the extra-hypothalamic vasopressinergic system of adult rats**

Ahumada, Catalina[1]., Bahamondes, Carolina[1].,Silva, Roxana[1].,Cruz, Gonzalo[1].,Sotomayor-Zárate, Ramón[1].,Renard, Georgina[1].,[1]Centro de Neurobiología y Plasticidad Cerebral - Instituto de Fisiología, Facultad de Ciencias, Universidad de Valparaíso. (Sponsored by FONDECYT N° 11140065 To GMR And Committee For Aid And Education In Neurochemistry (CAEN), ISN)

Drug addiction is a disease characterized by the need to use drugs despite adverse effects. It has been observed that women are more vulnerable to these effects than men. Medial amygdala (MeA) sends vasopressin (AVP) projections to the lateral septum (LS), nucleus involved in the addictive process. These projections regulate several behaviors altered by drug addiction and it is known that the density of the AVP fibers in the LS is higher in male rats than in females. So, AVP could be related with sex-dependent effects observed in the addictive process. The aim of this work was to study the effects of sensitization to amphetamine (AMPH) (behavioral paradigm of addiction) on the extrahypothalamic vasopressinergic system in male and female rats. Male and female Sprague Dawley rats (55-60 days old) were divided in 2 groups: Control rats (saline, n= 9 females and 7 males) and AMPH-treated rats (AMPH 1.5 mg/Kg i.p., n= 11 females and 7 males). The stage of the estrous cycle was determined daily by vaginal smears examination. At the induction phase of sensitization, animals received a daily injection during 5 consecutive days and locomotor activity was measured. The criterion for sensitization was a 20% increase in locomotor activity over 5-day injection period. Five days after the last injection, all rats were injected with AMPH (challenged) and locomotor activity was measured. After the protocol the animals were decapitate and the brain was removed to microdissect MeA used to quantify AVP mRNA expression by RT-Q-PCR and to microdissect LS to quantify AVP levels by ELISA. Our results showed that 75% of the AMPH-treated animals, females and males, sensitized to AMPH. At the expression of sensitization day, control female rats showed higher locomotor activity than males. No differences in LS AVP content of control and sensitized female and male rats was observed, but in the MeA there is a decrease in AVP gene expression in sensitized animals. AMPH treatment in female rats produces differences depending on phase of the estrous cycle in the AVP gene expression at the MeA. In conclusion, sensitization to AMPH causes a decrease in MeA AVP mRNA in both females and males rats, and we did not find sex-dependent effects in our results.

4) **Neonatal programming with Estradiol Valerate a vulnerability factor for Alcohol intake in adolescent female rats**

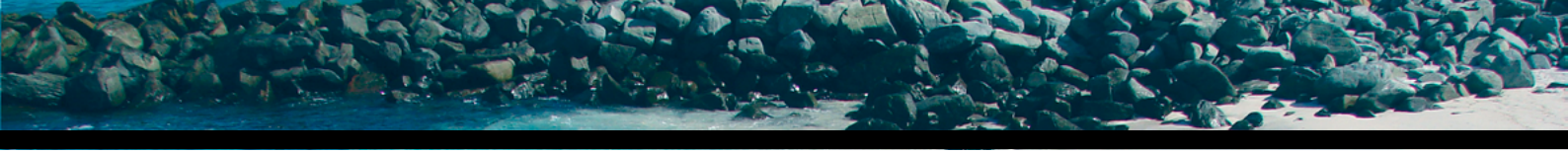
Venegas, Francisca[1]., Sanguinetti, Nicole[1].,Cruz, Gonzalo[1].,Acevedo, María[2].,Sotomayor-Zárate, Ramón [1].,[1]Institute of Physiology, Faculty of Sciences, Universidad De Valparaíso.[2]Laboratorio de Alcohol, Ontogenia y Aprendizaje Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC - CONICET). (Sponsored by This Work Was Funded By FONDECYT Grant N° 111-21205 For RS-Z)

Neonatal programming by sexual hormone such as estradiol valerate (EV) is a type of physiological reprogramming in which it has been displayed an increase in dopamine (DA) content in midbrain dopaminergic neurons in substantia nigra (SN) and ventral tegmental area (VTA) in adult rats. This increase of dopamine (DA) content has been associated to an increase in tyrosine hydroxylase (unpublished data) through the direct estrogenic effect of EV or through the aromatization of testosterone to estradiol. Nevertheless, the relationship between these results and alcohol intake behavior has not been studied yet.

In this work, EV (0.1 mg/50 µL s.c.) was injected in female and male Sprague-Dawley rats within the first 12 hours of life. In parallel sesame oil (50 µL s.c.) was injected to the control group. The protocol of intermittent access to ethanol from post-natal day (PND) 28 to 65 was used. Rats were allowed to drink from 2-bottles under the free choice paradigm. Every week the rats were exposed to 3 sessions of ethanol access. The first 2 weeks the animals were able to choice between water with sucrose and ethanol 5% solution with sucrose. Since the 3rd week the sucrose was taken out of the solutions for choice. In the last session at 4th week, the rats were forced to abstinence condition, and finally these were exposed to the challenge session for 24 hours to ethanol.

We have found that average ethanol consumption in female rats was 1.12 g/kg/day in 4 weeks, meanwhile in EV female rats was 6.59 g/kg/day. In the challenge session, the ethanol consumption was 1.60 g/kg/day in control female rats, while in EV female rats was 9.15 g/kg/day. In addition, it was observed that water and sucrose solution preference was 94% and 58% for control and EV female rats, respectively, in the first two weeks. Furthermore, the Ethanol preference during these sessions was 6% and 42% for control and EV female rats, respectively.

Our results show that early exposure to estrogenic compounds is a vulnerability factor for ethanol intake in adolescent female rats. New studies will be carried out to explain molecular mechanism implicated in this vulnerability factor.



5) **ACTIVATION OF THE TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE 1 (TRPV1) REDUCES THE INFLUENCE OF ANXIETY IN VISUOSPATIAL LEARNING OF MICE.**

Castillo, Amparo[1,2]., BURGOS, Héctor[3,4].,Cofré, Christian[5].,Hernández, Alejandro[6].,Morgan, Carlos[7].,Sáez-Briones, Patricio[8].,Burgos-Villaseca, Jorge[9,8].,Klagges, Jorge[10].,Morales, Bernardo[11].,Madrid, Rodolfo[11].,Zeise, Marc[12].,[1]Escuela de Psicología, Ciencias Sociales, Universidad Santo Tomás.[2]Programa de Postgrado, Doctorado en Psicología, Facultad de Ciencias Sociales, Universidad De Chile.[3]PSICOLOGIA, FACULTAD DE HUMANIDADES, Universidad De Santiago De Chile.[4]Escuela de Psicología, Ciencias Sociales, Universidad Central de Chile.[5]Escuela de Psicología, Laboratorio de Biopsicología, Facultad de Humanidades, Universidad De Santiago De Chile.[6]Laboratorio de Neurobiología, Facultad de Química y Biología, Universidad De Santiago De Chile.[7]Unidad de Nutrición Humana,, INTA, Universidad De Chile.[8]Laboratorio de Neurofarmacología y Comportamiento, , Facultad de Ciencias Médicas, Universidad De Santiago De Chile.[9]Departamento de Biología, Facultad de Ciencias Básicas, Universidad Metropolitana De Ciencias De La Educación.[10]Laboratorio de Neurofarmacología y Comportamiento, Facultad de Ciencias Médicas, Universidad De Santiago De Chile.[11]Laboratorio de Neurociencias, Facultad de Química y Biología, Universidad De Santiago De Chile.[12]Laboratorio de Biopsicología, Escuela de Psicología, Facultad de Humanidades, Universidad De Santiago De Chile. (Sponsored by ACT-1113)

The TRPV1 was first described in peripheral pain pathways, being sensitive to noxious stimuli such as capsaicin, temperature, acidity and physiological effects involving increased ATP. It is also present in neurons of the central nervous system, such as hippocampus, cortex, cerebellum, olfactory bulb, midbrain and rhombencephalic areas, among other limbic structures, and has been suggested to be involved in learning processes. To study the role of TRPV1 on visuospatial memory performance, two groups of eight C57/BL mice were injected with either 1.0 mg/kg i.p. of the TRPV1 agonist capsaicin or saline, and then subjected for 15 days to the Olton eight-arm radial maze to test short and long-term memory. Anxiety was assessed on day 1 and day 15 in the elevated plus maze.

There was no significant difference in the total number of cumulated errors committed between the capsaicin and the saline treated group during the whole 15-day period of testing. However, during the first 3 days of testing, short-term memory was significantly better in animals receiving capsaicin, while long-term memory performance had significantly improved later, on days 4-9. Concerning anxiety, there was no difference in scores (entry to open arms) between the capsaicin and the control group in the first session in the plus maze. However, while controls displayed higher scores in the session after day 15, the capsaicin group maintained their scores.

The data suggest that activation of TRPV1 by capsaicin initially improves working memory in mice and subsequently improves long-term memory, together with producing an anxiolytic effect.

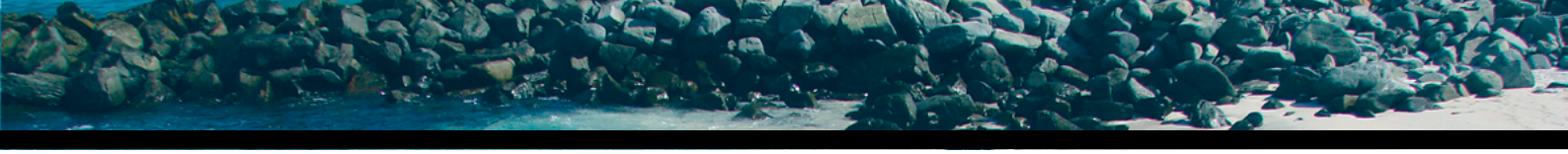
This study has been supported by ACT-1113.

6) **THE TRPV1 ANTAGONIST CAPSAZEPINE INHIBITS LONG-TERM POTENTIATION IN THE RAT PREFRONTAL CORTEX.**

Hernández, Alejandro[1].,BURGOS, Héctor[2,3].,Cofré, Christian[2].,**Castillo, Amparo[4,5].**, Sáez-Briones, Patricio[6].,Burgos-Villaseca, J[7].,Klagger, Jorge[7].,Morales, Bernardo[8].,Madrid, Rodolfo[8].,Morgan, Carlos[9].,Zeise, Marc[2].,[1]Laboratorio de Neurobiología, Facultad de Química y Biología, Universidad De Santiago De Chile.[2]LABORATORIO DE BIOPSICOLOGÍA, ESCUELA DE PSICOLOGIA, FACULTAD DE HUMANIDADES, Universidad De Santiago De Chile.[3]Escuela de Psicología, Facultad de Ciencias Sociales, Universidad Central De Chile.[4]Escuela de Psicología, Facultad de Ciencias Sociales, Universidad Santo Tomás.[5]Programa de Postgrado, Doctorado en Psicología, Facultad de Ciencias Sociales, Universidad De Chile.[6]Laboratorio de Neurofarmacología y Comportamiento,, Facultad de Ciencias Médicas, Universidad De Santiago De Chile.[7]Departamento de Biología, Facultad de Ciencias Básicas, Universidad Metropolitana De Ciencias De La Educación.[8]Laboratorio de Neurociencias, Facultad de Química y Biología, Universidad De Santiago De Chile.[9]Unidad de Nutrición, INTA, Universidad De Chile. (Sponsored by ACT-1113)

TRPV1 receptors are known to be expressed in peripheral pain pathways, but also in the spinal cord dorsal horn where they play a role in spinal cord long-term potentiation (LTP). More recently, it has been found that TRPV1 are also expressed in several brain regions, including the hippocampus and the cerebral cortex, suggesting that these channels be involved in neuroplasticity necessary for memory-related processes. To study the role of TRPV1 in LTP induction in the prefrontal cortex, two groups of Sprague-Dawley rats were injected either 5 mg/kg i.p. of the TRPV1 receptor antagonist capsazepine or saline, and thereafter submitted to a protocol of in vivo prefrontal cortex LTP elicited by transcallosal electrical stimulation. Rats treated with capsazepine exhibited decreased LTP, as compared to controls. This result is consistent with the inhibition of spinal LTP by capsazepine in rat and the reduction of hippocampal LTP in TRPV1o/o mice. Taken together, these results suggest that activation of TRPV1 may be necessary for synaptic plasticity in central synapses, being in line with possible supporting effects of TRPV1 activation in learning tasks.

This study was supported by ACT-1113.



7) **A series of brominated derivatives of the superpotent 5-HT$_2$ agonist 25B-NBOMe elicit anxiolytic-like responses in male Sprague-Dawley rats**

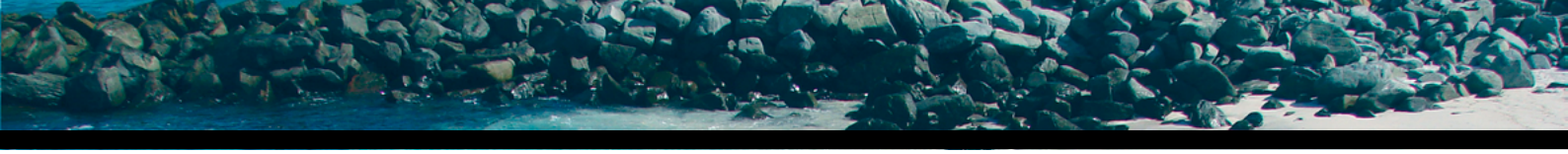
Burgos-Villaseca, Jorge[1,2]., Klagges-Troncoso, Jorge[1,3].,Benavente-Schonhaut, Sofia[1,3].,Malhue-Olmos, Valeska[1,3].,Hernández, Alejandro[4].,Burgos, Héctor[5].,Cassels, Bruce[6].,Sáez-Briones, Patricio[1,3].,[1]Laboratory of Neuropharmacology and Behavior, Faculty of Medical Sciences, Universidad De Santiago De Chile.[2]Department of Biology, Faculty of Basic Sciences, Universidad Metropolitana De Ciencias De La Educación.[3]School of Medicine, Faculty of Medical Sciences, Universidad De Santiago De Chile.[4]Laboratory of Neurobiology, Faculty of Chemistry and Biology, Universidad De Santiago De Chile.[5]School of Psychology, Faculty of Social Sciences, Universidad Central De Chile.[6]Department of Chemistry, Faculty of Sciences, Universidad De Chile. (Sponsored by FONDECYT Grant 1150868, DICYT-USACH Grant 021401SB)

The synthetic psychotropic amphetamine 2-(4-bromo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine, also known as 25B-NBOMe, is a N-methoxylated derivative of the hallucinogen 2C-B (2,5-dimethoxy-4-bromoamphetamine). Athough 25B-NBOMe exhibits high affinity for serotonergic 5-HT$_2$ receptors and very high in vivo potency, the underlying structure-activity relationships remain not fully understood. Moreover, reliable data regarding its behavioral effects in animal models are scarce and some relevant aspects of its effects in vivo, such as possible alterations in anixety-related responses, have not been described in detail. In the present work, a series of eight N-2-hidroxy-, N-2-methoxybenzylated and N-benzylbrominated 25B-NBOMe derivatives has been synthesized and behaviorally evaluated at the elevated plus maze in male Sprague Dawley rats (dose range 1 – 8 mg/kg). The percentages of entries and the corresponding percentages of time spent in the open arms measured after acute i.p. administration of doses ranging between 1 – 4 mg/kg are consistent with an anxiolytic-like response for the 25B-N-2-hydroxy- and N-2-methoxybenzylated derivatives. In contrast, the non-methoxylated N-benzylbrominated derivatives are not able to elicit any anxiolytic-like response. Interestingly, all compounds are indistinguishable from controls at 8 mg/kg. Therefore, the anxiolytic-like effects observed for the whole series of derivatives seem not to follow a rigid dose-response correlation. This behavioral profile is similar to that of the classical hallucinogen DOI (2,5-dimethoxy-4-iodoamphetamine), which was used as reference drug. Taken together, these results are in agreement with affinity and efficacy data obtained previously for this series of compounds. Nevertheless, they might indicate also that the anxiolytic-like response elicited could not be easily modulated by aromatic bromination, as already shown for other paradigmatic behavioral responses in the rat associated with a typical hallucinogenic profile. The latter highlights the notion that, regarless of the fact that superpotent serotonergic 5-HT$_2$ agonists may possess distinctive pharmacological properties, they also seem to share other effects with those of structurally related classic hallucinogens.

8) **Methylphenidate amplifies LTP by activation of β-adrenergic and D1/D5 receptors and increasing the AMPA currents of pyramidal cells.**

Contreras, D[1]., Carvallo, Claudia[1].,Farias, Ricardo[1].,Ugarte, Gonzalo[1].,Zeise, Marc[2].,Delgado, Ricardo[1].,Rozas, Carlos[1].,Morales, Bernardo[1].,[1]Biologia, Quimica y Biologia, Universidad De Santiago De Chile.[2]Psicología, Escuela de Psicología, Universidad De Santiago De Chile. (Sponsored by FONDECYT 1120580, FONDECY 11140430 And CONICYT, ACT1113. CONICYT Fellowship To C.C./D.C)

Methylphenidate (MPH) is a psychostimulant used in the therapy of the Attention Deficit/Hyperactivity Disorder and currently used also as a drug of abuse. It is known that MPH increases the TBS-dependent Long Term Potentiation (LTP) in the CA1 area of the hippocampus. Nevertheless, the cellular and molecular mechanisms involved in these synaptic mechanisms are still unknown. In this work, using electrophysiological recordings we show that MPH induces an increase of LTP involving the activation of β-adrenergic and D1/D5 dopaminergic receptors and an increase of AMPA-dependent current in CA1 area. 3 weeks old Sprague-Dawley rats were decapitated under Isoflurano 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 143.3 ± 3.1% (controls; n=5,6) to 146.2 ± 2.8% (3nM; n=3,3; p>0.05), 165.5 ± 6.6% (50nM; n=6,6; **p<0.001), 194.3 ± 5.8% (5µM; n=6,8, ***p<0.001), and 196.4 ± 4.2% (50µm; n=4,4; ***p<0.001). This effect was of nature postsynaptic since the paired-pulse curves remained unchanged after perfusion with MPH. We found that Timolol (5µM), a β-adrenergic receptor blocker, inhibited significantly the increase of TBS-dependent LTP by MPH from 194.3±5.8% (TBS+MPH, n=5,7) to 152.7±1.7% (TBS+MPH+TIM, n=4,4) (**p<0.01). Interestingly, LTP increase was also inhibited by 5 µM of SCH23390, a D1/D5 receptor blocker, from 195.5 ± 6.2% (TBS + MPH; n = 5, 7) to 144.4 ± 3.5% (TBS + MPH+ SCH: n = 5,7; *** p < 0.001). Both effects probably are post synaptic because the paired-pulse curve remains unchanged. Finally, using whole-cell recording in CA1 pyramidal cells we evaluate the effect of MPH on the AMPA-dependent currents. AMPA-dependent EPSCs were recorded at -65 mV through a short-term plasticity protocol in response to stimulus trains of 22 pulses to 30 Hz. We found that the amplitude of AMPA currents of MPH-treated neurons were significantly higher compared to those recorded in saline-treated neurons for all the responses evoked by the stimulation protocol (n=5,5; **p< 0.01). These results suggest that MPH increases TBS-dependent LTP in CA3-CA1 synapses through a polysynaptic mechanism involving activation of β-adrenergic and D1/D5 dopaminergic receptors and increasing the AMPA current by insertion of these receptors in the plasma membrane.



9) **Fasudil prevents depressive-like behavior and hippocampal dendritic spine loss promoted by stress in rats**

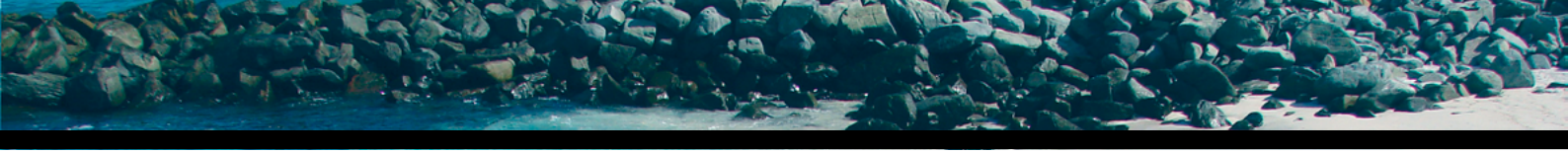
García-Rojo, Gonzalo[1]., Vilches, Natalia[1].,Fiedler, Jenny[1].,[1]Laboratorio de Neuroplasticidad y Neurogenética, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. (Sponsored by CONICYT 21120711)

Brain atrophy accompanied by dendritic arbor simplification and reduction in spine density of the hippocampus, a limbic structure implicated in mood disorders, are factors that seems to contribute to the symptoms of depression. It is plausible that these morphological changes imply alterations in the dendritic cytoskeleton dynamic. RhoA is an important regulator of actin dynamic through its effector ROCK. Transfection of hippocampal pyramidal neurons with constitutively active ROCK mutant produces dendritic simplification and dendritic spine loss. We propose that ROCK inhibitor as fasudil (also known as HA1077), may prevent both the stress induced depressive-like behavior in rats and the spine density reduction in the hippocampus. Adult male Sprague Dawley rats were injected with saline or fasudil (10 mg/kg) starting four days prior restraint stress procedure, which was conducted 2.5 hrs/day during 14 consecutive days. Control animals were injected with saline or fasudil during 18 days. In order to observe the effect of fasudil on depression-like behavior promoted by stress, we carried out the forced swimming test and conditioned avoidance test. Fasudil prevents the stress-induced immobility observed in forced swimming test. In addition, fasudil prevents the stress-induced reduction in conditioned avoidance responses. On the other hand, control animals treated with fasudil showed similar behavioral patterns as control saline animals. Furthermore, fasudil prevents the stress spine loss in the dorsal hippocampus promoted by chronic stress. Thus we proposed that Fasudil may prevent abnormal behavior promoted by stress probably blocking the ROCK activity with a concomitant prevention of the spine loss.

10) **Molecular modeling approaches to investigate corticotropin releasing factor receptor system structure-activity relationships**

Lagos, Carlos[1].,Gutiérrez-Maldonado, Sebastián[2].,Slater, Paula[3].,**Gysling, Katia[3]**., [1]Department of Endocrinology, School of Medicine, Pontificia Universidad Católica De Chile.[2]Computational Biology Lab (DLab) Fundación Ciencia y Vida.[3]Department of Cellular and Molecular Biology, Faculty of Biological Sciences, Pontificia Universidad Católica De Chile. (Sponsored by Supported By FONDECYT Grants 1110392 & 1150244, Programa De Financiamiento Basal PFB16 Grants, CONICYT PhD Thesis Research Grants To PGS And SEGM. Project NLHPC ECM-02 Supercomputing Infrastructure: Powered@NLHPC.)

Corticotropin-releasing factor (CRF) neuropeptide is the key mediator of the mammalian response to stressors. CRF acts through two subtypes of class-B G protein-coupled receptors, CRFR1 and CRFR2. The mammalian CRF system also comprises a CRF binding protein (CRFBP) and four different naturally occurring ligands, CRF and the related peptides urocortins I-III. Class B GPCRs represent a small GPCR subfamily encompassing 15 members, for which the activation mechanisms remains unexplored. On the other hand, CRFBP is a secreted protein without significant sequence homology to CRF receptors or to any other known class of proteins. In recent years, structure-function relationship studies have demonstrated that the N-terminal ectodomain (ECD) of CRFRs plays a pivotal role in natural ligand recognition, and more recently interactions with CRFBP have been identified. The recently solved crystal structures of the transmembrane domains (TMD) of the human glucagon receptor and human corticotropin releasing factor receptor 1 (CRFR1) have opened up new opportunities to study the structure and function of class B GPCRs. In this work we report the modeling of the entire CRF system as well as the interaction with CRFBP and peptide ligands. The complete receptor models including the ECD and the TMD were further studied using molecular dynamics simulations and protein-protein docking with two main goals: Firstly, structural investigations that help to understand the receptor function and the characterization of CRFBP binding to the CRFR2α receptor ECD domain, and secondly the identification of novel CRFR modulators as potential drugs. The present study provides a preliminary framework for further investigation of CRFR2α-CRFBP interactions and for the study of CRF receptor activation and regulation.



11) **Overexpression of LOXIN protects endothelial progenitor cells from apoptosis induced by oxidized low density lipoprotein.**

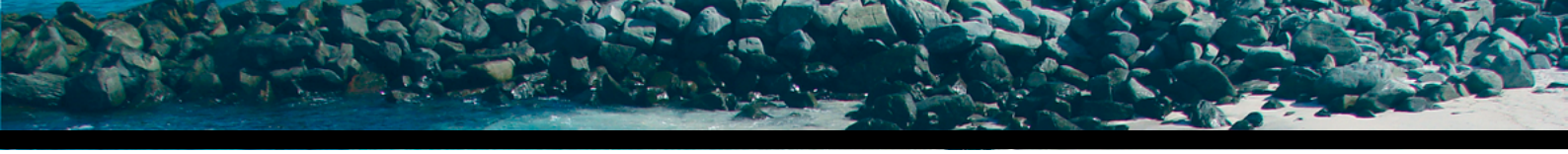
Carlos, Veas[4].,Willis, Naomi[1].,Gutierrez, Nicolas[2]., Radojkovic, Claudia[4].,Zuñiga, Felipe[4].,Toledo, Jorge Roberto[2].,Escudero, Carlos[3,5].,**Aguayo, Claudio [4,5]**., [1]Human Nutrition Research Centre, Institute of Cellular Medicine, Newcastle University.[2]Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad De Concepción.[3]Departamento de Ciencias Básicas, Facultad de Ciencias, Universidad del Bio-Bio.[4]Departamento Bioquímica Clínica e Inmunología, Facultad de Farmacia, Universidad De Concepción.[5]Group of Research and Innovation in Vascular Health (GRIVAS Health). (Sponsored by This Study Was Supported By INNOVA CORFO Chile (12IDL2-13351) And INNOVA BIOBIO, Chile (1245-EM.TES (12.21)), Dirección De Investigación, Universidad De Concepción (DIUC 211.072.034-1.0), Chile And Convenio De Desempeño, Universidad De Concepción, UCO1201)

Human endothelial progenitor cells (hEPC) are adult stem cells located in the bone marrow and peripheral blood. Studies have indicated that hEPCs play an important role in the recovery and repair of injured endothelium, however their quantity and functional capacity is reduced in several diseases including hypercholesterolemia. Recently it has been demonstrated that hEPC express lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and its activation by oxidized low-density lipoproteins (oxLDL) induces cellular dysfunction and apoptosis. This study aimed to investigate whether overexpression of LOXIN, a truncated isoform of LOX-1 that acts as a dominant negative plays a protective role against oxLDL-induced apoptosis in hEPC. Human endothelial progenitor cells exposed to oxLDL showed a significant increase in LOX-1 expression, and apoptosis began at oxLDL concentrations above 50 µg/mL. All hEPC apoptosed at 200 µg/mL oxLDL. High LOXIN expression was generated using adenoviral systems in hEPC and SiHa cells transduced with 100 colony-forming units/cell. Transduced LOXIN localized to the plasma membrane and blocked oxLDL uptake mediated by LOX-1. Overexpression of LOXIN protected hEPC from oxLDL-induced apoptosis and therefore maybe a novel way of improving hEPC function and quantity. These results suggest that adenoviral vectors of LOXIN may provide a possible treatment for diseases related to oxLDL and vascular endothelium dysfunction, including atherosclerosis.

12) **Circulating endothelial cells from patients with sepsis are source of activated fibroblasts**

Becerra, Alvaro[1]., Tapia, Pablo[2].,Gonzalez, Alvaro[1].,Simon, Felipe[1,3].,[1]Ciencia Biológicas, Ciencias Biológicas, Universidad Andrés Bello.[2]Medicina Intensiva, Medicina, Pontificia Universidad Católica De Chile.[3]IMII Millennium Institute on Immunology and Immunotherapy. (Sponsored by Fondecyt 1121078 And MII P09-016-F)

Introduction: Sepsis is mediated by immune system over-activation promoting an increased secretion of inflammatory mediators, which are released into the bloodstream. Impaired circulatory function is an important factor in sepsis pathogenesis, in which the endothelial cells (ECs) dysfunction is crucial to blood vessels damage. Several evidences have shown that inflammatory mediators induce conversion of ECs into activated fibroblasts through a process known as endothelialtomesenchymal transition. Septic patients exhibit increased circulating endothelial cells (CECs) levels, which include circulating mature endothelial cells (CMECs) and circulating endothelial progenitor cells (CEPCs). Thus, our aim was to study whether CECs from septic patients exhibit fibrotic features, which are characterized by the acquisition of fibrotic proteins and by the loss of endothelial markers. **Methods and Results:**CMECs and CEPCswere obtained from blood samples of septic patients and healthy volunteers using immunomagnetic bead capture (IBC). Endothelial fibrosis was demonstrated by detecting changes in the expression pattern, localization and cellular distribution of the endothelial/progenitors and fibrotic markers. Our results showed a decreased expression of endothelial and progenitors protein including VE-Cadherin, CD-31, VEGFR-2, and an increasedexpression of the fibroblast-specific proteins, α-SMA and vimentin. In addition, the amount of CMECs and CEPCs and the total CECs were increased in septic patients samples compared to healthy volunteers. **Conclusion:**Our data demonstrated that the expression of endothelial proteins in CMECs and CEPCs from septic patients is decreased when compared with ECs from healthy volunteers. Furthermore, CMECs and CEPCs from septic patients acquire expression of fibrotic markers. These results suggest that endothelial fibrosis may be a common mechanism of vascular endothelial dysfunction during sepsis. These data could be useful for diagnostic and sepsis treatment improvement.



13) ***IN VIVO* EVALUATION OF ATRIAL NATRIURETIC PEPTIDE ANP: CARDIOVASCULAR MEASUREMENTS IN CHRONICALLY HYPOXIC NEONATES IN THE *ALTO ANDINO*.**

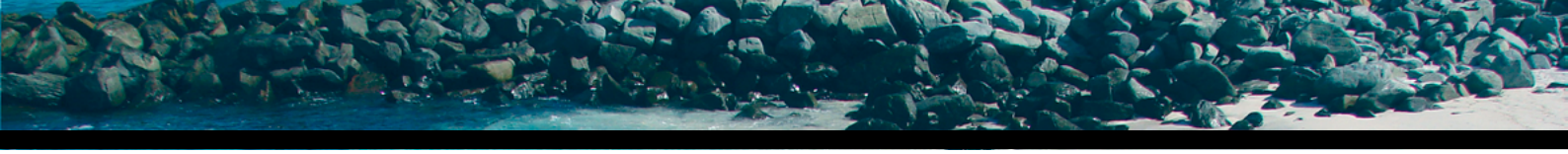
Beñaldo, Felipe[1]., Guzmán, Constanza[1].,Araneda, Felipe[1].,Araya, Claudio[1].,Poblete, Daniel[1].,Moraga, Fernando[2].,Herrera, Emilio [1].,Reyes, Víctor[1].,Ebensperger, Germán[1].,Llanos, Aníbal [1].,[1]Fisiopatología, Medicina , Universidad De Chile.[2]Ciencias Biomédicas, Medicina, Universidad Católica Del Norte. (Sponsored by Acknowledgments: Supported By FONDECYT 1140647, 1120605, 1130424 & 1151119.)

[1]Programa de Fisiopatología, ICBM, Facultad de Medicina, Universidad de Chile. [2]International Center for Andean Studies (INCAS), Universidad de Chile. [3]Departamento de Ciencias Biomédicas, Facultad de Medicina, Universidad Católica del Norte. **Background:** High altitude chronic hypoxia during development induces pulmonary hypertension of the neonate (1). Newborns in high altitude have a reduced lung sGC expression (1), central molecule involved in pulmonary vasodilatation. In contrast, no information is available regarding the particulate guanylyl cyclase (pGC), a membrane guanylyl cyclase, the receptor for ANP & BNP. Particulate GC is present in the lung (2) and could be the target for the ANP secreted by the right heart, eliciting pulmonary vasodilatation. We hypothesize that an acute dose of ANP reduces pulmonary hypertension in newborn lambs gestated, born and raised at high altitude. We aim to determine the effects of ANP on pulmonary (PAP) and systemic arterial pressure (SAP), pulmonary (PVR) and systemic vascular resistance (SVR), heart rate (HR) and cardiac output (CO). **Methods:** Four newborn sheep, born and raised at Putre Research Station, INCAS, University of Chile (3,600 m), were catheterized in pulmonary artery (Swan Ganz catheter) and abdominal aorta at the four days old. One day after surgery, the neonates were submitted to a 85 min protocol (15 min of basal, 10 min IV infusion of ANP (5 ug kg^{-1})and 60 min of recovery), while recording cardiovascular variables. **Results:** Basal hemodynamic variables were similar as previously described (1). ANP infusion decreased PAP and this reduction was maintained during recovery (Infusion: 17.6±5.8%, Recovery (R) 15: 21.5±2.9%, R30: 15.8±3.1%, R45:13.2±3.8, R60: 13.0±2.7 mmHg). However, PVR only decrease during the infusion period (13.5±3 %). SAP, SVR and HR did not change during the study, whereas CO did decrease only at R15 (13.2±1.7%). **Conclusions:** ANP did decrease PAP during acute infusion with no changes in systemic arterial pressure. These preliminary results offer a promising possibility to treat neonatal pulmonary hypertension, since no changes in systemic arterial pressure were observed**.** **References:** 1) Herrera et al. *Cardiovasc Res* **77**(1):197,2008. 2) Pandey KN. *Elsevier* **26**:901, 2005.

14) **CINACIGUAT (BAY-582667): A POTENTIAL TREATMENT FOR PULMONARY HYPERTENSION IN CHRONICALLY HYPOXIC NEONATES.**

Beñaldo, Felipe[1]., Araneda, Felipe[1].,Guzmán, Constanza[1].,Araya, Claudio[1].,Castillo-Galán, Sebastián[1].,Chen, Zhuoming[1].,Moraga, Fernando[2].,Herrera, Emilio[1].,Reyes, Víctor[1].,Ebensperger, Germán[1].,Llanos, Aníbal[1].,[1]Fisiopatología, Medicina , Universidad De Chile.[2]Ciencias Biomédicas, Medicina, Universidad Católica Del Norte. (Sponsored by Acknowledgments: Supported By FONDECYT 1140647, 1120605, 1130424 & 1151119.)

[1]Programa de Fisiopatología, ICBM, Facultad de Medicina, Universidad de Chile. [2]International Center for Andean Studies (INCAS), Universidad de Chile. [3]Departamento de Ciencias Biomédicas, Facultad de Medicina, Universidad Católica del Norte. **Background:** Gestational chronic hypoxia induces neonatal pulmonary hypertension (1). Inhaled nitric oxide (iNO) is the only approved treatment (iNO), which is effective only in 60% of the newborns (2). One reason of this failure, among others, is that guanylyl cyclase (sGC) is downregulated and/or the heme iron of the enzyme is oxidized, losing its activity, under chronic hypoxia (1). Therefore, enhancing its activity or expression can be a potential treatment. Cinaciguat is a sGC activator, even when the heme iron is oxidized, capable of inducing NO-independent vasodilatation (3). Hence, we hypothesized that an acute dose of Cinaciguat reverts pulmonary hypertension in newborn sheep gestated, born and raised at high altitude. We aim to determinate the effects of Cinaciguat on pulmonary (PAP) and systemic arterial pressure (SAP), pulmonary (PVR) and systemic vascular resistance (SVR), heart rate (HR) and cardiac output (CO). **Methods:** Five newborn sheep, born and raised at Putre Research Station, INCAS, University of Chile (3,600 m), were catheterized in pulmonary artery (Swan Ganz catheter) and abdominal aorta at the four days old. One day after surgery, the neonates were submitted to a 78 min protocol (15 min of basal, 3 min IV infusion of Cinaciguat (35 ug kg^{-1}) and 60 min of recovery), while recording cardiovascular variables. **Results:** Basal hemodynamic variables were similar as previously described (1). Cinaciguat infusion just increased CO during infusion. Further, Cinaciguat decreased PAP, SAP, PVR and SVR reaching a nadir at 30 min post-infusion with 34.7±5.5%, 19.1±1.6%, 36.6±8.0% and 17±2.9% decreases, respectively. In contrast, no changes were seen in HR along the protocol. **Conclusions:** Cinaciguat decreases pulmonary arterial pressure and resistance, with a slight fall in systemic pressure. Cinaciguat could potentially offer a new therapeutic approach for neonatal pulmonary hypertension either when iNO fails or is not available. **References:** 1) Herrera et al. *Cardiovasc Res* **77**(1):197,2008. 2) Walsh-Sukys et al. *Pediatrics* **105**:14, 2000. 3) Chester et al. *Am J Physiol* **301**: L755 2011.



15) **NLRP3 inflammasome in cardiac fibroblast**

Boza, P[1]., Tapia, Felipe[1].,Vivar, Raul[1].,Humeres, Claudio[1].,Díaz, Guillermo[1].,[1]Departamento de Farmacología y Toxicología, Ciencias Químicas y Farmacéuticas, Universidad De Chile. (Sponsored by Project Fondecyt 1130300 Scholarship. CONICYT Beca Doctorado Nacional 21120407. Grant CONICYT Gastos Operacionales 21120407)

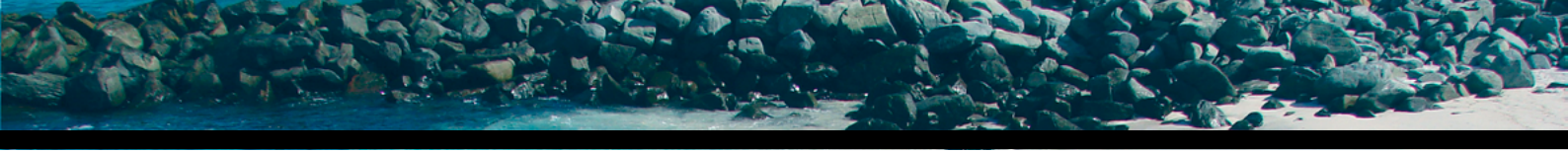
Introduction The sterile inflammation is a new topic in cardiovascular disease. After a stroke, resident heart cells express high levels of IL-1β. This cytokine is an inflammation master controller, its synthesis and secretion is a two-step process that concludes with the inflammasome activation and IL-1β secretion. We hypothesize that NLRP3 inflammasome in neonatal rat cardiac fibroblast (CF) can be activated and is functional. Materials and Methods Cells were seeded until passage 1 and fasted all night. The cells were stimulated with LPS 1µg/ml and/or ATP 3mM for 24h. Pro-IL-1beta, NLRP3, ASC and pro-caspase-1 were measured using Western blot (WB). Cells pretreated with 8h of LPS were stimulated with ATP for 16h. IL-1β secretion was measured by ELISA and also by protein precipitation followed by WB. Caspase-1 activity was measured using a Fluorometric assay. Results CF express NLRP3, ASC and pro-caspase-1. LPS induces NLRP3 expression. Pro-IL-1β is induced in a time depending manner. Both protein expressions are significant at 8h of LPS. LPS8h + ATP16h generate the synthesis and secretion of IL-1β to culture media (ELISA). However, protein precipitation followed by WB indicates that the secreted cytokine is a mix between IL-1β and pro-IL-1β. Caspase-1 activity assay shows an increase in the activity induced by ATP and LPS+ATP. Conclusion CF express the proteins that conforms the NLPR3 inflammasome. LPS can act like a first signal, inducing pro-IL-1β and NLRP3 synthesis. ATP can act like a second signal, inducing the NLRP3 inflammasome assembly and activating caspase-1. The assembled inflammasome is functional, can cleave pro-IL-1β into IL-1β. Both cytokines can be secreted, however the inflammasome is the pacemaker step.

16) **Key role of connexin hemichannels and pannexin channels in the PAF-induced Ca^{2+} signaling in endothelial cells of post-capillary venules**

Burboa, Pia[1]., Poblete, Ines[1].,Figueroa, Xavier[1].,[1]Phisiology, Ciencias Biológicas, Pontificia Universidad Católica De Chile.
(Sponsored by FONDECYT 1150530)

Endothelial cells constitute a permeability barrier between blood and tissue interstitium. Pro-inflammatory signals, such as platelet-activating factor (PAF), induce a Ca^{2+}-dependent increase of endothelial permeability to macromolecules in post-capillary venules. This Ca^{2+} signaling depends on Ca^{2+} release from the endoplasmic reticulum and Ca^{2+} influx from the extracellular space. However, the mechanisms involved in the Ca^{2+} influx have not been clearly determined. We analyzed the participation of the plasma membrane channels formed by connexin (Cx) proteins (i.e. hemichannels) or pannexins in PAF-elicited intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increase. We used the intact mesenteric vascular bed and primary cultures of mesenteric endothelial cells (EC) of resistance arteries (EC-A) and venules (EC-V). Changes in $[Ca^{2+}]_i$ were recorded by loading EC with the fluorescent Ca^{2+} indicator Fluo-4 and activity of connexin hemichannels or pannexin channels was evaluated by assessing ethidium uptake in EC and intact vessels. Stimulation with 10 nM PAF did not affect $[Ca^{2+}]_i$ or ethidium uptake in resistance arteries and EC-A. In contrast, PAF induced an increase in $[Ca^{2+}]_i$ and ethidium uptake in venules and EC-V. Both the increase in $[Ca^{2+}]_i$ and ethidium uptake were inhibited by treatment for 15 min with the connexin blocking peptide ^{37,43}Gap27 or the pannexin-1 blocking peptide ^{10}Panx, suggesting that connexin hemichannels and pannexin-1 channels contribute to the Ca^{2+} signal. Consistent with this, the expression of Cx37, Cx40, Cx43 and pannexin-1 was confirmed in EC-V by immunofluorescence analysis. Connexin and pannexin channels are permeable to Ca^{2+}, but they may also trigger Ca^{2+} signals through ATP release and blockade of purinergic receptors with PPADS blunted the PAF-induced $[Ca^{2+}]_i$ increase. These results suggest that the intracellular Ca^{2+} signaling activated by PAF is mainly mediated by ATP release through connexin hemichannels and/or pannexin-1 channels, which, in turn, leads to activation of purinergic receptors. However, direct Ca^{2+} influx via connexin hemichannels or pannexin-1 channels may also contribute to the response.

Proyecto FONDECYT 1150530.



17) **Pulmonary artery remodeling and mitogens are reduced in 2-aminoethyldiphenylborinate treated lambs**

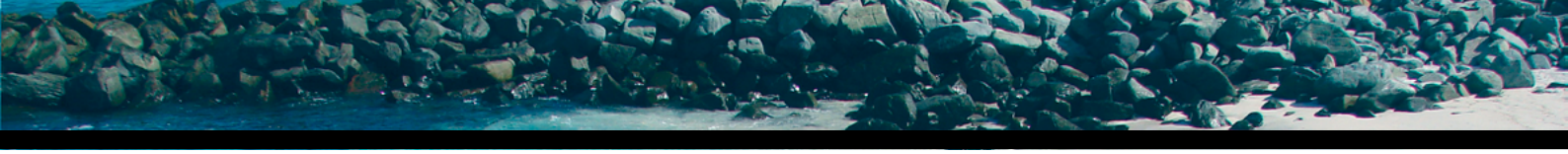
Castillo-Galán, Sebastián[1]., Quezada, Sebastián[1].,Beñaldo, Felipe[1].,Ebensperger, Renato[1].,Ebensperger, Germán[1].,Herrera, Emilio[1,2].,Llanos, Anibal[1,2].,Reyes, Roberto[1].,[1]Programa de Fisiología y Fisiopatología del Desarrollo, Facultad de Medicina Oriente, ICBM, Universidad de Chile, Universidad De Chile.[2]International Center for Andean Studies (INCAS) Universidad De Chile.
(Sponsored by Supported By FONDECYT 1120605, 1151119, 1140647, 1130424)

Perinatal chronic hypoxia induces an imbalance of vasoconstrictor and vasodilators mechanisms as well as a pathological pulmonary artery remodeling. This remodeling is characterized by a thickening of the medial layer due to a proliferation of pulmonary artery smooth muscle cells (PASMC) among other processes. These functional and structural changes result neonatal pulmonary hypertension (NPH), a disease in which persistent high pulmonary artery resistance (PVR) and pressure (PAP) are observed. The store operated channels (SOC), are calcium-permeable cationic channels, involved in the regulation of the pulmonary arteries reactivity and remodeling, particularly in response to hypoxia. We previously demonstrated that a ten-day blockade of these channels with 2-aminoethyldiphenylborinate (2-APB) reduces the basal PAP and its increase in response to hypoxia on newborn lambs with NPH induced by partial gestation under hypoxia. Here we studied if this treatment reverts the pathologic pulmonary artery remodeling in newborn lambs with NPH, and if this change was related with a reduction in the expression of the mitogen VEGF-A. Ten newborn lambs partially gestated at highlands (3600 m) and returned to lowlands immediately after delivery were treated for ten days with 2-APB, a putative SOC blocker, (10 $mg \cdot kg^{-1} \cdot day^{-1}$) or its vehicle (DMSO: saline 1:10). Two days after the end of the treatment, we evaluated the pathologic pulmonary artery remodeling, by measuring the area of medial layer thickness and the expression of the smooth muscle marker α-actin in the small pulmonary arteries. We also measured the pulmonary expression of VEGF-A isoforms -188, -164 and -120. Pulmonary arteries from animals treated for ten days with 2-APB, exhibited a thinner medial layer and lower expression of α-actin. In addition, 2-APB treatment reduced the pulmonary expression of VEGF-A -188 and -164 isoforms, whilst -120 isoform expression remained unchanged. A ten-day treatment with 2-APB reduces the pulmonary artery remodeling in newborn lambs with NPH induced by perinatal chronic hypoxia. This effect of 2-APB could be related with its ability to block SOC, and therefore to reduce the long term production of mitogens like VEGF.

18) **Increased potassium in the diet intensifies ATP release from rat mesenteric endothelial cells elicited by mechanical stimulation.**

Donoso, M. Veronica[1]., Huidobro-Toro, Juan [1].,[1]Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile. (Sponsored by Funded By FONDECYT Grant 1141132)

Diet has a critical influence in cardiovascular functioning. Humans fed diets rich in fresh fruits and vegetables have consistently been demonstrated to decrease the risk of vascular pathology, compared to humans that consume mainly processed foods. Reducing sodium intake and increasing consumption of potassium (K) containing foods prevents and reduces the development of hypertension and other vascular diseases. To elucidate the beneficial K effects in the control of diseases, we studied the release of ATP elicited by a mechanical stimulus from cultured endothelial cells derived from the rat arterial mesenteric bed of animal fed a control (1% K) and a diet supplemented with 4% K, during 4 weeks. We hypothesized that mechanical stimulation released ATP by endothelial cells which acts on autocrine purinoceptors, leading to an increased NO production, a potent vasodilator. Endothelial cell from control rats were plaqued (3-4 days) in media containing either 5.3 or 10.6 mM K. In parallel, endothelial cells from animals fed 4% K, were plaqued in10.6 mM K. Cultures were mechanically stimulated; the supernatant was collected and chemically derivatized to asses ATP/metabolites by fluorimetric detection. Basal ATP values from cells derived from animals ingesting either a control diet or high K diet did not change over time (0.5-15 min) nor showed differences when comparing values from control cells and those maintained in media with high K. Mechanical stimulation induced transient ATP release that peaked 1 min after stimulation and returned to baseline values after 15 min. The ATP ratio of the released/basal, 1min after mechanical stimulation, in control cells was 5.8±0.98 (n=16), a value that increased 2-fold (10.2±1.89 (n=16), $p<0.05$) in cells from the same animal maintained in 10.6 mM K during the days of cell culture. Likewise, an almost 2-fold increase in the ATP ratio was observed in cells of rats fed a high K diet and maintained in 10.6 mM K (10.4±1.8, n=16, $p<0.05$). The same increase in the ratio was observed for ADP, AMP and ADO in cells maintained in high K. Rats treated with a diet supplemented with K for 4 weeks showed a significant reduction in body weight compared to paired controls (282±22 vs 406±16 g, $p <0.001$, n= 5 each). In conclusion, increasing K in the diet, or the culture of cells in high K, increased significantly the ATP/metabolites released following mechanical stimulation, providing an explanation for the beneficial effects of K in preventing hypertension and other vascular diseases.



19) **Differential regulation of NO pathway in pulmonary circulation of neonatal sheep from low and high altitudes.**

Ferrada , Javiera[8].,Ebensperger , Renato[8].,Cerda , Tania[1].,Catriman , Danixsa[2].,Araya , Claudio[3].,Beñaldo, Felipe[3].,Díaz , Marcela[4].,Moraga, Fernando[5].,Castillo, Sebastian[8].,Herrera , Emilio[8,6].,Reyes, Víctor[7].,Llanos, Aníbal[8,6].,**Ebensperger, G[8].**, [1]Programa Fisioipatologia, Campus Oriente, ICBM, Facultad de Medicina, Universidad de Chile.[2]Programa de Fisiopatologia, Campus Oriente, ICBM, Facultad de Mdicina, Universidad de Chile.[3]Programa de Fisiopatologia, Campus Oriente, ICBM, Facultad de Medicina, Universidad de Chile.[4]Departamento de Promoción de la Salud de la Mujer y el Recién Nacido, Facultad de Medicna, Universidad de Chile.[5]Ciencias Biomedicas, Facultad de Medicina, Universidad Católica Del Norte.[6]INCAS Universidad de Chile.[7]Programa Fisiopatologia, Campus Oriente, ICBM, Facultad de Medicna, Universidad de Chile.[8]Programa Fisiopatologia, Campus Oriente, ICBM, Facultad de Medicina, Universidad de Chile.

Background. The neonatal pulmonary circulation needs to adapt to extrauterine life in few hours for neonatal survival. For this objective the induction of vasoactive pathways such as nitric oxide (NO) is imperative, producing a marked decrease in pulmonary arterial pressure (PAP) and vascular resistance (PVR). When the birth is carried out in a milieu with a low oxygen (such as chronic hypoxia at high altitudes, HA) the pulmonary transition is inefficient, resulting in neonatal pulmonary hypertension. Neonates of sea level (SL) species, such as the sheep, induce NO production when exposed to chronic hypoxia (1,2). We hypothesized that high altitude newborn sheep, not only induces NO production, but as well the NO signalling pathway (sGC & PKG-1).

Methods. Two weeks old newborn sheep from high altitudes (HANB, n=6; 3,600m) and sea level (SLNB, n=6; 580m) were instrumented in pulmonary artery with a Swan Ganz catheter for registering cardiopulmonary hemodynamic variables in the presence of eNOS inhibitor (L-NAME), in basal and hypoxemic conditions. The day after we extracted small resistance pulmonary arteries to assessed pulmonary vasodilator capacity in a wire myograph.

Results. HANB showed a basal pulmonary hypertension relative to SLNB (21±3 vs 12±1), as described previously (2). During basal conditions, LNAME induce increase in PAP in both group of neonates, reaching higher absolute values in HANB. However, during superimposed hypoxemia, both groups reached similar PAP levels (42±8 vs 38±2). The isolated vascular function experiments showed a marked endothelial dysfunction in HANB relative to SLNB, which is not recovered by the arginase inhibitor BEC. Further, LNAME fully blocked endothelial vasodilator function in both groups. In addition, sGC function in pulmonary arteries is depressed in HANB relative to SLNB. However, the PKG-1 and Rho kinase functions are increased in HANB relative to SLNB.

Conclusion. The NO pathway regulates the pulmonary circulation in newborn sheep from sea level and high altitude. High altitude exposition during development exacerbate the NO, PKG-1 and Rho kinase functions, as a compensatory response to sGC low expression and function.

Supported by Fondecyt Regular 1130424, 1110647, 1120605, 1151119.

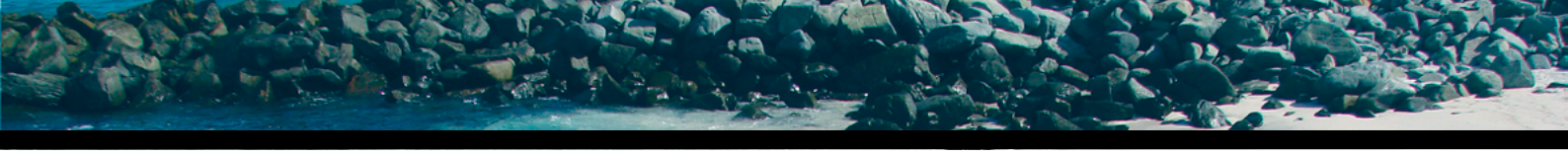
(1) Herrera *et al*. Cardiovasc Res 77(1):197, 2008.

(2) Herrera *et al*. Am J Physiol 292(6):R2234, 2007

20) **Effect of aging on calcium transients in rat cardiomyocytes: impact of NOX inhibition**

Gonzalez, Daniel[1]., Barrios, Guillermo[1].,[1]Departamento Ciencias Biomedicas, Facultad de Ciencias de la Salud, Universidad De Talca. (Sponsored by Funded By Proyecto Fondecyt 1150662 And Programa De Investigación De Excelencia Interdisciplinaria En Envejecimiento Saludable Universidad De Talca.)

Background. Cardiac aging is characterized by alterations in contractility and calcium handling. It has been suggested that oxidative stress may be involved in this process. The superoxide generating system NADPH oxidase (NOX) is expressed in the heart (isoforms NOX 2 and 4). We and other have previously observed that in some forms of cardiac failure, the NOX-derived reactive oxygen species are increased, with a negative impact on calcium transients and contractility, due to redox effects on the calcium release channel ryanodine receptor (RyR2) and the sarcoplasmic reticulum calcium pump SERCA2. **Aims.** The aim of this study was to analyze calcium transients and contractility in aged rat cardiomyocytes and to evaluate the impact of NADPH oxidase (NOX) inhibition. **Methods.** Cardiac myocytes were obtained form adult (5 months old) and aged (20 months old) Sprague-Dawley rats. Aged myocytes were treated with apocynin (50 µmol/L, 30 min). Cells were field-stimulated from 0.5 to 4 Hz, using Tyrode solution at 37˚ C. Contractility was evaluated as sarcomere shortening and intracellular calcium was evaluated loading the myocytes with fura-2. **Results.** Sarcomere shortening was similar in adult, aged and aged treated myocytes. Time to reach 50 % of peak shortening was increased in aged myocytes ($p < 0.05$), and was not improved by apocyinin treatment. The same was obtained for the time to reach 50% of relaxation. $[Ca^{2+}]_i$ transients (amplitude and fractional increase) were increased in aged cardiomyocytes ($p < 0.05$) and were further increased by apocynin treatment. Time 50 to peak Ca^{2+} was increased in aged myocytes ($p < 0.05$), suggesting impairment in the ryanodine receptor, but was improved (reduced) by the apocynin treatment. Time 50 to peak relaxation was increased in aged myocytes ($p < 0.05$) and reduced towards normal by apocynin treatment. Using thapsigargin to block SERCA function, we submitted myocytes to tetanic stimulation (40 Hz) to evaluate the myofilaments Ca^{2+} sensitivity. By comparing the amplitude of the tetanic contraction achieved with the level of $[Ca^{2+}]_i$ evoked, we found that myofilaments Ca^{2+} sensitivity was reduced in aged myoctes ($p < 0.05$). **Conclusions.** Contractility was preserved in aged myocytes, but at a higher $[Ca^{2+}]_i$ level, as a result of diminished myofilaments Ca^{2+} sensitivity. NOX inhibition with apocynin increased Ca^{2+} transients amplitude and improved Ca^{2+} kinetics, without changes in contractility. These changes suggest a redox effect at the level of ryanodine receptor and SERCA.



21) **Chronic exercise reduces fibrosis and hypertrophy but not oxidative stress in diabetic cardiomyopathy**

Novoa, Ulises[1].,Arauna, Diego[1].,Moran, Marisol[1].,**Gonzalez, Daniel[1].**, [1]Departamento Ciencias Biomedicas, Facultad de Ciencias de la Salud, Universidad De Talca. (Sponsored by Supported By Fondecyt 1150662 And Programa De Investigación De Excelencia Interdisciplinaria En Envejecimiento Saludable Universidad De Talca.)

Background: Diabetic cardiomyopathy refers to the cardiac manifestations observed in the heart as a result of altered glucose homeostasis that is reflected as fibrosis, cellular hypertrophy, increased sources of oxidative stress, apoptosis, and finally systolic and diastolic dysfunction. Exercise is known to exert salutary effects on cardiovascular function, mainly through the increase in the expression of nitric oxide synthase, particularly eNOS.

Aims: We tested the hypothesis that chronic exercise could reverse the cardiac maladaptations and oxidative stress that are produced by diabetes.

Methods. Diabetes was induced in rats by a single dose of alloxan (200/mg kg, i.p). Diabetic rats were randomly assigned to a sedentary group or submitted to a program of exercise on a motor-driven treadmill (80% of maximal aerobic capacity) 5 days/ weeks, for 4 weeks. Another group of normoglycemic rats was used as control. Cardiac fibrosis was evaluated by Picrosirius red staining and the levels of NOX and NOS enzymes were evaluated by real-time PCR and Western Blotting. Tetrahydrobiopterin levels were analyzed by HPLC.

Results. Chronic exercise reduced cardiac fibrosis and cellular hypertrophy in diabetic rats ($p<0.05$, ANOVA). On the contrary, exercise induced the expression of the NADPH oxidases NOX2 and NOX4, both at mRNA and protein level ($p<0.05$, ANOVA). Exercise induced no change in the levels of total and uncoupled eNOS. Furthermore, exercise was unable to restore the intracardiac levels of tetrahydrobiopterin, an essential cofactor for NOS activity, that were reduced in diabetic rats.

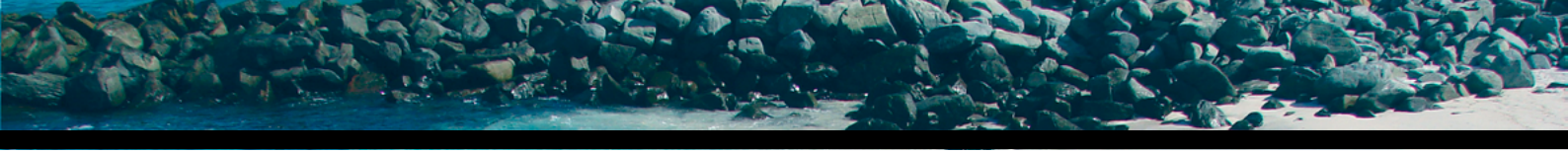
Conclusions. These results suggest that chronic exercise was able to reverse cardiac remodeling in the diabetic heart, but was unable to restore the nitroso-redox imbalance imposed by oxidative stress. This later could by restored by pharmacological manipulations.

22) **Nitric oxide releasing aspirin affects morphogenesis and increases Candida albicans susceptibility to fluconazole in clinical isolated.**

Madariaga, F[1]., Urzua, Blanca[1].,Fernandez, Roberto [1].,Ramires, Ricardo[1].,Jara, Jose[1].,Herreros, Eduardo[1].,Aguilera, Jocelyn[1].,Molina, Alfredo[1].,[1]instituto de investigación en ciencias odontologicas, odontologia, Universidad De Chile. (Sponsored by Project FONDECYT 11140227, U-inicia 195. Candida Spp Strains Were Kindly Given By Dr. Ximena Lee And Leyla Gomez, Facultad De Odontología, Universidad De Chile.)

Introduction: Candida albicans is able to form biofilms on denture prosthesis participating in denture stomatitis, a common oral pathology in elderly people. C. albicans biofilms function as an infection reservoir that in susceptible individuals could lead to invasive candidiasis. Biofilms acts as multiple resistance mechanism to antifungals. Because of the high resistance of C. albicans biofilms to antifungals, new pharmacological strategies to treat these infections are needed. In this sense, non-steroidal antiinflamatory drugs (NSAIDs), which inhibit prostaglandins synthesis, have shown to exert antibiofilm effect. Among them, aspirin not only prevents but also reverse biofilm formation. On the other hand, nitric oxide (NO) releasing drugs have shown similar results to aspirin and improve conventional antifungal effects on biofilms.

According to this information, we evaluated NO-releasing aspirin (NO-ASA) effect on *C. albicans* obtained from denture stomatitis patients alone and in combination with fluconazole. **Methods:** Candida spp. obtained from oral mucosa of denture stomatitis patients (n=60), were identified through CHROMagar Candida Medium BDTM and by sequencing the ITS1-5.8S rDNA-ITS2 region using the primers ITS1 and ITS4. To evaluate antifungal susceptibility, isolated strains were standardized to 0.5 McFarland and then grown on sabouraud agar plates and disk diffusion tests were performed in presence or absence of fluconazol, NO-ASA and their combination.*Candida albicans* Morphogenesis is a crucial step to biofilm formation so we evaluated effect of NO-ASA on hyphal induction. C. albicans hyphae were induced in standardized overnight cultured cells in RPMI-1640 medium (28°C, without agitation). After adjusting to $1x10^6$ cells/mL (Neaubauer chamber) samples were incubated at 37°C with agitation for 3 h. Percentage of Hyphae was obtained counting the cells by light microscopy. **Results:** from the 60 strains, 55% (n=33) were identified as C. albicans. In disk diffusion assay, 7 strains (21%) was classified as resistant to fluconazole (diameter <16 mm). NO-ASA had no effect when was added alone in the disks. However, in resistant strains combination with fluconazole it was observed 15-20% increase in inhibition zone diameter respect to fluconazole alone. For hyphae induction experiments, we selected the 7 strains resistant to fluconazole to evaluate NO-ASA effect. Results show that NO-ASA is able to decrease yeast to hyphae morphogenesis in all clinical strains tested to an extent of 70-80% ($p<0,05$ ANOVA). **Conclusion:** NO-ASA increases susceptibility to fluconazole in resistant strains and also affects ability to switch from yeast to hyphae. We are currently investigating possible mechanisms which explain these effects.



23) **Cytotoxic effect of lipophilic cation derived from polyhydroxy-benzoic acids in oral squamous cell carcinoma**

Aguilera, Jocelyn [1]., Molina, Alfredo [1].,Fernández, Ricardo[1].,Ferreira, Jorge [2].,Castro, Vicente[3].,Jara, José[1].,[1]Laboratorio de Farmacología y Farmacogenómica, Facultad de Odontología , Universidad De Chile.[2]Programa de Farmacología Molecular y Clínica, ICBM, Facultad de Medicina, Universidad De Chile.[3]Departamento de Química, Facultad de Ciencias Básicas, Universidad Metropolitana de Ciencias de la Educación. (Sponsored by Proyecto U-inicia VID N? 196 (Jara J), Financiado Por El Programa De Ayuda De Viajes Para El Fortalecimiento De La Investigación, Facultad De Odontología (Jara J).)

Oral cancer is a disease with hight impact worldwide, in fact, ranks sixth in the world in the category of most common cancers in men. As such, cancer is a disease in which cells undergo an imbalance between cell division and death. The uncontrolled growth of these cells come from a series of genetic alterations that allow them to multiply occurs out of context Normal tissue development.

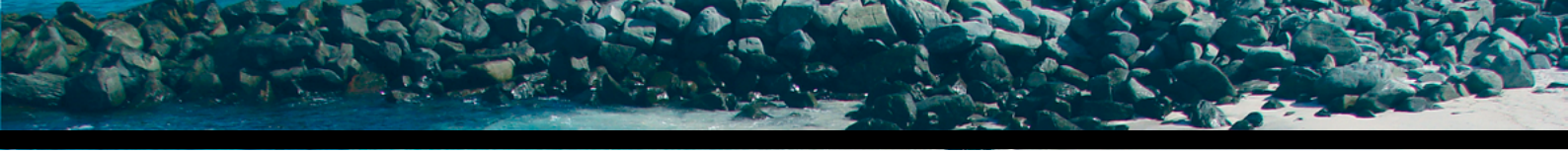
These cancer cells have striking features as unlimited replicative potential, sustained angiogenesis, evasion of apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals, invasiveness and metastasis. All these features provide selective advantages ending in the progression disease. Some of these functions are essentially relevant in the search for new therapeutic, for example, increased mitochondrial transmembrane potential, highly glycolytic activity and reduced mitochondrial mass tools. This characteristic makes mitochondrial an attractive drug target for the search for new molecules that increase the efficiency and selectivity of the treatments.

It wasused as cytotoxic agents derived from mono and/or poly-hydroxybenzoic acids attached to the triphenylphosphonium group, as there is evidence that this group can lead to pharmacophore to the mitochondria. We tested bromide salts: Triphenyl (10 - ((3,4,5-trihydroxybenzoyl) oxy) decyl) phosphonium, 10- ((2-hydroxybenzoyl) oxy) decyl) triphenylphosphonium 10 - ((2,5-dihydroxybenzoyl) oxy) decyl) triphenylphosphonium and 10- ((2,3-dihydroxybenzoyl) oxy) decyl) triphenylphosphonium on tumor cell lines CAL-27 and normal cells. MTT viability assay was used to measured cytotoxicity by calculating therespective IC_{50}, ATP content through luminescence, mitochondrial transmembrane potential by fluorescence andinduction of apoptotic deathwasdetermined by flow cytometry. The results indicate that the compounds exhibit enhanced cytotoxicity in the tumor cell line than normal cells. Furthermore, the compounds showed an uncoupling effect, and also triggered decreased transmembrane potential, falling ATP levels and induce apoptosis in a micromolar range of concentration. The results are promising in terms of cytotoxicity and selectivity and offer a pharmacological opportunity of treatment in oral cancer.

24) **Ar-c155858 decreases neutrophil extracellular traps formation and neutrophil adhesion onto endothelium induced by D-lactic acid**

Alarcon, Pablo[1]., Conejeros, Iván[1].,Munoz-caro, Tamara[2].,Taubert, Anja[2].,Hermosilla, Carlos[2].,Burgos, Rafael[1].,[1]Instituto de farmacologia y morfofisiologia Universidad Austral De Chile, Valdivia, Chile.[2]Institute of Parasitology Justus-Liebig University Giessen, Giessen, Germany. (Sponsored by Financed By FONDECYT 1151035, DID-UACh, MECESUP AUS1203)

In Cattle, ruminal acidosis occurs mainly by a highly fermentable carbohydrate overload, which leads to increased levels of D-lactic acid in the rumen and plasma. Also, rumen acidosis has been correlated with an increase in acute phase proteins in plasma and neutrophils infiltration in several tissues causing, rumenitis, laminitis, polysynovitis, suggesting the involvement of an extent pro-inflammatory response in ruminal acidosis. Neutrophils represent the first line of defense against pathogens but recent data also indicates that D-lactic acid interferes with neutrophil activation thereby decreasing the ROS production and the release of metalloproteinase 9 in bovine neutrophils stimulated with platelet-activating factor. In addition, D-lactic acid increased neutrophil extracellular traps (NET) formation and adhesion of bovine neutrophils to endothelium under flow conditions. We demonstrated the presence of monocarboxylate transporters (MCT-1, 4) in bovine neutrophils. In this work, we show for the first time D-lactic acid-triggered NET formation, colocalized with H_4 Citrullinated 3 and CD11b, through immunofluorescence analysis. Further aim of this study was to evaluate if blocking the MCT1 transporter with Ar-c155858 would be able to reduce the effect of D-lactic acid on NET formation as well as adhesion onto endothelium.We demonstrated that Ar-c155858 reduced the NET formation and adhesion of neutrophil onto endothelium induced by D-lactic acid. Indeed, neutrophils adhesion to endothelium induced by D-lactic acid was also reduced when DNAse I was added and measured under flow conditions. Moreover, when neutrophils treated with 5mM D-lactic acid plus anti-CD11b antibodies, and then perfused onto endothelium, there was a decrease of neutrophils adhesion under flow conditions which leads us to suggest that the endothelium-neutrophil adhesion induced by D-lactic acid CD11b-dependent process. Secondly, when the endothelium was perfused only with supernatant from neutrophils treated with 5mM of D-lactic acid for 10 minutes and later, perfused neutrophils without treatment, we observed an increase of neutrophil adhesion onto endothelium under flow conditions, and thus suggesting that there might be a soluble component released D-lactic acid-treated neutrophil which might have activated the vascular endothelium. Finally, we conclude that D-lactic acid is able to trigger NET formation and neutrophil adhesion and thus might be involved in the activation of the vascular bed which could contribute to neutrophil infiltration into locomotor apparatus during acute acidosis in cattle.



25) **Evaluation of leukocyte extravasation inhibition by *Ugni molinae* genotypes**

Avello, Zita[1]., Arancibia Radich, Jorge [1].,Seguel, Ivette [2].,Delporte , Carla[1].,[1]Química Farmacológica y Toxicológica, Ciencias Químicas y Farmacéuticas , Universidad De Chile.[2]Instituto de Investigación Agropecuarias, Instituto de Investigación Agropecuarias, INIA, Carrillanca, Temuco. (Sponsored by Acknowledgments: We Are Grateful To FONDECYT, Chile, Grant N° 1130155, CONICYT, Chile Grants N°21130672 And N°21120377. Thank You To INIA (Carillanca, Temuco) For The Genotypes.)

Ugni molinae Turcz is a Chilean evergreen shrub, it belongs to the Myrtaceae family and is native to central and southern Chile. Among its denominations are " murtilla ", " murta " or "Ugni". Research conducted in the Laboratory of Naturals Products at the Faculty of Chemical and Pharmaceutical Sciences at the University of Chile has recognized pentacyclic triterpenoid derivates of ursane,oleanane and lupane as : ursolic acid, oleanolic acid, corosolic acid, alphitolic acid, madecassic acid, asiatic acid and maslinic acid. Which have anti-inflammatory activity. In other studies the presence of phenolic compounds has been reported, such as catechin, epicatechin, myricetin, quercetin, kaempferol, myricetin glycoside, quercetin glycoside, rhamnoside, and gallic acid. The aim of this study was to evaluate comparatively the anti-inflammatory effect, through the inhibition of leukocyte extravasation in a mice model,of ethanol and ethyl acetate extracts from leaves of genotypes of *Ugni molinae* grown in the same soil and climate conditions and with the same agronomic management. The evaluated extracts were selected due to their anti-inflammatory activity *in vivo* in the TPA (phorbol 12 - myristate 13 -acetate) induced inflammation in mice edema ear model. A sample of 6 mm diameter was removed, fixed in 4% paraformaldehyde solution and treated with methanol solutions of increasing concentration to dehydrate it. Afterwards, xylene was added to the sample and finally embedded in paraffin. After this, histological cuts were done. For deparaffinize the sample was hydrated with methanol solutions of decreasing concentration. The sample was stained with hematoxylin and eosin that allowed for the observation and count of leukocytes in the optic microscope. The number of leukocytes in the treated samples and controls samples was determined. With these data, the inhibition percentage of extravasation in the inflamed site was calculated. In the evaluation of the anti-inflammatory effect through the inhibition of leukocyte extravasation, the results showed significant differences between the extracts from different *Ugni molinae* genotypes.

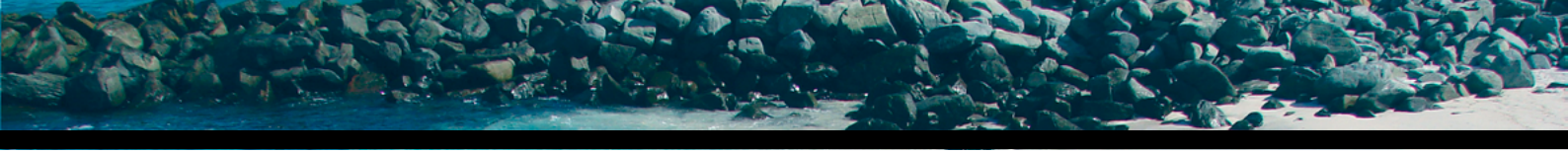
26) **Selective sympathetic plasticity in basal and evoked levels due to chronic sympathetic over-stimulation**

Pezo, Rafael[1]., Donoso, Maria Veronica[2].,Huidobro-Toro, Juan Pablo[1].,[1]Deparment of Biology, Chemistry and Biology, Universidad De Santiago De Chile.[2]Biology deparment, Chemistry and Biology, University of Santiago of Chile. (Sponsored by Postdoctoral FONDECYT To RB 3130573 And FONDECYT 1141132.)

Augmented sympathetic activity is associated with several chronic disorders like obesity, diabetes, etc. Nowadays there is a lack of basic information regarding the neurochemical adaptations associated to an altered sympathetic nerve terminals physiology, like described in. One of the common trades of the response to stress is the augment of the sympathetic nervous activity to blood vessels, including arteries and veins.

Based on this background, we hypothesized that rat chronic cold stress (CS) generates an increased noradrenergic secretion coupled to an altered sympathetic varicosity functioning. We subjected rats to CS without hypothermia, and assessed neurochemical changes in the sympathetic varicosities that innervate the rat arterial mesenteric bed. To this end, we perfused ex-vivo rat arterial mesenteric bed of control and rats exposed to CS for 3 hours every morning for 4 weeks. To evoke the release of co-transmitters, we electrically stimulated the perivascular mesenteric nerves (60Hz, 60 volts, 1 minute) and analyzed the secretion of sympathetic co-transmitters (ATP, NA and ir-NPY). ATP and metabolites were measured by fluorescence; NA was assessed by electrochemical detection and ir-NPY by RIA. Simultaneously, the perfusion pressure (PP) of the vascular bed was recorded via a grass transducer.

Present results consistently indicate that ATP and metabolites are lower in the group of CS rats compared to controls; basal ATP levels were 24.4±4.8 (n=10) vs. 119.6±39.8 pmol (n=6), $p \leq 0.05$. Likewise, ADP levels were 28.2 ± 10.8 (n=10) control 136.8 ± 51.8 (n=6) pmol $p \leq 0.05$. AMP; 27.6 ± 6.3 (n=10) vs. 161.5 ± 71.3 pmol (n=6) $p \leq 0.05$. ADO; 8.4 ± 0.6 (n=10) 38.7 ± 16.0 pmol (n=6) $p \leq 0.05$. Basal levels of NA were similar 4.424 ± 1.027 N=6 (CS) vs. 2.6 ± 0.9 N=5 (Control). In addition, we found that the NA secreted by electrical stimuli was higher in the CS vs. control 47.0 ± 15.7 (n=6) vs. 9.4 ± 4.6 (n=5) $p \leq 0.05$), while ATP values were 144.5 ± 50.4 (n=10) vs. 130.9 ± 77.4 (n=6), for CS and Control respectively. In agreement with the co-transmitters released, we observed an increase in the electrically evoked rise in PP in the animals submitted to CS compared to the respective controls (114.9 ± 10.8(n=6) vs 78.9 ± 12.0 vs. (n=7) $p \leq 0.05$). In sum, we discovered that CS induces neurochemical changes in the sympathetic varicosities. We interpret the present data indicating that CS causes a selective plasticity of the sympathetic nerves in such a way that basal release of ATP is lowered but increased following the evoked release of NA. This is the first report of specific co-transmitter changes between the basal and evoked release levels; the mechanics of these changes remain as yet unknown.



27) **Interferon beta (IFN-β) activates the JAK-STAT pathway in cardiac fibroblasts and produces anti-inflammatory and anti-fibrotic effects**

Bolivar, Samir[1]., Humeres, Claudio [1].,Vivar, Raul [1].,Boza, Pia[1].,Muñoz, Claudia[1].,Díaz-Araya, Guillermo [1].,[1]Farmacologia y Toxicologia, Ciencias Quimicas y Farmaceuticas, Universidad De Chile. (Sponsored by FONDECYT Project 1130300, CONICYT PhD Scholarship For Foreigners, Operating Expenses 63130233 CONICYT Scholarship.)

Introduction: Interferon beta (IFN-β) is a cytokine that activates the signaling transduction pathway, Janus kinase (JAK) and the transducer and activator of transcription (STAT), and causes a variety of responses. Cardiac Fibroblasts (CF) are the most abundant resident cells in the heart, and regulate the maintenance of extracellular matrix. The CF plays a central role in cardiac remodeling against certain injuries; however, it remains unknown if there are any potential anti-inflammatory and anti-fibrotic of IFN-β in CF actions. This work proposes that IFN-β and the activation of the canonical pathway in CF, could be novel drug targets in the cardiovascular area.

Objective: Determine whether activation of the JAK-STAT pathway in CF, produces anti-fibrotic and anti-inflammatory effects.

Materials and Methods: Primary cultures of adult rat FC in passage 2 were maintained in serum-free medium for 24 hours and stimulated by IFN-β in the presence or absence of Ruxolitinib (JAK inhibitor) for 1 hour. The expression of STAT proteins, pro-interleukin 1 beta (IL-1β pro), alpha smooth muscle actin (α-SMA) and 1 collagen (Col I) was evaluated by western blot. Immuno-precipitation by forming homo and heterodimers of STAT proteins after stimulation with IFN-β was determined.

Results: The activation of the signal transduction pathway JAK/STAT by IFN-β, induced formation of homodimers p-Stat1/p-Stat1 and p-Stat3/p-Stat3-p and heterodimer p-Stat1/p-Stat2. CF stimulation with IFN-β decreased the expression of pro-IL-1β, Col I and α-SMA.

Conclusion: These results demonstrate that IFN-β produces anti-fibrotic and anti-inflammatory effects in CF, therefore, it could reduce injuries and improve cardiac remodeling.

28) **Oil essential from *Cryptocaria alba* and *Peumus boldus* with anti- *Helicobacter pylori* activities**

Bravo, Jessica[1]., Touma, Jorge[1].,Venegas, Alejandro[1].,Navarro, Myriam[1].,Delporte, Carla[2].,Sepulveda , Betsabet[3].,[1]CIB, Medicina, Universidad Diego Portales.[2]Farmacognosia, Química y Farmacia , Universidad de Chile.[3]CEPEDEQ Universidad de Chile .

Resistance of *H. pylori* strains to common antibiotics has been developed in different parts of the world and continues to increase. It is important to investigate the novel and efficient anti-*H. pylori* drugs, among which the plants would be suitable sources.

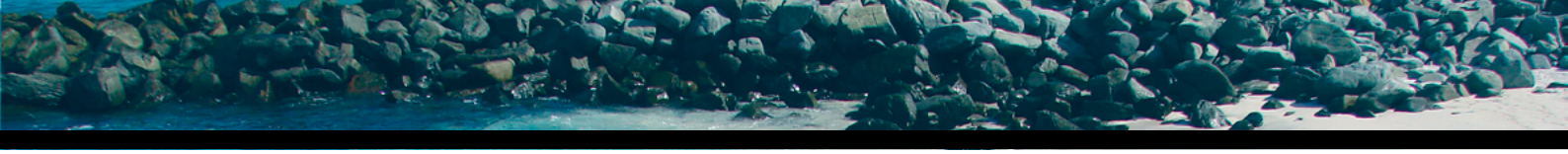
The study aims to the detection, purification and characterization of oils from native vegetable species like Peumo (*Cryptocaria alba*) and Boldo (*Peumus boldus*), in search of an effective antimicrobial activity against *Helicobacter pylori*, the causative bacterial agent of gastritis, ulcers and gastric cancer in humans. The characterization of the oils is to define their structure and eventually their possible mechanisms of action. The contribution of the project is to find natural alternatives to antibiotics and to reduce significantly their use due to the increasing emergence of antibiotic resistant strains. We expect to find compounds that also have a low level of toxicity, therefore making human administration safe and effective.

In this study, we evaluated the antibacterial activity of *Cryptocaria alba* and *Peumus boldus* essential oil against 3 clinical isolates of *Helicobacter pylori* by disc diffusion and agar dilution methods. *Cryptocaria alba* essential oil showed strong antibacterial activity against clinical isolates of *H. pylori* (MIC 0.00124µg/ml). The chemical composition of essential oil was analyzed by GC and GC–MS. a-Terpineol (27.38%), eucalyptoL (23.27%) and fellandrene (16.28%) were the primary constituents of oil. Terpineol, as the first main component, had a significant role in this effect (MIC 0.00093µg/mL).Therefore, *Cryptocaria alba*essential oil can be applied as an alternative agent for treatment of *H. pylori* infections. More studies would be required to better clarify its mechanism of action on *H. pylori*.

Reference

1. Montenegro, G. 2002. Nuestra flora útil. Universidad Católica de Chile. 267 pp.

2. Miyamoto T, Okimoto T, Kuwano M. 2014. Chemical composition of the essential oil of mastic gum and their antibacterial activity against drug-resistant *Helicobacter pylori. Nat Prod Bioprospect.* 4:227–31.



29) **Activation of μU-opioid receptor by Salsolinol, a brain metabolite of alcohol**

Bravo, Alex[1]., Berríos-Cárcamo, Pablo[2].,Herrera-Marschitz, Mario[3].,Quintanilla, María Elena[2].,Rivera-Meza, Mario.,[1]Departamento de Ciencias Farmacéuticas, Facultad de la Salud, Universidad Arturo Prat.[2]Programa de farmacología Molecular y Clínica, Facultad de Medicina, Universidad De Chile.[3]Iniciativa Cientifica Milenio Instituto de Neurociencia Biomédica . (Sponsored by Supported By: FONDECYT #11130241: BNI P09-015-F)

Ethanol is metabolized in the liver by alcohol dehydrogenase (ADH) to acetaldehyde, which is in turn metabolized to acetate by mitochondrial aldehyde dehydrogenase (ALDH). In the brain, there is no expression of ADH, therefore ethanol is metabolized by the action of catalase to acetaldehyde, which is then converted to acetate by ALDH.

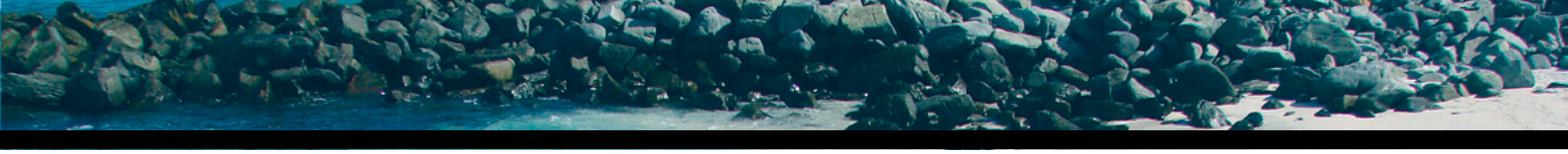
In the brain, ethanol-derived acetaldehyde can condense with dopamine to generate salsolinol. There are reports showing that salsolinol is a reinforcing molecule, since rats self-administer this substance in the ventral tegmental area (VTA), an area of the brain involved in the reward system (pleasure). The VTA is composed by dopaminergic neurons that project to the nucleus accumbens and the prefrontal cortex. VTA dopaminergic neurons are negatively controlled by GABAergic inter-neurons. In vitro studies suggest that salsolinol can inhibit the inhibitory effects of GABA neurons by activating μ-opioid receptors; however, there is no direct evidence of this action.

It has been found that the activation of the μ-opioid receptor by agonist such as morphine or DAMGO can induce a phosphorylation of specific sites in its intracellular domain (e.g., serine 375). These modifications are essential for subsequent receptor desensitization and its recycling into the cell. The objective of this study is to demonstrate the hypothesis that salsolinol is able of acting as an agonist of μ-opioid receptor, through the study of the phosphorylation that this molecule can induce in the μ-opioid receptor. To confirm this hypothesis we propose: (i) to clone the rat gen (cDNA) of the μ-opiod receptor; (ii) to express the rat mu-opioid receptor in HEK-293T cells by transient transfection; (iii) to expose the transfected cells to different concentration of salsolinol and (iv) using a specific antibody for the phosphorylated mu-opioid receptor (S375-P), to determine by western blot if salsolinol is able to act as an agonist of the μ-opioid receptor.

30) **Agonists of HCA2 receptor induce calcium mobilization and increase chemotactic response in bovine neutrophils**

Carretta, Daniella[1]., Hidalgo, Maria[2].,Burgos, Rafael[2].,[1]Laboratory of Inflammation Pharmacology, Institute of Pharmacology and Morphophysiology, Faculty of Veterinary Science, Universidad Austral De Chile.[2]Instituto de Farmacología y Morfofisiología, Facultad de Ciencias Veterinarias , UACH. (Sponsored by Fondecyt 1151035)

In dairy cattle elevated concentrations of the ketone body β-hydroxybutyrate (BHB) during lactation are associated with an increased incidence of inflammatory diseases such as metritis and mastitis, however it remains unclear how BHB could affects the inflammatory response in dairy cows. BHB and the short chain fatty acid butyrate was identified as endogenous ligands of the Hydroxycarboxylic acid receptor 2 (HCA2), a G protein-coupled receptor. HCA2 also is activated by nicotinic acid (NA), a lipidlowering drug, thus this receptor is better known for its antilipolytic effect in adipocytes. However, recent studies suggest that activation of HCA2 can modulate the inflammatory response in human macrophages, monocytes and neutrophils, where this receptor is highly expressed. Neutrophils are undoubtedly the major effectors of acute inflammation and are characterized by the ability to directed migrate to the site of infection or inflammation in a processes called chemotaxis. Because the critical function of neutrophil chemotaxis to many inflammatory diseases in humans as well in cattle the chemoattractants receptors are targets of intense investigation. HCA2 expression and the response of specific ligands on bovine immune cells have not been demonstrated yet. In this work, we have preliminarily observed the mRNA expression of HCA2 receptor in bovine neutrophils. Besides, the treatment with MK-1903, a selective full agonist of HCA2 elicit a transient rise of intracellular Ca^{2+}levels, suggesting that bovine neutrophils express a functional HCA2 receptor. Also, we observed that MK-1903 increase the bovine neutrophil chemotactic response induced by PAF, a potent chemoattractant for bovine neutrophils. So we propose that activation of HCA2 receptor enhance the chemotactic response of bovine neutrophils**.** First we will characterize the pharmacology of this receptor using the endogenous (BHB) and synthetic (NA and MK-1903) agonists. We will estimate de EC50 of each one by their ability to induce Ca^{2+} flux. We will evaluate if HCA2 agonists induce the chemotaxis of bovine neutrophils or increase the chemotactic response elicit by chemoattractants such as PAF. We observed that the addition of BHB, NA and MK-1903 to fura-2-loaded neutrophils led to rapid and transient changes in Ca^{2+} levels that were concentration dependent. Also, we observed that activation of HCA2 receptor, increases bovine neutrophils chemotaxis alone or induced by PAF. In summary, these results will contribute to improve our knowledge about the novel modulatory mechanism of HCA2 in the innate immune system that could be involved in various pathophysiologic situations in cattle.



31) **Alkylhydroxy-benzoate derivatives as new compounds with cytotoxic effect in human colon cancer cells**

Catalán, Mabel[1,2]., Rojas, Diego[2].,Guzmán, Daniela[2].,Jara, José[3].,Castro-Castillo, Vicente[4].,Rebolledo, Solange[4].,Madrid-Rojas , Matías[4].,Pavani, Mario[2].,Ferreira, Jorge[2].,[1]Programa de Farmacología Molecular yClínica, Medicina, Universidad De Chile.[2]Programa de Farmacología Molecular y Clínica, Medicina, Universidad de Chile.[3]Programa de Farmacología y Farmacogenética, Odontología, Universidad De Chile.[4]Química, Ciencias básicas, Universidad Metropolitana De Ciencias De La Educación. (Sponsored by FONDECYT Regular N °1130772 (Ferreira J), Proyecto Inserción En La Academia (Catalán M) N° 791220004)

Cancer cells have interesting features, resistance to cell death, high invasiveness and metastasis, induction of angiogenesis and metabolic reprogramming. Some characteristics are especially relevant in the search of new treatment: i) higher transmembrane potential due to an inner membrane modified composition, highly glycolitic activity and ii) reduced mitochondrial mass. These properties make the mitochondria a convincing pharmacology target in the search and design of new molecules targeting to bioenergetics of cancer cells. Data exist about triphenylphosphonium group application to drive pharmacophore moieties towards mitochondria. Previously, we have used 3,4,5- tri-hydroxybenzoic acid derivatives linked to triphenylphosphonium group as a mitochondriotrophic cytotoxic agent with successful results regarding their cytotoxic activity and selectivity. It was also established that these molecules exercised uncoupling effect. Now, we assess mono- and di-hydroxy benzoates linked by an aliphatic chain of 10 carbon atoms to the triphenylphosphonium group, to thereby establish the cytotoxic activity in relation to the number and position of hydroxyl groups in the benzoic acid ring. We tested bromide salts: 10-((2-hydroxybenzoyl)oxy)decyl)triphenylphosphonium, 10-((2,3-dihydroxybenzoyl)oxy)decyl)triphenylphosphonium and 10-((2,5-dihydroxy benzoyl)oxy)decyl)triphenylphosphonium over percentage survival of HCT-15 and COLO-205 tumor cells lines by MTT assay. Mitochondrial uncoupling effect by polarography, ATP content by luminescence and transmembrane potential by spectrofluorography were also evaluated in intact cells. Thus, the falling of NAD(P)H level by autofluorescence, and induction of apoptotic death through flow cytometry were determined. The results indicated compounds exhibited increased cytotoxicity in both cell line types, establishing IC50 values. The compounds showed uncoupling effect, also they triggered the decrease of both the NAD(P)H level and the transmembrane potential, falling ATP levels, and the induction of apoptosis in both cell types.

32) **Comparative study of Inhibitory activity of the protein tyrosine phosphatase 1B of Ugni molinae leaves genotypes**

Bugueño, Italo[2].,Arancibia-Radich, Jorge[2].,Peña-Cerda, Marcelo[2].,Cortez , Giovanni[2].,Seguel, Ivette[1].,**Delporte, Carla[2]**., [1]Instituto de Investigaciones Agropecuarias, Instituto de Investigaciones Agropecuarias, INIA, Carillanca, Temuco, Chile.[2]Química Farmacológica y Toxicológica, Ciencias Químicas y Farmacéuticas, Universidad De Chile.

Ugni molinae, Myrtaceae, is used against diabetes in folk medicine. In our previous *Ugni molinae* studies with wild murtilla leaves, both ethyl acetate and ethanolic extracts have shown anti-inflammatory and analgesic properties. We have additionally identified triterpenoid acids in murtilla leaves having anti-inflammatory activity, namely, oleanolic-, ursolic-, betulinic-, alphitolic-, corosolic-, maslinic-, asiatic- and madecassic acids. Protein tyrosine phosphatase 1B (PTP1B) is an effective target for the treatment of both type 2 diabetes and obesity.

The aim of this study was to assess comparatively the inhibitory activity of PTP1B of the ethyl acetate and ethanol extracts from leaves of ten genotypes of this species at the single concentration, grown in the same soil and climate conditions and with the same agronomic management.

Methodology: ten *Ugni molinae* leaves genotype cultures from the germplasm bank of INIA de Carillanca. Five genotypes were selected due to their agronomic potential in respect to the production of fruits according to previous studies made by the INIA, and another 5 were selected due to their capacity to produce a large number of leaves.

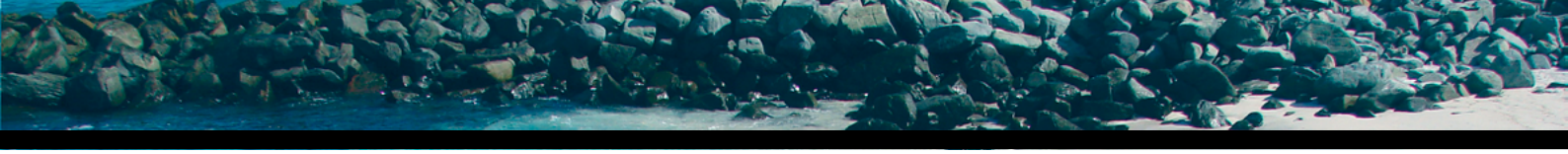
Dried and ground leaves (2 kg) of each genotype were successively extracted by maceration at room temperature with hexane, dichloromethane, ethyl acetate and ethanol (6 L of each solvent); after removing the solvents, the extracts were completely dried at 30°C, to obtain dry extracts (HE, DME, EAE and ETE, respectively)

Protein tyrosine phosphatase 1B (PTP1B) assay. The enzyme activity will be measured

using p-nitro-phenyl phosphate (pNPP) as described Yi-ming Ma et al. (2011). The product, p-nitrophenol will be estimated by measuring the absorbance at 405 nm. The nonenzymatic hydrolysis of 2 mM pNPP will be corrected by measuring the increase in absorbance at 405 nm obtained in the absence of PTP1B enzyme. Sodium orthovanadate was the reference drug. Statistical significance was evaluated using ANOVA followed by Tukey tests.

Results: *Ugni molinae* leaves of different genotypes showed significant differences in their inhibitory activity of PTP1B. The ETEs (1µg/mL) were more active than EAEs (2µg/mL). Both extracts were more active than sodium orthovanadate (2µg/mL).

Acknowledgments: We are grateful to FONDECYT, Chile, Grant N° 1130155, CONICYT, Chile Grants N°21130672 and N°21120377. Thank you to INIA (Carillanca, Temuco) for the genotypes.



33) **New isoxazole compounds: Activity on α7 nicotinic receptors and toxicity in endothelial cells**

Espinoza, Hilda[1]., Sepúlveda , Evelyn [1].,Álvarez, Rocío[1].,Vallejos, Gabriel[2].,Cortés , Magdalena[1].,[1]Pharmacy Faculty Universidad of Valparaiso .[2]Chemistry Institute Austral University of Chile . (Sponsored by DIPUV Project 59/2009.)

Introduction: The isoxazole molecules (ISO) exhibit numerous pharmacological properties, one that has attracted increased interest in recent times; it is its effect on the homomeric α7 nicotinic acetylcholine receptor (α7 nAChR). Recent evidences suggest that α7 nAChR participate in angiogenesis and would be a new endothelial target on therapeutic angiogenesis. Therefore, it's of great interest study new molecules that behave as α7 nAChR agonists. Previous studies have shown that two ISO, ABT-418 and PNU-120596, behave as agonist and positive allosteric modulator of α7 nAChR respectively. In addition to the bioactivity evaluation, always is necessary to rule out the possible cytotoxic effects that would make not viable its subsequent therapeutic use. In this sense, the toxicity assessment of the compound PNU-120596 demonstrated that it doesn't present toxic effects on rat cortical neurons and pheochromocytoma cells of rat adrenal medulla. In the same way, ABT-418 doesn't induced cytotoxicity and even exhibited neuroprotective effects on rat cortical neurons and neuroblastoma cells IMR32. **Objetives:** The objectives of the study were determinate the effects of three ISO, ISO-1, ISO-2 and ISO-3 on the cytosolic Ca^{2+} signal, determining the involvement of α7 nAChR; and evaluate the possible cytotoxic effects of these compounds in three biomarkers on HUVEC. **Methodology:** The effect on $[Ca^{2+}]i$ were assessed using epifluorescence microscopy. The involvement of a7 nAChR was evaluated using with non selective nAChR antagonists, mecamylamine (MECA) and hexamethonium (HEXA), and the selective α7 nAChR antagonist methyllycaconitine (MLA). The possible cytotoxic effects were evaluated using concentrations of ISO from 10^{-9} to $10^{-3,5}$ M and incubation times of 6 and 24 hours on the lysosomal function (NRU assay), mitochondrial activity (MTS assay) and total protein content (SRB assay) in HUVEC. **Results:** The three ISO induced a concentration-dependent increase on the $[Ca^{2+}]i$ in HUVEC. ISO-1 at 10 µM induced the highest increase on the $[Ca^{2+}]i$ (6 times higher than at 100 nM; $p<0,001$). The effect of ISO-1 was significantly inhibited by MLA (10 nM; $p<0,05$) and in 100% by MECA. Meanwhile, the effect of ISO-2 and ISO-3 at 10 and 100 nM weren't inhibited by MLA, however were completely inhibited by HEXA (100 mM). In relation to the cytotoxicity evaluation, no statistically significant toxic effects were found in any of the biomarkers in relation to the negative control in HUVEC. **Conclusions:** Taken together the results indicate that ISO-1, ISO-2 and ISO-3 increase the $[Ca^{2+}]i$ in HUVEC in a concentration-dependent manner and doesn't exhibit cytotoxic effects in HUVEC. According to the results obtained for ISO-1, it would behave as α7 nAChR agonist, and therefore it would be interesting its assessment on the angiogenesis process in HUVEC.

34) **Increased intestinal permeability induced by diet modification: a novel animal model for pharmacological studies of the gut barrier**

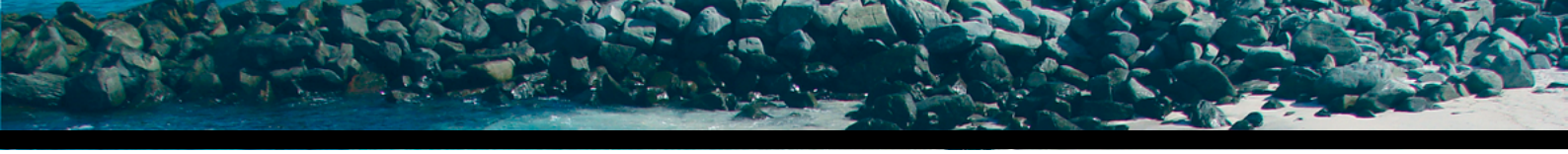
Eyzaguirre-Velásquez, Johana[1]., Olavarría- Ramírez , Loreto[1].,González-Arancibia, Camila[1].,Escobar-Luna, Jorge[1].,Díaz-Merino, Camila[1].,Barrera-Bugueño , Camila[1].,Bravo, Javier[1].,Julio-Pieper, Marcela[1].,[1]Grupo de NeuroGastroBioquímica, Laboratorio de Química Biológica. Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica De Valparaíso. (Sponsored by Funding: PUCV DI 037.470/2015, Fondecyt 1130213)

Evaluating drugs directed to the protection of the intestinal barrier requires suitable animal models. Most of these models involve stress, which may be unfavorable if the drugs under study have different pharmacological targets, being one of them the central nervous system.

The objective of this work was to generate an animal model with increased intestinal permeability, by means of diet modification. A diet low in protein (LP) where 4% of calories come from protein, was applied to 40 day male Sprague Dawley rats. Control animals received a diet where 26% of calories come from protein. The effect of LP diet was assessed at 5, 10 or 20 days, after which plasma samples, colon and ileum were taken to analyze ex vivo: 1) the tissue permeability to 40 and 4.4 kDa fluorescent macromolecules, 2) the transepithelial electrical resistance (TEER) and 3) plasma corticosterone levels as a physiological marker of the stress response.

In the colon and ileum of animals treated for 5, 10 or 20 days with LP diet, the tissue permeability to macromolecules was increased compared to that observed in the controls, although it did not reach statistical significance. LP diet induced a significant decrease in TEER at 10 days only in colon while at 20 days a decrease in TEER was observed in both colon and ileum, suggesting a general increase in gut permeability compared to controls. Finally, corticosterone levels of rats in all treatment groups showed no difference from controls.

The results show that 20 days of LP diet result in increased intestinal permeability without affecting the physiological response to stress in the rat. The animal model proposed allows for the study of drugs at intestinal level, independent of actions in the central nervous system.



35) **Cryopreservation induces alterations in the mitochondrial function of Atlantic salmon spermatozoa (*Salmo salar*).**

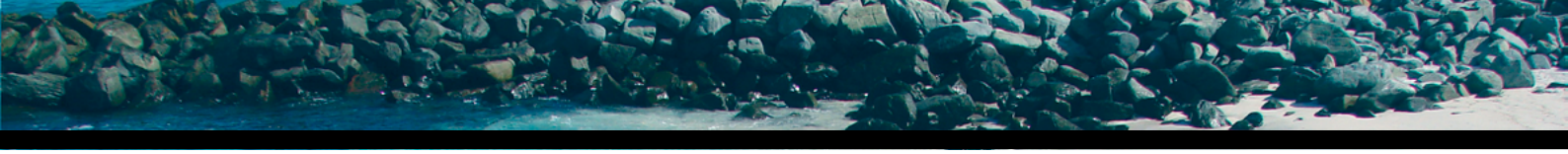
Farias, Jorge[1]., Figueroa, Elias[1].,Valdebenito, Ivan[2].,Risopatron, Jennie[3].,Short, Stefania[4].,Zepeda, Andrea[1].,Figueroa, Carolina[1].,[1]Departamento de Ingeniería Química, Facultad de Ingenieria y Ciencias, Universidad de La Frontera.[2]Escuela de Acuicultura Universidad Catolica de Temuco.[3]BIOREN?Center for Biotechnology in Reproduction Universidad de La Frontera.[4]Departamento de Ingeniería Química, Facultad de Ingenieria y Ciencia, Universidad de La Frontera. (Sponsored by FONDECYT 1151315 (F.JG);, FONDEF D10I1064 (I.V). CONICYT Doctorate Grant (F.E; S.SE))

To date there are few data on the effect of cryopreservation on the mitochondrial dynamic in fish spermatozoa. The objective of this work is to assess the effect of cryopreservation on the mitochondrial dynamic in Atlantic salmon spermatozoa. The sperm were frozen in Cortland® medium + 1.3M DMSO + 0.3M glucose + 2% BSA for the treatment (T); fresh semen was used for the control (C). We determined [ATP] with the CellTiter-Glo® kit and [O_2] with the MitoXpress® Xtra kit. In these analyses we used electron transport chain inhibitors and uncouplers, namely: rotenone (R, 10µM), antimycin A (A, 10 µM), cyanide (C, 0.5 µM) and 2,4 dinitrophenol (D, 10µM). In the cryopreserved spermatozoa (T), the base [ATP] was 5.7±1.2 nmoles/10^9sp presenting significant differences from the control (7.4±0.64 nmoles/10^9sp, $p<0.05$); likewise the cells incubated with R (2.9±0,78 nmoles/10^9sp), A (3.98±0.92 nmoles/10^9sp), C (1.37±0.66 nmoles/10^9sp) and D (1.59±0.48 nmoles/10^9sp) presented statistically significant differences during the first 10 seconds of incubation as compared to the control (5.5±0.84 nmoles/10^9sp; 6.1±0.56 nmoles/10^9sp; 4.1±0.99 nmoles/10^9sp and 4.9±0.79 nmoles/10^9sp respectively, $p<0.05$). The base [O_2] in control spermatozoa was 4230±520 RFU/10^9sp, presenting significant differences from T (3040 RFU/10^9sp); the treatments incubated with R (3508±320 RFU/10^9sp), A (3627±480 RFU/10^9sp) and D (4290±429 RFU/10^9sp) presented significant differences from the control (R: 2704±298 RFU/10^9sp; A:2852±570 RFU/10^9sp and D:3442±612 RFU/10^9sp respectively, $p<0.05$). The changes in the [O_2] rate in spermatozoa in the presence of inhibitors and uncouplers occurred after 10 seconds of incubation. Preliminary results suggest that cryopreservation induces alterations in the mitochondrial function of Atlantic salmon spermatozoa.

36) **Differential effect of NO-aspirin on susceptibility to fluconazole in *C. glabrata* and *C. tropicalis* obtained from denture stomatitis patients**

Fernández, Roberto[1]., Madariaga, Francisco[1].,Urzúa, Blanca[2].,Fernández-Ramires, Ricardo[1].,Jara, José[1].,Herreros, Eduardo[1].,Molina-Berríos, Alfredo[1].,[1]Instituto de Investigación en Ciencias Odontológicas, Laboratorio de Farmacología y Farmacogenética, Facultad de Odontología, Universidad De Chile.[2]Instituto de Investigación en Ciencias Odontológicas, Laboratorio de Biología y Bioquímica Oral, Facultad de Odontología, Universidad De Chile. (Sponsored by Project FONDECYT 11140227, U-inicia 195. Candida Spp Strains Were Kindly Given By Dr. Ximena Lee And Leyla Gomez, Facultad De Odontología, Universidad De Chile.)

Candida spp. biofilms have been pointed as the main cause of denture stomatitis, one of the most frequent conditions in elderly people wearing denture. Although *C. albicans* is the most frequent, it has been described an increase in prevalence of *C. glabrata and C. tropicalis* in human oral flora, and their biofilms are associated with high resistance to conventional antifungal treatment. Because of the high resistance of *Candida spp.* biofilms to antifungals, new pharmacological strategies to treat these infections are needed. Aspirin, an antiinflamatory drug without antimicrobial activity, has shown to inhibit *C. albicans* biofilm formation. On the other hand, nitric oxide (NO) releasing molecules have shown to inhibit *C. albicans* biofilms and to potentiate the effect of conventional antifungals. NO releasing aspirin (NO-ASA) has been proposed for treatment of different cardiovascular affections and inflammatory conditions, since maintain the anti-inflammatory effects of aspirin, besides the beneficial effects of NO such as endothelial protection. According to this, we evaluated the effect of NO-ASA alone and in combination with fluconazole on *C. glabrata and C. tropicalis* clinical isolates obtained from denture stomatitis patients. **Methods:** *Candida spp.* obtained from oral mucosa of denture stomatitis patients (n=60), were identified through CHROMagar *Candida* Medium BD™ and by sequencing the ITS1-5.8S rDNA-ITS2 region using the primers ITS1 and ITS4. To evaluate antifungal susceptibility, isolated strains were standardized to 0.5 McFarland and then grown on sabouraud agar plates and disk diffusion tests were performed in presence or absence of fluconazole (Sigma Aldrich, USA), NO-ASA (Sigma-Aldrich, USA) and their combination. **Results:** from the 60 strains 27% corresponded to *C. tropicalis* (n=16) and remaining 18% was identified as *C. glabrata* (n=18). *C. glabrata* strains were all classified as resistant to fluconazole, with inhibition zone diameters , C. tropicalis strains were all classified as fluconazole susceptible, with inhibition zone diameters ranging from 16 mm to 27 mm. Combination of NO-ASA with fluconazole produced a significant increase of at least 20% of inhibition zone diameters as compared with fluconazole alone ($p<0,05$, ANOVA) for *C. tropicalis* strains. **Conclusion:** Although NO-aspirin has no antifungal effect by itself, it can potentiate the effect of fluconazole in *C. tropicalis* strains. However, this effect appears to be dependent of the characteristics of the strain assayed. We are currently working to elucidate the possible mechanisms associated with these effects.



37) **Synthetic coumarins able to inhibit α-glucosidase and exhibiting antioxidant activity**

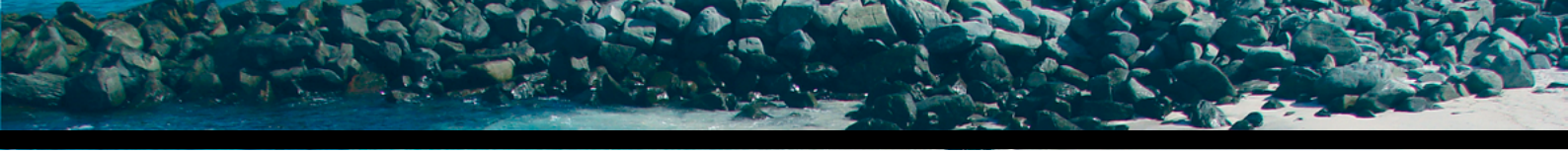
Figueroa, Catalina[1]., Rivera, Constanza[1].,Olea, Claudio[2].,Delporte, Carla[1].,[1]Química Farmacológica y Toxicológica, Ciencias Químicas y Farmacéuticas, Universidad De Chile.[2]Química Inorgánica y Analítica, Ciencias Químicas y Farmacéuticas, Universidad De Chile. (Sponsored by Grant, Beca Doctorado Nacional CONICYT Nº 21141172 And FONDECYT N° 1130155)

INTRODUCTION: The diabetes mellitus type 2 (T2DM) is a metabolic disease that is characterized by a chronic increase of glycemia and oxidative imbalance leading to the appearance of pathophysiological complications. The α-glucosidase is an enzyme that catalyzes the hydrolysis of disaccharides to absorbable monosaccharides, thus its inhibition suppresses the influx of glucose from the intestine to the blood vessels and therefore, is considered as an important target for handling the hyperglycemia linked to T2DM. METHODOLOGY: From 19 synthetic coumarins, a preliminary study of inhibition against α-glucosidase was performed, to a single dose. According to the obtained inhibitory capacity, 10 compounds were selected to determine the inhibitory potency (calculation of half maximal inhibitory concentration [IC50]) and also antioxidant capacity, determined by the assay of oxygen radical absorbance apacity (ORAC-FL). INHIBITION OF α-GLUCOSIDASE: A buffered sodium phosphate solution at pH 6.8, ρ-nitrophenyl-α-D-glucopyranoside (substrate) and the enzyme to 0.1 U / mL, was used. Absorbance at 400 nm was determined by a microplate reader. Acarbose was considered as standard inhibitor (Lordan et al., 2013). ORAC-FL: The fluorescence emitted by fluorescein (FL) was read every 1 min in a microplate reader at an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The reaction was performed in phosphate buffer at pH 7.0 and was added 2,2-azobis (2-methylpropionamidine) dihydrochloride (AAPH) as peroxyl radical source. The standard molecule was 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). The antioxidant capacity was quantified by integrating the area under the curve of FL decay (Perez et al., 2013). RESULTS AND DISCUSSION: From ten coumarins studied, six were able to inhibit α-glucosidase, all of them showing a great inhibitory potency, even higher than the standard inhibitor acarbose. As regards to the antioxidant capacity, no coumarin excelled, giving ORAC indexes lower than those obtained by other studies. However, most of coumarins ORAC indexes presented were higher than trolox index. By analyzing both assays, coumarin 18 highlights, because it has an IC50 of 5.96 g / mL against α-glucosidase and 2.06 ORAC. CONCLUSION: Several coumarins that are able to inhibit α-glucosidase exhibited antioxidant capacity determined by ORAC-FL. This is useful since it could reduce the hyperglycemia associated with DMT2 and secondly, it could reduce oxidative stress that accelerates the progress of the disease. In this regard, coumarin 18 could be considered as a molecule with potential capacity to treat T2DM. Lordan et al. (2013). The α-amylase and α-glucosidase inhibitory effects of Irish seaweed extracts.Food Chemistry.

38) **Protective effect of ascorbic acid on recombinant *Pichia pastoris*.**

Figueroa, Carolina[1,2]., Zepeda, Andrea[1,2].,Figueroa, Elías[1].,Abdalla, Dulcineia[3].,Pessoa, Adalberto[2].,Farías, Jorge[1].,[1]Department of Chemical Engineering, Faculty of Engineering and Sciences, University of La Frontera.[2]Departamento de Tecnologia Bioquímico-Farmacêutica, Faculdade de Ciências Farmacêuticas, Universidade de Sao Paulo.[3]Departamento de Análises Clínicas e Toxicológicas, Faculdade de Ciências Farmacêuticas, Universidade de Sao Paulo. (Sponsored by PhD Scholarship N°21110913, CONICYT. Coordination Of Superior Level Staff Improvement (CAPES, Brazil), National Council For Scientific And Technological Development (CNPq, Brazil) And São Paulo Research Foundation (FAPESP, Brazil).)

Pichia pastoris is a methylotrophic microorganism used as an expression system for the production of heterologous proteins. It uses a methanol inducible-promoter, alcohol oxidase. Methanol oxidation has been related with reactive oxygen species (ROS) generation in yeast peroxisomes and the main ROS generated during methanol oxidation is hydrogen peroxide (H_2O_2). Otherwise the increased culture temperature could also generate higher levels of ROS. The main aim of this work was to expose the recombinant *Pichia pastoris* cells to ascorbic acid and to evaluate the protective effect on ROS generation. The yeast culture conditions were Control: 30°C - 1% (v/v) methanol and AA: 30°C - 1% (v/v) methanol and 6.7 mM ascorbic acid, both cultures were incubated at 250 rpm for 72 hours in shaking incubator. Intracellular H_2O_2 was evaluated using 2´,7´dichlorofluorescein diacetate (DCFH-DA), antioxidant proteins expression were evaluated using indirect immunofluorescence and cell viability using propidium iodide by flow cytometry. Intracellular H_2O_2 in control group at 72 h was higher than 0 h. At the end of incubation an increased protein expression of catalase and glutathione peroxidase was observed in both cultures. However, the superoxide dismutase expression did not change in any culture. Meanwhile at 72 h in the control culture was observed decreased cell viability compared with 0 h. In conclusion these results suggest that ascorbic acid supplementation during induction phase of recombinant *Pichia pastoris* could protect cells and it could prevent oxidative stress-induced cellular senescence.



39) ***Ulva Compressa*, a chlorophyte algae releases extracellular ATP and metabolizes the nucleotide through multiple ATPases**

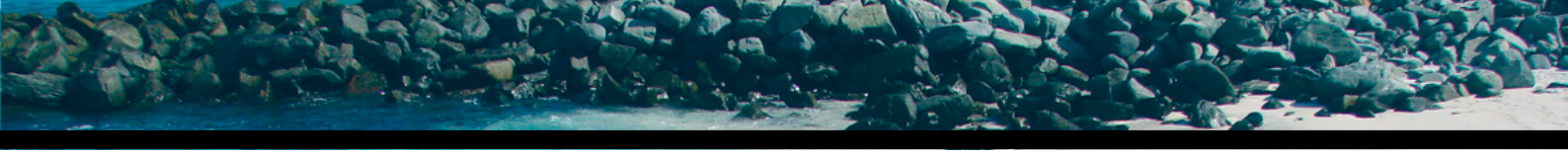
Garcia-Huidobro, J Pablo[1]., Donoso, M Verónica[1].,Gómez, Melissa[1].,Moenne, Alejandra[1].,[1]Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile. (Sponsored by Funded CEDENNA, Fondecyt Grants 1141132, 1130118)

Recent reports indicate that ATP induce coalescence in spores of red algae, suggesting a role of nucleotides as cell messengers in these cells, which appeared almost 1.5 billion years ago. ATP has been recognized as an early signaling molecule present from unicellular to multicellular organisms. It follows that if ATP is relevant as a signaling molecule, there must be a mechanism(s) to turn off the signal. Based on this premise, we hypothesized the presence of ectoATPases in these organisms. To test whether purines are extracellular signals in green algae, we assessed whether fresh cultures of *Ulva compressa* (UC), a common chlorophyte (green algae of the central Chilean coast) release and metabolize extracellular ATP. We assessed ATP/metabolites in UC cultures (1 g of UC in 30 ml sea water). Media samples from controls or UC exposed to extracellular ATP/metabolites were chemically reacted with chloroacetaldehyde to obtain the corresponding etheno-fluorescent derivatives. The purines were separated by HPLC coupled to fluorometric detection. Stirring UC released to the cell media 1.3±0.6 nM ATP (n=4), a value that was reduced by addition of exogenous apyrase to 0.01±0.01 nM (n=4, $p< 0.05$). Samples incubated with 3mM orthovanadate (inhibitor of ectoATPases) tripled sea water ATP to 4.1±0.4 nM (n=3, $p< 0.05$). Application of 1 µM exogenous ATP to UC cultures was rapidly metabolized; 30 and 60-min after exogenous ATP addition, the ATP content in the cell media was reduced to 80.8 ± 2.9 and 96.8 ± 0.9 %, respectively, (n=7). Likewise, exogenous applications of 1 µM ADP, or 1 µM AMP or 1 µM adenosine showed a similar decay, an indication of multiple ecto enzymatic activity. We further demonstrate not only the decrease of the enzyme substarte, but the accumulation of enzyme products, particularly after 1-10 mM orthovanadate addition, a finding consistent with multiple ectoATPase activity. To examine whether ectoATPase is soluble, the UC supernatant was stirred for 30-min in sea water, the supernatant was filtered free of algae and examined for ATPase activity. Results consistently showed that ATP was metabolized by supernant enzymes; this enzyme activity was inhibited by 3 mM orthovanadate. In conclusion, stirring UC results in extracellular ATP release; the signal is rapidly metabolized by multiple soluble ecto-ATPases, suggesting an extracellular role of ATP in this primitive organism. Red, and brown algae will be examined to assay for ATP signaling mechanism(s).

40) **A comparative study of the *in vitro* antioxidant capacity of ethanolic extracts from leaves of different *Ugni molinae* genotypes.**

Pérez-Arancibia, Rodrigo[1]., Valenzuela-Bustamante, Paula[1].,Peña-Cerda, Marcelo[1].,Seguel, Ivette[2].,Delporte, Carla[1].,[1]Departamento de Farmacología y Toxicología, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile.[2]Centro Regional de Investigación Carillanca Instituto de Investigaciones Agropecuarias (INIA). (Sponsored by FONDECYT N°1130155, Beca CONICYT N°21120377, INIA Carillanca, Chile.)

Murtilla (*Ugni molinae* Turcz, Myrtaceae) is a wild shrub native from south-central Chile that contains different polyphenolic compounds on its leaves, including several flavonoids like mirycetin and quercetin glycosylated derivatives. The polar extracts of murtilla's leaves are a significant antioxidant source which could prevent oxidative stress, generated when the balance between free radicals and the endogenous antioxidant systems is lost. Oxidative stress is implicated in the development of different pathologies, contributing to cellular aging, mutagenesis, carcinogenesis, and coronary heart disease through membrane disruption, DNA damage and low density lipoprotein oxidation. Due to the mentioned above and because of the influence of the genotype on the chemical composition, the aim of this work was to comparatively evaluate the antioxidant capacity of 10 serial ethanolic extracts (EET) from murtilla leaves, cultivated under the same conditions by the Instituto de Investigaciones Agropecuarias (INIA) in Carillanca, Temuco, assessing its DPPH and peroxyl (ORAC-FL) radical scavenging capacity. Results showed that the ZF-18 genotype had the lowest EC_{50} on the DPPH assay (9.3 ± 0.6 mg dry EET/L), and the 19-1 genotype had the highest ORAC value (23.8 ± 1.7 µmol Trolox equivalents/mg dry EET). Furthermore, total phenolics were determined by the Folin-Ciocalteu method, expressed as gallic acid equivalents (EAG), being the ZF-18 genotype the one that exhibited the highest phenolic content (260.6 ± 3.7 mg EAG/g dry EET). Finally, quercetin, myricitrin, and gallic acid were identified by HPLC-MS and quantified by HPLC-DAD in every sample. These results suggest that murtilla leaves are a good source of antioxidants and that the different antioxidant capacities of their EETs, mediated by free radical scavenging, are influenced by the differences on polyphenol concentration due to the genotype.



41) **Chronic Ketamine treatment during adolescence induces long-term impairment of prefrontal cortex in adult rats.**

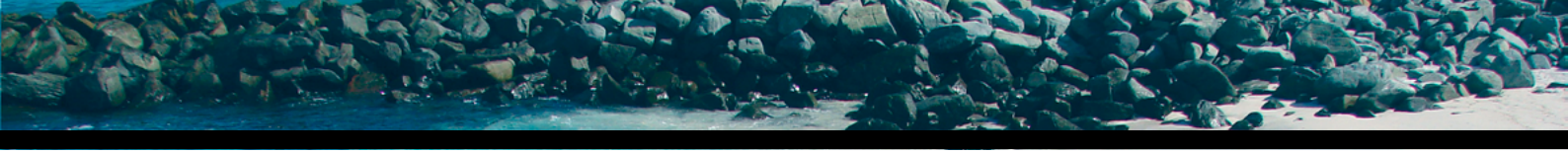
MORALES, CAMILA[1,2,3]., PÉREZ, MIGUEL[1,2,3].,ARRIAGADA, JORGE[1,2,3].,FUENZALIDA, MARCO[3,1,2].,[1]Neural Plasticity Lab Universidad De Valparaíso.[2]Centro de Neurobiología y Plasticidad Cerebral, Instituto de Fisiología Universidad De Valparaíso.[3]Millennium Nucleus in Neuropsychiatric Disorders NU-MIND Universidad De Valparaíso. (Sponsored by This Work Was Supported By Grants From Millennium Nucleus NU-MIND NC-130011 (M.F), FONDECYT 1130614 (M.F.) Master Fellowship CONICYT 20877 (CMM).)

Ketamine (Ket), an NMDA-receptor antagonist, has been widely used as an animal model of schizophrenia (SZ), due its capability to induce molecular, cellular, synaptic and behavioral SZ-like impairment. However, there is only few evidence related to inhibitory synaptic dysfunction and even less about the effect of systemic Ket-treatment over specific brain structures. Using Behavioral tasks and electrophysiological recordings we studied the cognitive performance and GABAergic synaptic transmission of two brain structures involved in SZ, the hippocampus (HPC) and prefrontal cortex (PFC) of adult rats, which were chronically treated (i.p.) with Ket (30 mg/kg) or Vehicle (Veh, NaCl 0.9%) during adolescence. First, we evaluated the executive function through working memory (WM) assays, which differentially assessed HPC and PFC. We applied the spontaneous alternation task in a Y-maze with visual clues to test HPC function, founding no differences between groups. Furthermore, to evaluate PFC dependent WM, we used the delayed non-match to sample task, where Ket-treated rats displayed a worse performance compared to Veh. Interestingly, our behavioral results were according to the intracellular recordings of pyramidal neurons from both structures. We found no differences in the evoked and spontaneous inhibitory post-synaptic currents (eIPSC and sIPSC, respectively) between Ket- and Veh-HPC slices, whereas in PFC the eIPSC and sIPSC were changed. The analysis of paired pulse ratio (PPR) allowed us to recognize two groups of synapses in Ket-PFC slices; a group in which the PPR was reduced compared to Veh, and another group that showed an increase in the PPR. Additionally, we observed that, unlike to mIPSC, the frequency of sIPSC in Ket-PFC slices was diminished. Nevertheless, the amplitude of both, sIPSC and mIPSC was increased. Our results indicate that chronic Ket-treatment in adolescent rats is able to induce specific PFC impairment and suggest that change in PFC inhibitory transmission disrupts the excitatory/inhibitory balance, triggering the cognitive dysfunction.

42) **A failure in ascorbic acid recycling and release from striatal astrocytes is responsible for the metabolic impairment in Huntington's disease.**

Beltran, Felipe[1]., Troncoso-Escudero, Paulina[1].,Valverde-Porras, Naizmi[2].,Rojas, Patricio[3].,Li, Xiao-Jiang[4].,Li, ShiHua[4].,Castro, Maite[1].,[1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile.[2]Biotecnología TEC.[3]Departamento de Biología Universidad De Santiago De Chile.[4]Department of Human Genetics EMORY University.

Introduction: Huntington's disease (HD) is a neurodegenerative disorder characterized by an impairment in glucose metabolism in the basal ganglia and in the cerebral cortex of symptomatic HD patients. When HD animal models become behaviorally active, the levels of ascorbic acid in striatal extracellular fluid is abnormally low in relation with the levels found in their littermate controls. We have demonstrated that intracellular ascorbic acid inhibits glucose transport and stimulates lactate transport in synaptically active neurons. In this work, we studied the ability of ascorbic acid to modulate neuronal glucose consumption in YAC128 mice. Additionally, we evaluated the ability of striatal astrocytes of YAC128 mice to recycle and release ascorbic acid. Finally, to evaluate the contribution of astrocytes to the impairment in ascorbic acid metabolic modulation, we used transgenic mice that express N-terminal mutant huntingtin only in astrocytes.**Materials and Methods:** Synaptic activity was measured as recordings from striatal medium spiny neurons. Excitatory postsynaptic potentials (EPSPs) were evoked by stimulating the cortico-striatal pathway. Using qPCR and Western blot analyses, we explored protein and mRNA levels of proteins involved in ascorbic acid recycling in astrocytes. Ascorbic acid release and recycling was evaluated by HPLC. **Results:** Ascorbic acid was not able to modulate the ability of glucose to function as an energetic fuel sustaining glutamatergic synaptic activity in presymptomatic YAC128 mice. We observed a failure in ascorbic acid recycling and release from striatal astrocytes from YAC128 mice. Finally, similar results were observed in mice that express mutant huntingtin only astrocytes.**Discussion:** Abnormalities observed in the ascorbic acid-dependent modulation of neuronal metabolism of presymptomatic HD mice, suggests that ascorbic acid homeostasis failure could be important in the progression of HD. Our findings demonstrate that astrocyte mutant huntingtin can contribute to the neuronal metabolic failure and glial function could be an effective route to therapies for HD. FONDECYT 1151206; FONDECYT 1110571; MECESUP AUS1204; Beca CONICYT Doctorado; Gastos Operacionales CONICYT; DID-UACh and CISNE-UACh.



43) **Iron Regulatory Protein 1 (IRP1) dysregulation mediates neuroblastoma cell death induced by mitochondrial complex I inhibition**

Aguirre, Pabla[1,2]., Urrutia, Pamela[1,2].,Mena, Natalia[1].,Tapia, Victoria[1,2].,Esparza, Andrés[1].,Núñez, Marco[1,2].,[1]Biología, Facultad de Ciencias, Universidad de Chile.[2]Biología, Facultad de Ciencias, Research Ring on Oxidative Stress in the Nervous System.

Inhibition of mitochondrial complex I results in decreased iron-sulfur cluster (ISC) synthesis, which may lead to activation of IRP1, a key regulator of cellular iron homeostasis. This process may be relevant for the understanding of Parkinson's disease (PD) neuropathology, where mitochondrial dysfunction, iron accumulation and oxidative stress are pathognomonic signs. Here, we report the effects of mitochondrial dysfunction on IRP1 activity and iron homeostasis, and its role in the death of SH-SY5Y dopaminergic neuroblastoma cells. Mitochondrial complex I was inhibited with rotenone and iron dyshomeostasis was evaluated by changes in Transferrin Receptor 1 (TfR1), Divalent Metal Transporter 1 (DMT1), Ferroportin 1 (FPN1), ferritin, ^{55}Fe uptake and oxidative modification of proteins. Resistance to apoptotic cell death induced by oxidative insults and Complex I inhibition was evaluated in IRP1 knockdown cell lines. Complex I inhibition associated with increased IRP1 IRE binding activity, increased levels of TfR1 and DMT1, and decreased levels of FPN1, together with increased ^{55}Fe uptake activity. Silencing of IRP1 abolished the rotenone-induced increase in iron uptake activity and protected cells from death induced by complex I inhibition. IRP1 knockdown cells demonstrated increased resistance to cysteine oxidation and decreased loss of cell viability induced by oxidative stimuli. These results support the idea that IRP1 is an oxidative stress biosensor that when deregulated by mitochondrial dysfunction mediates iron accumulation and cell death. IRP1 activation, secondary to inhibition of ISC synthesis, may underline the events leading to the accumulation of iron observed in PD.

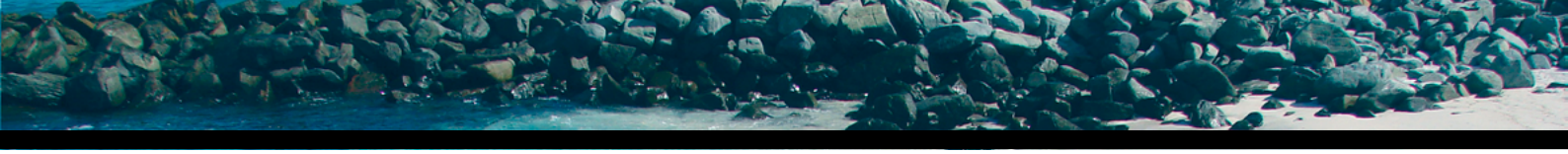
Financed by PIA grant ACT1114 and FONDECYT grant 1130068.

44) **CHANGES ON PURINERGIC RECEPTOR P2X2 AND PROTEIN FE65 EXPRESSION, AND ITS EFFECT ON MITOCHONDRIAL FUNCTIONS**

Barra, Karen[1]., Silva-Grecchi, Tiare[2].,Godoy, Pamela[1].,Celis, Teresa[1].,Panes, Yessica[1].,Fuentealba, Jorge[2].,[1]Fisiología, Ciencias Biológicas , Universidad De Concepción.[2]Fisiología Universidad De Concepción. (Sponsored by This Work Has Been Funded By The 1130747 FONDECYT Project)

Alzheimer's Disease (AD) is a neurodegenerative disorder and there are different hypothesis for explain its pathogenic mechanism. The amyloidogenic theory has been deeply studied, and place the Amyloid-β peptide (Aβ) as one of most important responsible of AD, that is generated from the proteolitic processing of the Amyloid precursor protein (APP); which also generates the Amyloid intracellular domain (AICD). The Aβ peptide has many toxic effects such as the alteration of function and expression of different proteins and dishomeostasis of intracellular Ca^{+2}, which alters the mitochondrial activity and finally produces the neuronal death. Additionally, changes on the purinergic receptors P2X (P2XR) and Fe65 (a multidomain adaptor protein) levels have been reported by us and others. In our group, we have observed that treatments with Aβ increase the P2X2 expression, which could suggest a higher interaction with Fe65 that could affect the function or availability of this protein. On the other side, the Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) expression, key molecule in the cellular energy metabolism and mitochondrial biogenesis, is regulated by different signals as the interaction between Fe65 and AICD, since these proteins form a complex which activates this cofactor expression. Meanwhile, PGC-1α interacts with varied factors, which allow the transcription of several genes that participates in the mitochondrial biogenesis.

The aim of this work was to evaluate the changes on P2X2R and Fe65 levels in mouse hippocampal slices, after acute and subchronic treatments (1 and 5 hours respectively) with Aβ peptide. Using immunohistochemistry techniques we have observed changes in immunoreactivity and distribution of these proteins, shown variations depending on the zone that was evaluated (dentate gyrus, CA1 and CA3 hippocampal zones) and these changes shown to be dependent of treatment time. Besides, we studied changes on PGC-1α and observed a decrease on its immunoreactivity. These results suggest that a higher interaction between P2X2R and Fe65 could be playing a key role in the expression of the coactivator PGC-1α; furthermore, they could participate in the potentiation of Aβ toxicity inducing deep alterations on mitochondrial metabolism and biogenesis.



45) **Characterization of neuroprotective peptidomimetics based on the C terminal region of the β amyloid peptide (Aβ)**

Bascuñán, D[1]., Peters, Christian[1].,Perez, Nelson [1].,Burgos, Felipe[1].,Aguayo, Luis[1].,[1]Departamento de Fisiología, Ciencias Biológicas, Universidad De Concepción. (Sponsored by Work Supported By Fondecyt Grant 1140473.)

Alzheimer´s disease (AD) is a neurodegenerative disorder of high prevalence that mainly affects elderly individuals. The principal neurotoxic agent corresponds to oligomers of the β amyloid peptide (Aβ) that associate to the neuronal membrane and form "pore-like" structures. These membrane perforations alter calcium homeostasis and ultimately lead to synaptic failure. Current therapies are not effective at curing or deterring disease progression, thus further research is needed to find more effective therapeutic targets to revert AD. We recently demonstrated the importance of the C terminal region of Aβ in the association and perforation of the neuronal membrane. Specifically, we blocked Aβ aggregation, association, membrane insertion, and synaptotoxicity with the pentapeptide $G_{33}LMVG_{37}$ derived from the Aβ peptide. We are currently examining a library of synthetic molecules as the basis for *in silico* screening in order to find small peptidomimetic molecules that have similar neuroprotective effects, but with higher pharmacological potential. A group of molecules having a higher interaction with Ab were selected and analyzed for their effects on Ab aggregation, association, mitochondrial function and membrane perforation in PC12 cells and/or rat hippocampal neurons. The results showed that a benzopirolic derivative at micromolar concentrations was able to inhibit Ab aggregation (70±5% of control) and subsequent membrane association (55±3% of control) without producing an intrinsic toxic effect (97±3% of control). These preliminary results indicate that it is possible to develop peptidomimetic molecules that exert protective actions against Ab similar to the those observed with the $G_{33}LMVG_{37}$ pentapeptide.

46) **Ventilatory arrest is the primary event that leads to sudden death after heat-induced seizures in a Dravet mouse model.**

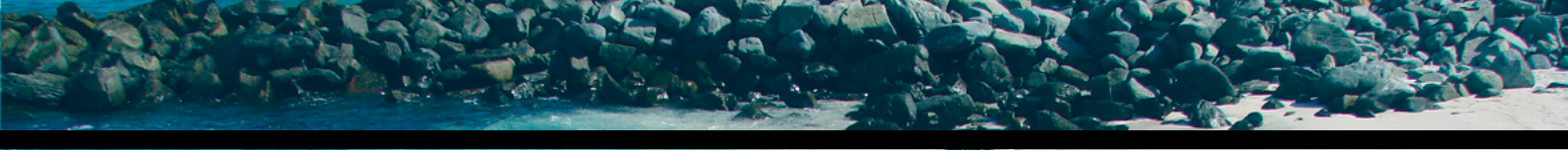
Bravo, E[1]., YuJaung, Kim[1].,Richerson, George[1].,[1]Neurology University of Iowa.

In patients with Dravet Syndrome (DS) and intractable epilepsy, Sudden Unexpected Death in Epilepsy (SUDEP) is the most common cause of death. Compared to the general population, DS patients are up to 40 times more likely to die from premature death. Several types of postictal arrhythmia have been associated with seizures, leading some investigators to conclude that cardiac arrest is the principal cause of SUDEP. The recent MORTality in Epilepsy Monitoring Unit Study (MORTEMUS) reported the largest series of SUDEP cases in epilepsy monitoring units (EMUs), and included video, EEG, and EKG. Respiratory activity was assessed indirectly by observing the video recording, but ventilation was not measured directly. In a mouse model of DS (Kalume et al, J Clin Invest, 2013), heat induced seizures are followed by progressive bradycardia and death, which has been proposed to reproduce the events that occur in human DS patients with SUDEP. However, postictal breathing has not been measured in these mice. Here we studied a mouse model of DS to determine the relationship between cardiac activity, respiratory output and death after heat-induced seizures.

A mouse EMU was used to continuously record EEG, EMG, EKG, whole-body plethysmography (breathing), body temperature, room temperature, humidity and video. Heterozygous mice with a knockin mutation (R1407X) of SCN1A (*Scn1a*$^{R1407X/+}$) and wild-type (WT) littermates on a C3H background were bred and genotyped as previously described (Auerbach et al, PLoS ONE, 2013). WT and *Scn1a*$^{R1407X/+}$ mice were exposed to a heat lamp to cause a continuous increase in body temperature from 37°C to 43°C. For those mice that did not die on the first trial, a second trial was performed two days later.

In response to an increase in body temperature to 43°C, 100% of *Scn1a*$^{R1407X/+}$ mice had at least one seizure on the first trial, with 84% of these mice having at least one convulsive seizure. 78% of *Scn1a*$^{R1407X/+}$ mice died, and this always occurred after a convulsive seizure. Two days later, a second trial induced death in 100% of the remaining *Scn1a*$^{R1407X/+}$ mice. In contrast, none of the WT mice had any seizures and 100% survived the two heating trials. In *Scn1a*$^{R1407X/+}$ mice, when death occurred the first abnormality was always complete cessation of all respiratory effort that did not return. Heart rate remained normal for 20 seconds, and then subsequently began to decrease progressively over the next 3 minutes until asystole occurred.

These results indicate that postictal death in *Scn1a*$^{R1407X/+}$ mice is due to respiratory arrest, and that the subsequent bradycardia is likely secondary to an increase in parasympathetic output due to hypoxia.



47) **The transcription factor Nuclear receptor related 1 (Nurr1) is down-regulated by iron and mitochondrial complex I inhibition.**

Carrasco, Carlos M.[2,1]., Aguirre, Pabla[2,1].,Gonzalez-Billault, Christian[1,2].,Nuñez, Marco[1,2].,[1]Santiago, Chile Research Ring on Oxidative Stress in the Nervous System.[2]Biology, Science, Universidad De Chile.

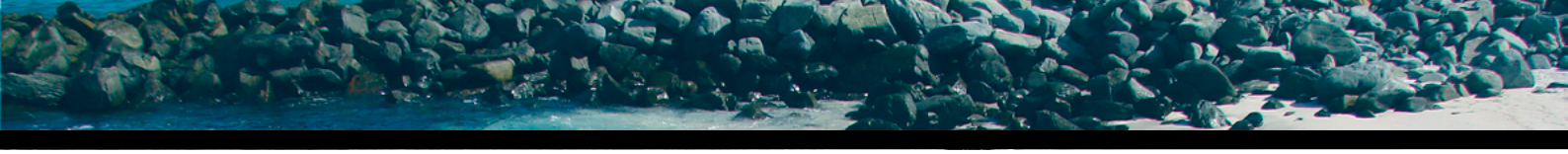
Parkinson´s disease (PD) is characterized by the loss of motor control as a consequence of denervation in the striatum of dopaminergic neurons of the *substantia nigra pars compacta*. Hallmark of PD that leads to neurodegeneration are inflammation, elevated iron levels and oxidative stress. Recent data from our laboratory show that treatments with either iron chelators or antioxidants protect dopaminergic neurons from MPTP-induced degeneration, both in vitro and in vivo, suggesting that reactive oxygen species (ROS) resulting from iron accumulation are critical in this degenerative process. Nurr1, a transcription factor involved in development and maintenance of midbrain dopaminergic neurons, decrease its activity under oxidative conditions. In this work, we studied the interplay between iron and inhibition of mitochondrial activity (1-methyl-4-phenylpyridinium (MPP+)) in determining Nurr1 transcriptional activity. We used mesencephalic cells in culture and SH-SY5Y cells. We tested the effect of iron,MPP+, rotenone and iron chelators on i) dopaminergic neuron death, ii) the nucleus/cytoplasm distribution of Nurr1 and iii) Nurr1 transcriptional activity. In addition, we analyzed the mRNA expression of proto-oncogene RET, a transcriptional target of Nurr1, upon treatment with MPP+ or iron, in the absence or presence of iron chelators M30, DFO, or the novel chelator Q11. We found that co-treatment with chelators protected dopaminergic neurons from MPP+-induced death. Treatments with iron or MPP+ resulted in decreased levels of Nurr1 in the nuclei and decreased RET expression. Furthermore, iron chelation, both as a pre- or as a post-treatment, reverted RET expression to control levels. We conclude that in the MPP+ PD model, iron accumulation is crucial for MPP+-induced death, and that Nurr1 exclusion from nuclei could be a downstream consequence of iron accumulation.

Financed by PIA grant ACT1114, FONDECYT grant 1130068 and CONICYT Fellowship 21130574.

48) **Amyloid-β peptide induces an increase in P2X2 receptor levels and changes mitochondrial dynamic-related protein expression."**

Celis, Teresa[1]., Barra, Karen[1].,Godoy, Pamela[1].,Panes, Jessica[1].,Fuentealba, Jorge[1].,[1]Physiology, Biological Sciences, Universidad De Concepción. (Sponsored by This Work Has Been Funded By FONDECYT Project 1130747)

Mitochondrial dysfunction has been associated with neurodegenerative disorders, such as Alzheimer's disease (AD). This highly dynamic organelle controls several cellular processes that involve energetic supply, metabolic pathway regulation and intracellular calcium signaling, entailing grand repercussions on neurons. All of these features rely on fission and fusion forces that control shape, size, distribution and maintenance of this organelle. Fission and fusion balance, is mediated by specific GTPases, like Drp1 (dynamin-related protein) which controls fission events, inducing mitochondrial fragmentation via outer membrane constriction. Disturbed mitochondrial dynamics has been observed on AD brains, where increased intracellular concentration of Amyloid β peptide (Aβ) appears to be responsible of fission and fusion imbalance. On the other hand, amyloid intracellular domain (AICD) generated from amyloid precursor protein (APP) proteolytic processing (along with Aβ) displays a nucleus translocation along with Fe65, a multidomain adaptor protein, enhancing Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) expression, a renowned mitochondrial transcriptional coactivator. Fe65 interacts with the purinergic receptor P2X2 whose expression after Aβ treatments increases according to previous results from our work; nonetheless, Fe65 expression remains stable but decreases its presence in the nucleus. The aim of this work was to study mitochondrial dynamics related to Aβ toxicity and P2X2R overexpression, through PGC1-α and Drp1 expression quantification on PC12 cells employing western blot and immunofluorescence techniques. Immunoreactivity for PGC1-α showed decreased expression levels after chronical treatments with Aβ (0,5μM), PPADS (10μM) -a P2X receptor antagonist- and PPADS+Aβ (C: 100 ± 4%; Aβ: 57 ± 7%; PPADS+Aβ: 47 ± 5%). Additionally, we observed a significant decrease on Drp1 expression after P2X2R transfection and Aβ treatments; furthermore, simultaneous conditions revealed additive effects (C:100%; Aβ:76 ± 9%; P2X2: 85 ± 5%; Aβ+P2X2: 46 ±11%). Taken together, these results suggest that enhanced P2X2R expression could be sequestrating Fe65 protein, reducing its availability to interact with AICD along with their capability to modulate PGC1-α expression levels, entailing mitochondrial consequences, such as mitochondrial dynamics perturbation on a calcium-independent mechanism. This observation represents a potential target for new biomarker development.



49) **Study of locomotor activity in *Octodon degus*: a potential natural model for neurodegeneration Parkinson type**

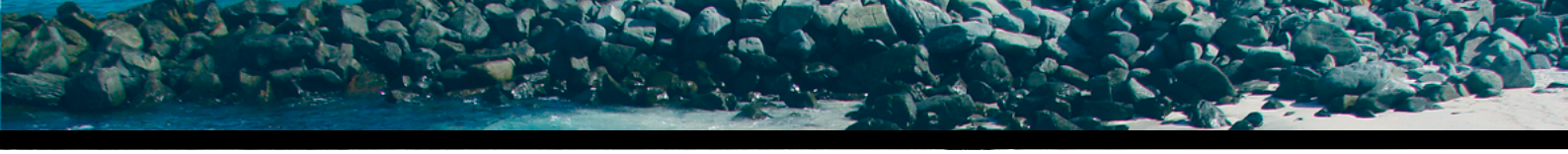
Gajardo, Ivana[1]., Palacios, Adrián [1].,[1]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad De Valparaíso. (Sponsored by Millennium Institute ICM-P09-022-F)

We have recently shown that *Octodon degus* develops during aging the main signs of a neurodegeneration Alzheimer disease type (degu AD), including the accumulation of Aβ (amyloid) soluble and phosphorylated Tau protein; decreased of postsynaptic proteins, as well reduced spatial memory and object recognition, and a decrease in locomotors activity. Parkinson's disease (PD) represents a progressive neurological disorder with loss of dopaminergic neurons (substantia nigra), leading to a reduction in dopamine, which is related to the onset of motor rigidity, tremor, slowness or decreased movement. We have previously shown in degu a decrease of locomotor exploration during aging and prompt us to ask if degu would be a potential animal model to study PD. In a large group of degus we first characterized the locomotor activity through the open field test (OF). Our preliminary results show, independent of ages, that individual degus show high and lower locomotor activity. From this, we evaluated the effect of levodopa administration on locomotor activity, in two experiments, to different concentrations of the drug and to different time's post-injection levodopa through of the test OF. Results of both experiments show that old animals with low locomotor activity have a positive response to the drug increasing its activity, unlike the other animals. Based on our results we can predict that the decrease of locomotor activity of some old animals may be due to a deficit of the nigrostriatal dopaminergic pathway.

50) **Single unit activity in the dorsolateral striatum of amphetamine treated rats: preliminary results**

Gatica, R.I.[1]., Aguilar-Rivera, M.I.[2].,Fuentealba, J.A.[1].,[1]Laboratorio de Neuroquímica, Departamento de Farmacia, Facultad de Química, Pontificia Universidad Católica De Chile.[2]Department of Bioengineering University of California, San Diego. (Sponsored by Sponsored By FONDECYT N° 1141088)

Drug addiction is a chronic disease associated with physiological, cognitive and behavioral impairments. The transition from voluntary drug use to a compulsive habit by repetitive drug intake characterizes the developed of this pathology. The dorsolateral striatum (DLS) has a key role in the habits learning. It has been reported that the increase of dopamine levels in the DLS, after psychostimulants administration, is correlated with the establishment of habitual drug seeking. However, data with regard changes in the electrophysiological properties of DLS neurons, after psychostimulant administration, are still lacking. In this work, we studied the effect of repeated amphetamine (AMPH) administration on the firing rate of DLS single units. Rats received repeated injections of AMPH (1.0 mg/kg) before the single units recording experiment. An array of eight tetrodes was lowered to the DLS in urethane-anesthetized rats. Neural activity was recorded using the following protocol: 20 min of basal activity, 20 min after saline injection and 40 min after an acute injection of AMPH (1.0 mg/kg). Preliminary result shows a non-significant decrease of basal firing rate in AMPH group relative to control group (0.088 + 0.04486 Hz AMPH group v/s 0.479 + 0.195 Hz control group). We observed a trend in the DLS neurons of AMPH treated rats to increase their firing rate in response to an acute dose of AMPH in comparison to control rats (33% v/s 17% respectively). These preliminary data suggest that an acute exposure of AMPH would change DLS neural activity.



51) **Use of deep transcranial magnetic stimulation (deep TMS) as add-on treatment for Parkinson's disease.**

Linsambarth, Sergio[1]., Villalon, Esteban[2].,Moraga-Amaro, Rodrigo[1].,Zangen, Abraham[3].,Poblete, Patricio[3].,Stehberg, Jimmy[1].,[1]Laboratorio Neurobiologia Universidad Andrés Bello.[2]Neuroclinic NeuroMagnetics.[3]Neuroscience Laboratory University of the Negev.

The standard pharmacological treatment for Parkinson's disease (PD) is the administration of levodopa, which is very effective, but requires progressive increments in the dosage leading to concomitant increases in adverse effects. The development of novel therapies that can improve PD symptoms is critical. The non-invasive repetitive transcranial magnetic stimulation (rTMS) has been proposed as an add-on for the treatment of Parkinson´s disease. Recently, a special coil (Deep TMS or dTMS) capable of stimulating deeper brain areas including the complete cortical thickness has been developed. The objective of the present study is to evaluate the safety and efficacy of combined 1Hz primary motor cortex and 10Hz prefrontal cortex stimulation using the dTMS H-coil as an add-on treatment for PD. 45 patients were treated with dTMS and showed significant improvements in UPDRS, gait speed, depressive symptoms, balance, autonomic symptoms and a 73% increase in daily ON time in response to levodopa. In the cohort, dTMS was well tolerated with only minor adverse effects. The dTMS induced significant improvements in motor, postural, and motivational symptoms of PD patients and may potentiate concurrent levodopa treatment, demonstrate that dTMS may have a much wider spectrum of beneficial effects than previously reported for TMS. The present results suggest that future clinical trials with dTMS should include a broader range of symptom measurements.

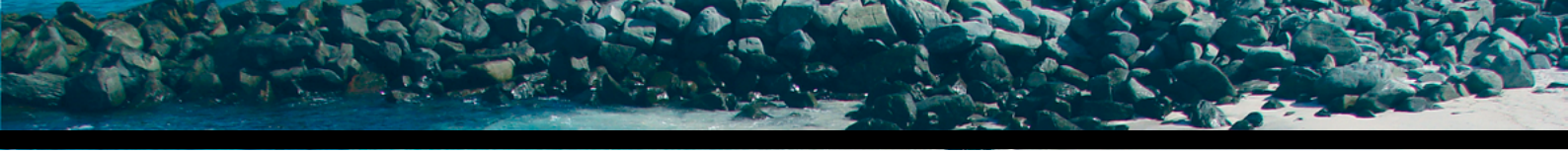
52) **Role of $Ca_V1.2$ calcium channel as gene regulation in a depression-like model**

Moreno, C[1,2]., Hardy, Paulina [1].,Hermosilla, Tamara [2].,Varela, Diego[2].,Rojas , Patricio[2].,[1]Biología, Química y Biología, Universidad De Santiago De Chile.[2]Instituto Ciencias Biomédicas, Medicina, Universidad De Chile. (Sponsored by Fondecyt Regular 1130904 And 1120240; Beca Doctorado Nacional 21130549.)

Mayor depression has a high prevalence and is serious public health problem in most countries. The study of depression to a biological level has elucidated partially the development of this condition. Previous studies demonstrate that neurons from depressive patients show a reduction in dendritic arborization and number of dendritic spines, however, the mechanisms, at the molecular level, responsible for these changes are still to be defined. $Ca_V1.2$ calcium channels are the principal pathway for calcium influx in neuronal soma, and have been associated to several cellular processes such as changes in neuronal morphology, activation of Calmodulin, nuclear translocation of Nuclear Factor Activated cellules T (NFAT) and nuclear translocation of C-terminus region of the $Ca_V1.2$. These last three processes involve changes in gene expression.

Our working hypothesis is that changes in morphology and function in hippocampal neurons from animal models of depression are due to regulation in gene expression dependent on signaling through $Ca_V1.2$. In order to test this hypothesis we use chronic restraint stress to generate a model of chronic depression. Behavioral parameters such as anhedonia, changes in social interaction and behavioral despair, demonstrate that these animals share characteristic of mayor depression, as has been reported for this model previously. By using electrophysiological and molecular biology techniques, we are currently studying changes in $Ca_V1.2$ calcium channel expression and subcellular localization that could be related to the described pathways that modulate gene expression.

If our hypothesis is true, we would be able to establish that $Ca_V1.2$ is a key player in the development of depression.



53) **Evaluation of Obsessive Compulsive Disorder- related behaviors in a mouse model with altered Eaat3 expression in GABAergic neurons**

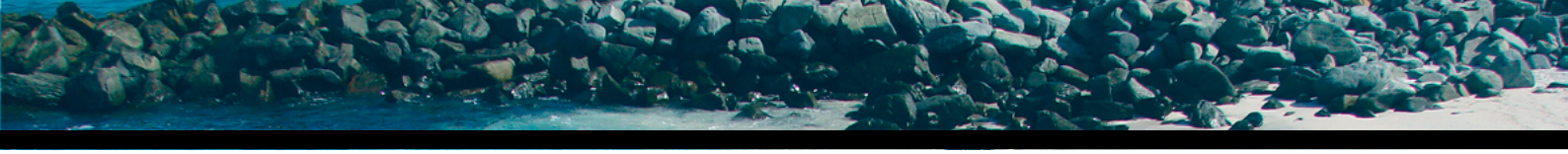
Delgado, Claudia[3]., Martinez, Jonathan[3].,Henriquez, Francisca[3,1].,Gonzalez, Luis[2].,Moya, Pablo[3].,[1]Bioquimica Universidad Catolica de Valparaiso.[2]Quimica y Farmacia, Farmacia, Universidad De Valparaíso.[3]Fisiologia, Ciencias, Universidad De Valparaíso.
(Sponsored by This Work Was Funded By Millennium Nucleus NU-MIND NC 130011 (PRM) And FONDECYT Grants 1141272 (PRM))

Obsessive-Compulsive Disorder (OCD) is a neuropsychiatric disorder characterized by persistent, intrusive thoughts (obsessions) and repetitive, intentional behaviors (compulsions). Extensive evidence suggests altered glutamatergic synapses in cortico-striato-thalamo-cortical (CSTC) circuitry. Glutamatergic system genes emerge then as good candidates for studies in OCD, particularly the *SLC1A1* gene encoding EAAT3, the neuronal glutamate transporter. EAAT3 is expressed in soma and dendrites of glutamatergic and GABergic neurons, and prominently in the CSTC circuit. EAAT3 plays multiple roles in regulating neuronal function, removes glutamate from the extracellular space limiting the activation of extrasynaptic neurotransmitter receptors and consequent excitotoxicity, and regulates cysteine transport -an essential rate-limiting step in the production of the endogenous antioxidant glutathione. Moreover, when present in GABAergic neurons, EAAT3 provides the precursor for the synthesis of GABA. Therefore, it is of great interest to find out whether altered EAAT3 expression is related to OCD-like behaviors. Thus, restricted EAAT3 expression manipulations in specific neuronal types, particularly in GABAergic neurons, are needed to evaluate the potential role of this transporter. To accomplish this aim, conditional Knock out (KO) and conditional overexpressing (OE) mice were generated, aiming to manipulate Eaat3 in a cell-specific manner using the Cre/loxP system. Here, we describe the current status of validation and characterization of GAD65-Cre-mediated mouse models with altered Eaat3 expression. Eaat3-OE mated with GAD65-Cre mice were generated and genotyped by PCR. Anxiety and OCD-related behaviors are currently underway using several paradigms including open field test, elevated plus maze, and marble burying tests. We expect this study will contribute to help understand the potential role of Eaat3 in neuropsychiatric disorders and particularly in OCD.

54) *** Strategies to identify neuroprotective molecules in the diet: From genome to behavior.**

Prado, L[1]., [1]Centro de Genómica, Facultad de CIencias, Universidad mayor.

Diet impacts the physiology of organisms at molecular, metabolic and systemic levels. However, the individual components of food that determine individual changes in phenotype and behavior are unknown. The bacterivore nematode C. elegans is an excellent model to study the effect of diet on phenotype and behavior because both the animal and its food are genetically tractable. We have previously shown that different types of bacterial diet change the degeneration rate of mechanosensory neurons that have been triggered to die by the constitutive expression of the degenerins MEC-4d and DEG-1. Animals feeding on E. coli OP50 degenerate their neurons in 72 hours, from birth to adulthood. However, when animals feed on E. coli HT115, neurons are largely protected even three days after adulthood. To dissect the individual components of the protective diet, we first sequenced the genomes and transcriptomes of both E. coli strains and generated a list of unique and differentially expressed genes. Subsequently we generated bacterial vectors expressing the unique genes from E. coli HT115 under inducible promoters and introduced them into E. coli OP50. Finally E. coli OP50 expressing unique genes from E. coli HT115 is fed to mec-4d animals and three days later, the morphology of the neurons is tested under a fluorescent microscope and a functional behavioral test is performed to test the integrity of the touch circuit. This work establishes a powerful strategy to discover the contribution of individual components of the bacterial diet to neuroprotection, by functional supplementation of each gene product.



55) **Role of chloride co-transporters in animal models of Schizophrenia**

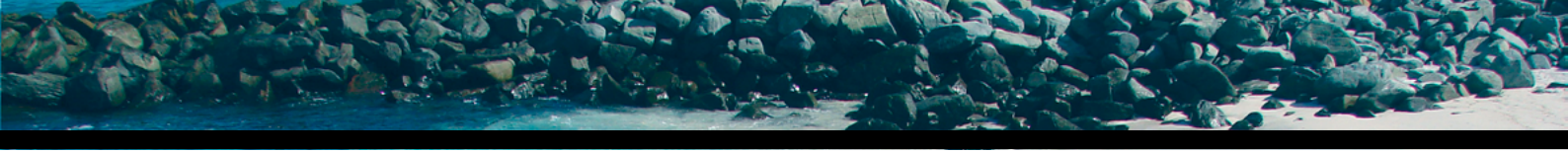
Lorca, Enrique[1]., Lara, Marcelo[1].,Rojas, Patricio[1].,[1]Biología, Química y Biología, Universidad De Santiago De Chile.

Schizophrenia is a neuropsychiatric disease that primarily affects the social and cognitive characteristics of the subject through an altered perception of the reality and a disruption of formal thought. The main hypothesis about the cause of the disease is an alteration of dopamine neurotransmission. However, several studies have shown that the neurotransmitter GABA would be directly involved in the disease, resulting in a diminution of inhibition. Recently, it has been shown that chloride co-transporters NKCC1 and KCC2, which regulate the intracellular concentration of this anion, and thus the excitation and inhibition balance, exhibit altered expression. The aim of this work is to study the role of these co-transporters in the hippocampal circuits, one of the areas of the brain affected by the disease. We studied the changes in excitability of the hippocampal circuits in two animal models of schizophrenia, Ketamine administration and maternal deprivation. We demonstrated that there is a decrease in the glutamatergic excitability in the CA3-CA1 circuit and an increase in dentate gyrus. Otherwise, the GABAergic response was only found altered in dentate gyrus in both models. Pharmacologically block of NKCC1 with its selective inhibitor Bumetanide, produces a decrease in GABAergic response only in deprivation model. On the other hand, after the Bumetanide administration in ketamine animal model and subsequent behavioral studies, a decrease was observed in the positive symptoms of the disease, so this drug could be a potential antipsychotic. We can conclude that NKCC1 function is altered in one model of schizophrenia, being responsible of changes in GABAergic response.

56) **Identity and Characteristics of Nitric Oxide synthesizing Bipolar Cells in the Retina**

Agurto, Adolfo[1]., Vielma, Alex[1].,Schmachtenberg, Oliver[1].,[1]Neuroscience, Science, Universidad De Valparaíso. (Sponsored by Fondecyt 1120513 And Millenium Institute CINV)

Nitric oxide (NO) is a neuromodulator involved in both physiological and pathological processes in the mammalian retina. Its synthesis is established for certain amacrine cells which modulate inner retinal synapses, but bipolar cells, the only connection between the inner and outer retina, have also been reported to produce NO. However, identification and characterization of this alternative NO source are not complete. We incubated rat retinal slices with the NO-fluorescent probe DAF-FM and morphologically identified labeled bipolar cells by injection of an intracellular dye. Under dark and light adaptation, immunohistochemistry of neuronal NO synthase (nNOS) was performed in retinas with previously identified NO-positive bipolar cells to establish their NO source and to assess a putative nNOS expression dependence on light conditions. Bipolar cell type 8 was found to be the most frequent NO-positive cell. On the other hand, immunohistochemistry showed that at least one ON type bipolar cell and one OFF type bipolar cell express nNOS. One of the ON bipolar cells corresponds to BC type 9, a morphologically distinguishable nNOS expressing bipolar cell. Most other bipolar cell types do not contain nNOS, and the overall expression pattern of nNOS did not change depending on dark or light adaptation. Finally, NO fluorescence was most frequently present in axonless bipolar cells, suggesting that NO is also synthesized in response to injury or pathological conditions, possibly by the inducible isoform of NOS.



57) **Carotid chemosensory responses to acute hypoxia are reduced by chronic phenytoin treatment**

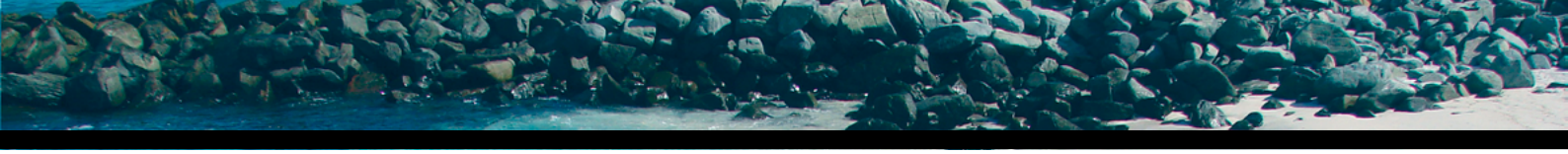
Alcayaga, Julio[1]., Oyarce, María[2].,Del RIo, Rodrigo[3].,[1]Biología, Ciencias, Universidad De Chile.[2]Ciencias Fisiológicas, Ciencias Biológicas, Pontificia Universidad Católica De Chile.[3]Unidad de Control Cardiorrespiratorio, Centro de Investigación Biomédica, Universidad Autónoma De Chile. (Sponsored by Supported By Grant FONDECYT 1130177)

Carotid body (CB) chemoafferents are the main peripheral signal that participates in ventilatory control. In the rat, the sensory neurons that innervate CB receptor cells present a persistent Na^+ current (I_{NaP}), which blockade reduces both normoxic afferent activity and increases in frequency discharge induced by acute hypoxia. Because there is no data available on the effects of prolonged I_{NaP} blockade on chemoafferent activity, we recorded carotid nerve discharges and responses to acute hypoxia from animals treated with phenytoin for two to four weeks. Male Sprague-Dawley rats (204 ± 6 g; n = 18), under isoflurane anesthesia, were implanted subcutaneously with an osmotic pulp filled with vehicle (control) or with phenytoin (10 mg daily dose); the animals received antibiotics and anti-inflammatory after surgery. After sixteen to 28 days, the rats were anesthetized with sodium pentobarbitone (60 mg/Kg) placed in a thermoregulated pad. The neck was opened through the midline, the trachea cannulated, the carotid bifurcation exposed and the carotid nerve severed at its origin in the glossopharyngeal nerve. The nerve was placed in paired Pt/Ir electrodes, connected in turn to an AC preamplifier, and covered with mineral oil. The recorded signal was amplified, band-pass filtered (10-1000 Hz) and digitally counted to assess the chemoafferent discharge (fx), in Hz. Basal chemoafferent discharges in normoxia (fraction of inspired oxygen, F_IO_2 = 21%) as well as well as the changes in chemoafferent discharges (Δfx) induced by 30 s changes in F_IO_2 (0 – 100% range) were recorded. The animals were sacrificed with an anesthetic overdose at the end of the recording. The basal chemoafferent discharges were not significantly different (P > 0.3; Student´s t-test) between controls (76.5 ± 8.6 Hz; n=10) and phenytoin treated (93.4 ± 14.5 Hz; n= 8) animals. However, responses induced by changing F_IO_2 were significantly (P < 0.02; 2 way ANOVA) reduced by phenytoin treatment. Moreover, responses induced by the lowest values of F_IO_2 in the range, were significantly lower (P < 0.05; Bonferrroni test after 2 Way ANOVA) in treated animals (F_IO_2 5 %, Δfx = 96.5 ± 22.4 Hz; F_IO_2 0 %, Δfx = 92.0 ± 14.4 Hz) than in control ones (F_IO_2 5 %, Δfx = 161.2 ± 17.3 Hz; F_IO_2 0 %, Δfx = 167.3 ± 20.0 Hz). Thus, afferent discharges in normoxia are not modified by phenytoin treatment, while responses to short acute hypoxic challenges are significantly reduced by phenytoin treatment. This reduction in hypoxic sensibility may alter ventilatory responses after chronic phenytoin treatment.

58) **STUDYING THE ROLE OF AMINERGIC RECEPTORS EXPRESSED IN *DROSOPHILA* MUSHROOM BODIES IN BEHAVIORAL RESPONSES TO AN AVERSIVE STIMULUS**

Fuenzalida-Uribe, Nicolás[1]., Campusano, Jorge[1].,[1]Biología Celular y Molecular, Facultad de Ciencias Biológicas , Pontificia Universidad Católica De Chile. (Sponsored by Supported By Fondecyt 1141233)

The Mushroom Body (MB) is a brain integration center key in processing several sensory stimuli and in defining locomotor behavior in insects. Aminergic systems innervate and modulate the activity of neurons in the MB. Through the regulation of different neuronal populations in *Drosophila* MB, these systems become relevant in the modulation of locomotor behavior in insects. Here we have studied the role of different aminergic receptors on locomotor behavior in response an aversive stimulus (Benzaldehyde, Bz). We directed the expression of specific RNAi for different Dopaminergic and Octopaminergic receptors to *Drosophila* MB, by using the Gal4-UAS technique. We recorded and analyzed fly behaviors in a circular arena, when flies are exposed or not to an aversive odorant, Benzaldehyde (Bz). Our results show that one Dopamine receptor analyzed (DAMB-R) contributes to locomotor behavior in response to Bz in *Drosophila* while, Octopamine receptors do not have major effects on innate locomotor behavior. Interestingly though, one of these receptors (Octb3-R) has a marked effect on centrophobism, a parameter reflecting the way flies explore the recording arena. This data suggests that complex behaviors associated to anxiety are modulated by this receptor in MB. Our results show a differential contribution of aminergic receptors expressed in MB in different behaviors.



59) **On the spatial extension of the correlations on a retinal ganglion cells population: dependence on the stimuli.**

Herzog, Rubén[1]., Palacios, Adrian[2].,Escobar, Maria-Jose[3].,[1]Biología, Ciencias, Universidad De Chile.[2]CINV, ciencias, Universidad De Valparaíso.[3]Electrónica Universidad Técnica Federico Santa María. (Sponsored by Fondecyt #1150638, Millennium Institute ICM-P09-022-F., ANR_CONICYT ANR-47 ECOS-Conicyt C13E06 Fondecyt #1140403, Basal Project FB0008)

The retina is an extension of the brain, a neural network working as a visual sensory interface between the nervous system and the world. The retina is organized in cellular and plexiform layers, and composed by different cell types. One of the types forming the retina is retinal ganglion cells (RGC), which receives inputs from the other cell types and sends retina activity to the brain through the optic nerve. Nowadays, the development of Multi-electrode Array systems allows the simultaneous recording and analysis of hundreds of RGCs, shifting the focus of the questions and observations towards the network (population) level, instead of studying single neurons activity. In this context, we recorded several *in vitro* retinas patches from the Chilean diurnal rodent *Octodon degus* under different natural and artificial stimuli conditions, obtaining the response of more than 700 retinal ganglion cells.

Taking into account the network nature of the retina we asked whether the concerted spiking activity of RGCs depends on the visual stimuli and the local connectivity of the several recorded sties: So, what is the spatial extension of the correlations on the RGCs? Do this correlations and its spatial extension depend on the stimuli? To address these questions, we characterize those cells using spike-triggered average, which allow us to estimate the linear receptive field and its spatial position in the retina. Additionally, we computed the cross-correlation function between all pairs of cells and then, with the position of each cell, we computed the distance between all the pairs of neurons, obtaining, at the end, a function that relates how the correlation depends on the physical distance between two recorded neurons. Usually, we observe an exponential asymptotic decay of the correlations with the distance between cells.

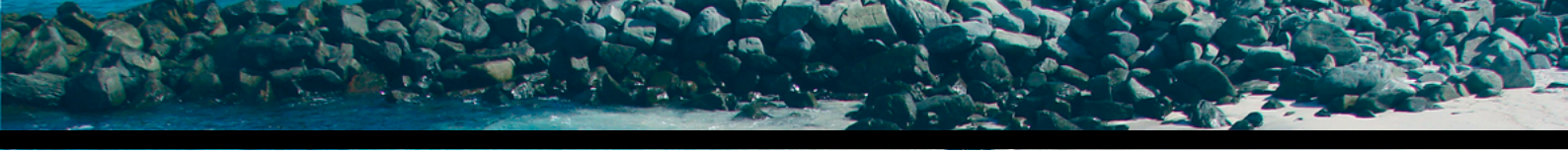
Using data recorded from natural stimulus we observed that both, baseline correlations and spatial extent of this correlations reach the highest values, when compared to other experimental conditions. The spatial extension in the case of natural stimulus extends beyond the mere receptive field overlap between neurons, suggesting the presence of a lateral mechanism on the retina that could be coordinating the RGC activity on a constrained locality.

60) **EFFECT OF 4-METHYL-THIOAMPHETAMINE ON OLFACTORY RESPONSES IN DROSOPHILA IS EXPLAINED BY AN INCREASED SEROTONIN RELEASE IN THE FLY BRAIN.**

Hidalgo, Sergio[1]., Molina, Daniela[1].,Fuenzalida-Uribe, Nicolás[1].,Campusano, Jorge[1].,[1]Biología celular y molecular , Ciencias biológicas , Pontificia Universidad Católica De Chile. (Sponsored by Acknowledgement To Dr. Iturriaga-Vasquez. Supported By Fondecyt 1141233)

4-MTA (4-Methylthioamphetamine) is a “designer drug” which induces prolonged stimulation and euphoria, and has been associated with neurotoxicity and even death. It was designed to specifically block the serotonergic plasma membrane transporter (SerT), leading to an increase in the extracellular content of this amine. However, it has been also shown to act on other two molecular targets: the dopamine transporter and MAO-A. Thus, the behavioral consequences of 4-MTA exposure in an animal depend on the effects induced by this drug on all these targets. Invertebrates exposed to drugs of abuse display a set of behaviors that depend on the activation of aminergic systems, which are highly conserved when compared to vertebrate counterparts. In our lab, we are using some of the behavioral, physiological and genetic tools available in the fly *Drosophila melanogaster* to dissect out the contribution of different amine systems to the behavioral effects induced by 4-MTA.

Our data show that 4-MTA at different concentrations induce differential effects on fly olfaction and motor responses. Chronoamperometry studies indicate that 4-MTA induces the release of endogenous BAs in the fly brain, with a slow kinetics as compared to the effects observed for nicotine. Experiments in mutant flies suggest that the amine whose release is being modified by 4-MTA is serotonin. We further show that the effects on olfaction are not observed in animals expressing a mutation for SerT. Altogether, this data supports the proposition that 4-MTA induces the release of serotonin to modulate olfactory responses in *Drosophila*.



61) **Synchrony of neural oscillations in the olfactory system of the rainbow trout**

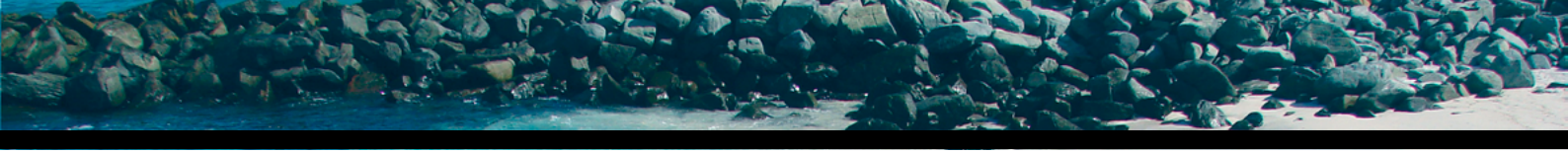
Olivares, Jesús[1]., Herzog, Rubén[1].,Orio, Patricio[1].,Schmachtenberg, Oliver[1].,[1]Neurociencia, Science, Universidad De Valparaíso.
(Sponsored by FONDECYT 1120513, Instituto Milenio CINV, Beca De Estudios De Doctorado CONICYT)

The olfactory system is evolutionarily conserved and shows a similar general operating scheme across invertebrates and vertebrates. Two regions of the telecephalon, the ventral nucleus of the ventral region of the telencephalon (Vv) and dorsal posterior region of telencephalon (Dp), are related to the analysis of olfactory information in the rainbow trout. Neural oscillations can be observed in the local field potential (LFP) in specific frequency bands within these areas. Fourier analysis shows that the olfactory bulb, Vv and Dp oscillate with frequencies around 9-10 Hz during the olfactory response. By performing the calculation of coherence between these two zones, a high degree of coherence is observed at frequencies belonging to bands known as Theta (t) (9-12 Hz) Beta (b) (about 15 Hz) and Gamma (g) (about 25 Hz). Neural oscillations in these frequency bands can be found in the olfactory systems of many species and are thought to be related to olfactory learning and alertness, but their meaning at the level of olfactory information processing remains to be investigated. In order to determine whether responses in the telencephalon show differences depending on the type of odorant, we stimulate with different concentrations of artificial mixtures of amino acids (AA) and bile salts (BS) and with two natural odors, human skin rinse (HSR) and trout skin extract (TSE). Equipotent concentrations of these odors were established based on the electroolfactogram amplitude. These experiments allow the comparative analysis of differences in amplitude, delay, duration, frequency, and envelope shape for each odor among other parameters of neural oscillations.

62) **Inatentively viewing a bistable stimulus induces simultaneous processing of both alternative percepts**

Rodriguez, Eugenio[1]., Campos, German[1].,Artigas, Claudio[1].,Morales, Ricardo[1].,[1]Escuela de Psicologia, Facultad de Ciencias Sociales, Pontificia Universidad Católica De Chile. (Sponsored by Proyecto Fondecyt 1120752)

In bistable perception a single sensory stimulus give rise to two mutually exclusive alternating perceptual states. A case model of self-organizing properties of brain activity, bistable perception has been extensively studied. However many aspects of bistable perception are still poorly understood. One of such is 'what happens when attention is diverted away from the bistable stimulus?' Does the perceptual alternation goes on? Does it stay fixed in one state? Or there is no perception whatsoever? We hypothesize that when attention is diverted from the bistable stimulus, the brain simultaneously processes both perceptual states. In order to test this hipothesis we presented subjects with the Rubin Vase illusion (vase-face picture). Vase and face areas were tagged by flashing them at 12 and 15 Hz respectively. The EEG activity of 4 subjects was recorded while they were looking at the Rubin vase but either attending to or attending away from the stimulus. Results suggest that during inatentive wieving of the stimulus both perceptual states are simultaneously procesed to higher cognitive levels as shown by tagged activity up to frontal brain areas. By contrast when attention is turned to the stimulus the tagging frequencies change with one of them being enhanced while the other is atenuated in a paralel way to perception were the corresponding percepts is selected while the other one vanishes.



63) **Serotoninergic modulation of synaptic strength in rat dentate gyrus**

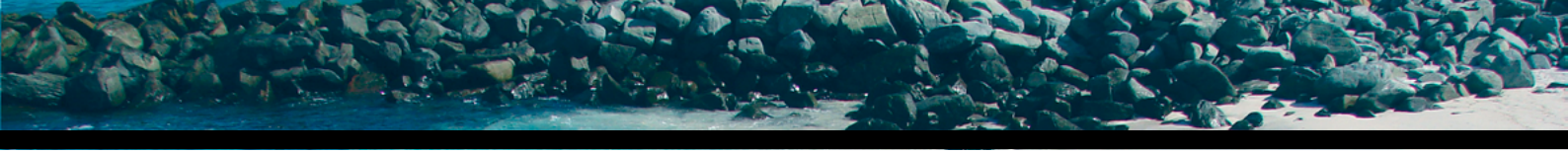
Chavez, Andres[1,2]., Castillo, Pablo[2].,[1]Instituto de Neurociencias, Ciencias, Universidad de Valparaiso, Centro Interdisciplinario de Neurociencias.[2]Dominick P. Purpura Department of Neuroscience, Neuroscience, Albert Einstein College of Medicine. (Sponsored by This Work Was Supported By NIH Grants MH081935 And DA017392 (to P.E.C.) And By Nucleo Milenio Nu-MIND NC 130011 (to A.E.C).)

Several lines of evidence indicate that the complex action of serotonin (5-HT) in synaptic function is determined by diverse 5-HT receptor (5-HTR) subtypes. However, the precise contribution of each receptor subtype to brain function regulation is not fully understood. In the inferior olive, it has been reported that the $G_{q/11}$-coupled 5-HT_2R can indirectly suppress excitatory synaptic transmission by mobilizing endocannabinoids (eCB) that activate presynaptic cannabinoid receptors (CB1Rs) to suppress glutamate release. More recently, we reported that the eCB anandamide acts on postsynaptic transient receptor potential vanilloid 1 (TRPV1) to suppress synaptic transmission presumably by reducing the number of AMPA-type glutamate receptors (AMPARs) at the synapse. Given that 5-HT_2Rs, CB1Rs and TRPV1 channels are expressed in the dentate gyrus (DG), we sought to examine whether 5-HT_2Rs, via eCB production and activation of presynaptic CB1R and/or postsynaptic TRPV1 channels, could regulate excitatory synaptic transmission in dentate granule cells (DGCs). To this end, we monitored two distinct glutamatergic inputs on dentate granule cells (DGCs) in acute hippocampal slices: mossy cell fiber (MCFs) inputs, which are modulated by retrograde eCB signaling and CB1Rs, and medial perforant pathway (MPP) inputs, which are known to be modulated by non-retrograde eCB signaling and TRPV1 channels. Using selective pharmacology for 5-HTRs in acute rat hippocampal slices, we found that bath application of 5-HT (50 µM - 10 min) depresses MPP-EPSCs, but not MCF-EPSCs via activation of 5-$HT2_{a/c}$Rs. This effect was observed at AMPAR-EPSCs, but not NMDAR-EPSCs, indicating that changes in synaptic efficacy were not due to a direct modulation of transmitter release. Moreover, the 5-HT-mediated depression required postsynaptic Ca^{2+} rise and internalization of AMPARs, strongly suggesting a postsynaptic mechanism of action. Consistent with this idea and the postsynaptic localization of TRPV1 channels at the MPP synapse, pretreating slices (10–20 min) with two different TRPV1 antagonists, capsazepine (CPZ, 10 µM) or AMG9810 (AMG, 3 µM) eliminated 5-HT-mediated depression of MPP-EPSCs. Furthermore, 5-HT-mediated depression was still present in CB1Rs knockout mice, but was abolished in TRPV1 knockout mice. Taken together, our findings reveal a novel form of 5-HT-TRPV1 mediated regulation of excitatory synaptic transmission at central synapses. The potential contribution of eCBs signaling in this novel form of 5-HT-mediated depression is currently under investigation.

64) **Prostaglandin E2 decrease inhibitory post-synaptic current in CA1 pyramidal neurons of hippocampus**

Ahumada, Juan[1]., Bonansco, Christian[1].,Fuenzalida, Marco[1].,[1]Fisiología, Ciencias, Universidad De Valparaíso. (Sponsored by This Work Was Supported By Grants From Millennium Nucleus NU-MIND NC-130011 (MF), FONDECYT 1130614 (M.F.) And 1130491 (CB), CONICYT PhD Fellow Nº 21130547 (JA).)

The nervous system operates in a balanced regime of excitation and inhibition. Several neurotransmitter and neuromodulator systems are essential to maintaining the excitation and inhibition (E/I) ratio, which is critical for cognitive processes such as learning and memory. Increasing evidence indicates that central component of the immune response, such as cyclooxygenase-2 (COX-2), can regulate the physiological function in the brain. COX-2 mediates changes in neurotransmission and synaptic plasticity in the CNS through its chemical products, principally by prostaglandin E2 (PGE2), which activates four subtypes of seven transmembrane G-protein coupled receptors (EP1-EP4). Although several studies has described the effects of PGE2 activation on glutamatergic transmission and their possible consequences in the excitatory long-term potentiation (LTP), the cellular mechanism through which the PGE2 can regulate the synaptic efficacy of GABAergic synapses and E/I balance is poorly known. Using electrophysiological tools we investigated whether the activation of the EP3 and/or EP4 receptors can modulated the GABAergic synapses on pyramidal neurons of CA1 region. According to literature, we showed that PGE2 induces LTP of field excitatory post-synaptic potential in CA3-CA1 synapses, interestingly, without block GABAergic receptors. Remarkably, we found that the activation of EP3 and/or EP4 receptors can decrease the amplitude of evoked inhibitory post-synaptic current (eIPSC) on CA1 pyramidal neurons. Taken together, these results suggest that PGE2 could be an important signaling pathway in modulation of both glutamatergic and GABAergic transmission and synaptic plasticity as well as the maintenance of E/I balance in the CNS. Furthermore, PGE2 signalling could be an important component of cognitive deficits observed in different neuropathologies such as traumatic brain injury, epilepsy and schizophrenia, when it has been described that COX-2 activity is impaired.



65) **Brevican and reelin regulate neuronal refinement in hippocampal neurons.**

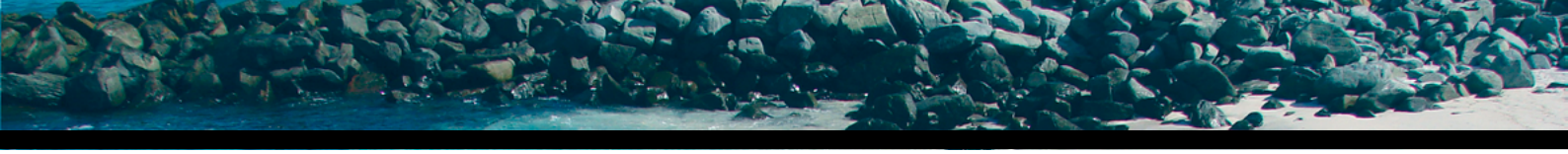
Ampuero, Estibaliz[1]., Jury, Nur[1].,Marzolo, María Paz[2].,Jaramillo, Karen [1].,Montecino, Martín [3].,Van Zundert, Brigitte[1].,[1]Laboratorio de Plasticidad Neuronal , Centro de Investigaciones Biomédicas, Universidad Andrés Bello.[2]Laboratorio de Tráfico Intracelular y Señalización, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile.[3]Laboratorio de Regulación Génica, Centro de Investigaciones Biomédicas, Universidad Andrés Bello. (Sponsored by Fondecyt-3130582 (E.A.), Fondecyt-1140301 (B.v.Z.), Anillo ACT1114 (B.v.Z.).)

Introduction: The extracellular matrix proteins (ECM) brevican and reelin are secreted by astrocytes and GABAregic cells, respectively. Both proteins are found surrounding the excitatory synapses where they contribute to synapse maturation and restricting receptor mobility. It has been reported that reelin binds to the lipoproteins receptors ApoER2 and VLDLR, hereby inducing an intracellular signaling pathway that modulates neuronal plasticity. It is believed that brevican restrict synapses by forming a perineuronal networks. Here we studied how interference of reelin-mediated signaling or degradation of brevican in mature hippocampal neurons impacts dendritogenesis and the distribution of the synaptic proteins like NMDA receptor subunits (NR2B and NR2A), the scaffolding protein postsynaptic density 95 (PSD95) and the presynaptic proteins bassoon and synapsin-I. We hypothesized that interfering the presence/signaling of reelin and brevican can reactivate neuronal plasticity in mature neurons by increasing the synaptic expression of NR2B and PSD95 that allow spine remodeling concomitant with dendritogenesis. Material and Methods: Expression of brevican and reelin was analyzed by immunostainings using permeablization and non-permeablization conditions during development of cultured hippocampal neurons (0-20 days *in vitro* (DIV)). Mature hippocampal neurons (15 DIV) were transfected with GFP by magnetofection and chronically treated during 5 days with GST-RAP or CR-50 to decrease reelin signaling, or chondroitinase ABC (ChABC) for brevicandegradation. At 20 DIV we analyzed the dendritic architecture and the expression of synaptic proteins using double immunostainings. To evaluate if degradation of ECM also regulates the architecture of hippocampal neurons *in vivo,* CR50 or ChABC were injected into the dentate gyrus of adult mice. HSV-GFP was co-injected to visualize the morphology of neurons. Results: With hippocampal maturation *in vitro* gradually more neurons ($MAP2^+$) showed an increment in extracellular brevican and reelin immunoreactivity. We found that interference of reelin-mediated signaling or degradation of brevican resulted into a significant increase in dendritogenesis relative to control neurons. Increased dendritogenesis was associated with an exchange of synaptic proteins, with an increment in the clustering of the NR2B subunit, concurrent with a reduction of PSD95 clusters. Discussion: Our results show that the modification of critical ECM proteins in mature neurons leads to an immature phenotype of the neurons, potentially favoring formation of new contact.

66) **Methylphenidate amplifies LTP in hippocampus CA1 area involving the insertion of AMPA receptors by activation of β-adrenergic and D1/D5 receptors**

Carvallo, Claudia[1]., Contreras, Darwin[1].,Farias, Ricardo[2].,Rozas, Carlos[2].,Zeise, Marc[2].,Morales , Bernardo[2].,[1] Biología, Quimica-Biología, Universidad De Santiago De Chile.[2]Biología, Química y Biología, Universidad De Santiago De Chile. (Sponsored by Supported By FONDECYT 1120580, Anillo ACT-1113 And CONICYT Fellowship To C.C./D.C)

Methylphenidate (MPH, Ritalin©) is widely used in the treatment of Attention Deficit Hyperactivity Disorder and recently as a drug of abuse. The effect of MPH in hippocampus has received relatively little attention considering that it plays a prominent role in memory and learning processes. It is known that MPH increases the TBS-dependent Long Term Potentiation (LTP) in the CA1 area. However, the cellular and molecular mechanisms involved in this process are still unknown. Using an electrophysiological approach and Western blot analysis we studied the transduction mechanism involved in the effect of MPH on Long-Term Potentiation (LTP) in rat hippocampus slices. 3-4 weeks old Sprague-Dawley rats were decapitated under halothane anesthesia, and hippocampus slices (400 µm thick) were prepared. LTPs were induced by applying theta burst stimulation (TBS, 5 trains, 100 Hz) at the Schaeffer collaterals and recorded in the striatum radiatum of the CA1 area. Superfusion of hippocampus slices during 20 min with MPH enhances LTP in CA3-CA1 synapses in a dose-dependent manner with an EC_{50} of 73.44 ± 6.32 nM and a maximum response of 52.93 ± 0.63 %. Paired-pulse facilitation (PPF) curves remained unchanged after perfusion with MPH, suggesting that the effect of MPH is at postsynaptic level. Using specific antagonists and PPF protocols, we found that the MPH-dependent increase of LTP involves not only β-adrenergic receptors activation but also post-synaptic D1/D5 dopamine receptors. The inhibition of PKA with PKI, suppressed the facilitation of LTP induced by MPH consistent with an involvement of PKA dependent cascade downstream of the activation of D1/D5 receptors. To evaluate whether the increase of LTP induced by MPH involves the insertion of new AMPA receptors in the post-synaptic membrane, we collected CA1 areas from hippocampal slices used in LTP experiments and performed Western blot analysis of the phosphorylation state of Ser845 and Ser831 residues in GluA1. Samples of CA1 areas taken from slices potentiated with MPH presented an increase in the phosphorylation of the Ser845 residue of the GluA1 subunit of AMPA receptors compared to control slices. This effect was inhibited by SCH23390, antagonist of D1/D5 receptors. Moreover, using cross-linking essay we found an increase of AMPA receptors in the surface. These results suggest that MPH increases TBS-dependent LTP in CA3-CA1 synapses through a polysynaptic mechanism involving activation of β-adrenergic and D1/D5 dopaminergic receptors and promoting the trafficking and insertion of AMPA receptors to the plasmatic membrane.



67) **Cannabinoid receptor activation modulate the temporal properties of scotopic visual signal in rat retina**

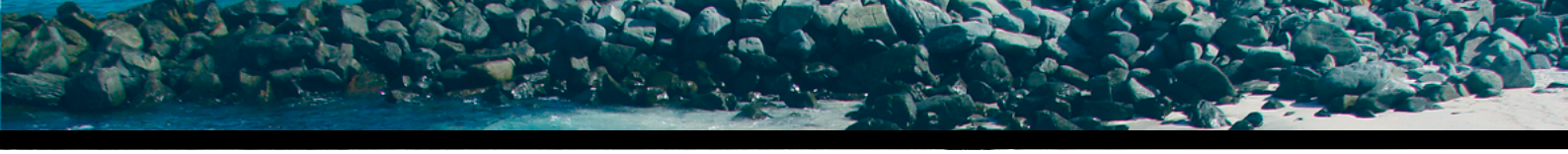
Palacios-Muñoz, Angelina[1]., Vielma, Alex[2].,Palacios, Adrian[3].,Chavez, Andres[4].,[1]Instituto de Neurociencias, Facultad de Ciencias, Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso.[2]Instituto de Neurociencias, Facultad de Ciencias, Universidad de Valparaíso, Centro Interdisciplinario de Neurociencia de Valparaíso .[3]Instituto de Neurociencias, Facultad de Ciencias, Universidad de Valparaiso, Centro Interdisciplinario de Neurociencias.[4]Instituto de Neurociencias, Facultad de Ciencias, Universidad de Valparaiso, Centro Interdisciplinario de Neurociencia. (Sponsored by This Work Was Supported By FONDECYT Grant # 1151091 (AEC), Iniciativa Científica Milenio ICM-P09-022-F (AEC And AGP) And Núcleo Milenio Nu-MIND # NC 130011 (AEC).)

Extensive distributions of type-1 cannabinoid receptor (CB1R) in major retinal neurons suggests a role as modulators of retinal network, but how activation of these receptors can modulates visual activity remain largely unexplored. Using *in vivo* extracellular electroretinogram (ERG) recordings, we examine the functional consequences of CB1R activation on rat retinal circuitry under scotopic (dark) and photopic (light) conditions. Intravitreal injection of the CB1R agonist WIN 55,212-2 (WIN, 1 µM) enhanced the amplitude of ERG a- and b-wave under scotopic conditions, without altering the gain of ERG a- and b-waves under photopic conditions. In addition, WIN also prolonged the decay time of the scotopic ERG b-wave, which reflects ON bipolar cell depolarization, but not the photopic ERG b-wave, which reflects cone ON bipolar cells. Importantly, WIN-mediated effect on ERG a- and b-wave depends on CB1R activation as the changes in the amplitude and kinetics were eliminated by blocking CB1Rs with AM251 (5 µM). Remarkably, WIN-mediated effects on ERG waves were occluded by blocking inhibitory GABAergic synaptic receptors ($GABA_A$ and $GABA_C$ type), strongly suggesting that activation of CB1Rs may play a role in modulating the signal transfer through the rod/dark pathway by reducing GABAergic inhibitory inputs onto second order ON rod bipolar cell (RBC) terminals. To test this possibility, whole-cell recordings were made from ON RBCs in acute retinal slices, while GABAergic feedback IPSCs mediated by A17 amacrine cells were elicited by step depolarizations in the RBCs. WIN (5 µM) significantly reduced voltage step-evoked IPSCs recorded in RBCs, an effect that was largely blocked by the CB1R antagonist AM251 (5 µM). Altogether these results suggest that cannabinoid receptor activation modulate signal transfer through the rod pathway by reducing GABAergic inhibitory feedback onto RBC terminals.The precise mechanism underlying this effect on GABAergic feedback inhibition is currently under investigation.

68) **Role of NADPH oxidase (NOX) in spatial memory formation and synaptic function in rat hippocampus**

Contreras, Marcela[1]., Chávez, Andrés[2].,Sanchez, Gina[3].,Hidalgo, Cecilia[4].,Haeger, Paola[1].,[1]Departamento Ciencias Biomédicas, Facultad de Medicina, Universidad Católica Del Norte.[2]Centro Interdisciplinario de Neurociencias de Valparaíso, Facultad de Ciencias, Universidad De Valparaíso.[3]Programa de Fisiopatologia, ICBM, Facultad de Medicina, Universidad De Chile.[4]BNI, CEMC & ICBM, Facultad de Medicina, Universidad De Chile. (Sponsored by Funded By Fondecyt #1140855 (P.H), Fondecyt #1151091 & Millennium Nucleus NU-MIND NC-130011 (AEC), # Fondecyt 1140545 & BNI P-09-015F (CH))

The NOX family encompasses several enzymatic complexes that transfer electrons across biological membranes and which are responsible for superoxide production, a cellular reactive oxygen species (ROS). Increasing evidence suggest that activation of NMDA receptors (NMDARs) increases ROS levels through activation of the NOX2 isoform, whereas NOX inhibition reduces NMDAR-dependent ERK activation. Moreover, studies in male knockout mice demonstrated that NOX2 activity is necessary for the induction of long-term potentiation (LTP) in the hippocampus as well as for spatial memory formation. However, direct involvement of NMDAR function in these processes remains unclear. Here, using the Morris Water Maze paradigm, we evaluated the role of NOX activity in hippocampal memory formation in adult rats. Our results indicate that rats orally treated with the NOX2 inhibitor apocynin (5 mM), spent more time to reach the hidden platform in the last days of training compared with controls, without changes in the swimming speed. Additionally, we investigated the role of NOX on synaptic function by assessing excitatory synaptic transmission at the CA3-CA1 synapse in acute rat hippocampal slices, measuring extracellular field potentials (EPSP) and using whole-cell recording (EPSC) techniques. We found that bath application for 20 min of VAS2870 (VAS, 10 mM, a specific NOX family inhibitor) did not alter the slope of AMPA receptor-mediated EPSP, but reduced the magnitude of NMDAR-dependent LTP compared with control slices. To test whether this effect on LTP could be due to a VAS-dependent alteration of NMDAR-dependent basal transmission, we evaluated the effects of VAS on isolated NMDAR-mediated currents. No difference was found in the amplitude of NMDAR-mediated EPSC compared to baseline. Taken together, these results suggest acute NOX activity involvement in hippocampal LTP and spatial memory formation, presumably via modulation of activity-dependent NMDAR function. The precise mechanisms underlying NOX effects on LTP and memory formation are currently under investigation.



69) **ATP a probable mediator of the respiratory response at caudal medullary chemosensitive nuclei.**

Gómez, Karina[1]., Olivares, María José[1].,Beltran-Castillo, Sebastián[1].,Donoso, María Verónica[1].,Huidobro-Toro, Juan Pablo[1].,Eugenin, Jaime[1].,[1]Biología, Química y Biología, Universidad De Santiago De Chile. (Sponsored by Fondecyt 1130874 Y Fondecyt 1141132)

ATP, released by astrocytes at the retrotrapezoid nucleus (RTN) in response to hypercapnia, increases the frequency of fictive respiration in rodents. However, in other chemosensitive nucleus, like the raphe nucleus (RN) and the nucleus tractus solitarius (NTS), the ATP role as a mediator of the respiratory response to hypercapnia is questioned. In this work, we wanted to know whether ATP can be released by hypercapnic stimulation from other regions of the medulla located caudally to the RTN.

Eight medullary slices (700 µm width)from CF1 mice at postnatal days 1-4 (P1-P4) containing the caudal portion of the ventral respiratory column (VRC), NTS and RN, but not containing the RTN were acutely incubated in 30 ml of artificial cerebrospinal fluid (aCSF) equilibrated with 10% CO_2 and 90% O_2 for 10 minutes (hypercapnia-conditioned medium, h-cm). Similar protocol was followed to obtain a normocapnia-conditioned medium (n-cm, control), that is, 8 slices were incubated with 30 ml aCSF equilibrated with 5% CO2, 90% O2 for 10 minutes. Conditioned media were collected and equilibrated with 5% CO2, 95% O2, and superfused to another medullary slice in which fictive respiration was recorded at basal conditions with a suction electrode placed on the VRC.

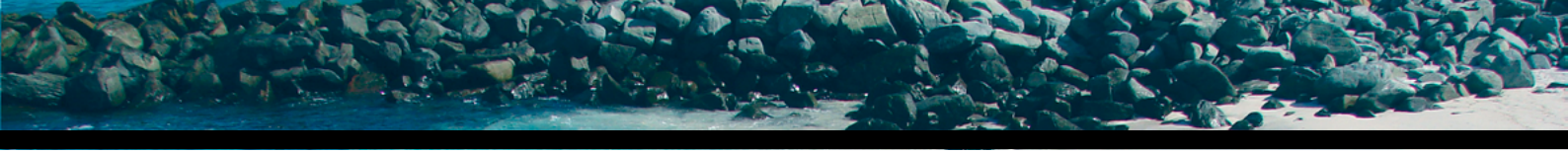
H-cm, but not n-cm, increases the frequency of inspiratory bursts up to 180% of the basal rate. This increase was abolished by apyrase, enzyme that degrades ATP. Results are consistent with the HPLC detection of augmented ATP concentration in the conditioned medium after the hypercarbic acidosis.

These data strongly suggest that ATP is released not only by the RTN but also by other caudal medullary nuclei during hypercarbia, and it would contribute at RN, NTS or VRC, likely as mediator, to the respiratory response to hypercapnia.

70) **Adaptation of CA1 pyramidal neuron excitability to chronic inactivity: role of CaMKII**

Karmelic, D[1]., Palma, Verónica[1].,Sanhueza, Magdalena[1].,[1]Departamento de Biología, Facultad de Ciencias, Universidad De Chile.
(Sponsored by Financial Support: CONICYT Scholarship 21110650 And FONDECYT Grants 1140700 And 1140697.)

“Hebbian” synaptic plasticity processes, such as long-term potentiation, generate positive feedback loops that can result destabilizing for neuronal circuits. This is thought to be prevented by a set of slower plasticity phenomena, termed ‘homeostatic plasticity’, acting in opposition to the changes in neural activity. These mechanisms are not entirely understood, but can include synaptic changes and changes in intrinsic neuronal excitability through modification of different membrane conductances. In the present work, we study the adaptation to chronic inactivity of the excitability of CA1 principal cells in cultured slices. Using whole-cell recording we evaluated, as a first step to describe such plasticity, the spiking frequency in response to depolarizing current injection in CA1 neurons from slices incubated in 1 µM TTX for 3 days compared to neurons from control slices. Neurons from TTX-treated slices show significantly higher excitability, with increased firing frequencies at lower stimulation levels. We then measured the firing threshold using a current ramp and found no significant differences in this parameter, suggesting that changes in voltage-gated sodium channels do not explain the observed rise in excitability. Membrane resistance, on the other hand, increased significantly with chronic inactivity. Searching for possible mechanisms for the expression of these adaptations in excitability triggered by chronic inactivity, we assessed the role of CaMKII activity by co-incubating TTX-treated slices with 10 µM KN-93, a well-characterized inhibitor of CaMKII activation by calcium. The studied excitability variables in CA1 neurons from these slices are indistinguishable from TTX-treated cells. On the other hand, chronic incubation with KN-93 alone induces an important excitability increase in terms of firing frequency vs. injected current, along with a significant rise in membrane resistance. Several lines of evidence in murine models of epilepsy link CaMKII inhibition with increased seizure occurrence. The higher neuronal excitability observed in the present work is consistent with those epileptic phenomena. The fact that the effects of CaMKII inhibition and chronic inactivity have virtually the same magnitude and are not additive, points towards an occlusion of the effects due to the physiological constraints of a shared mechanism.



71) **Sorting determinants of Corticotrophin Releasing Factor Binding Protein towards the Regulated Secretory Pathway**

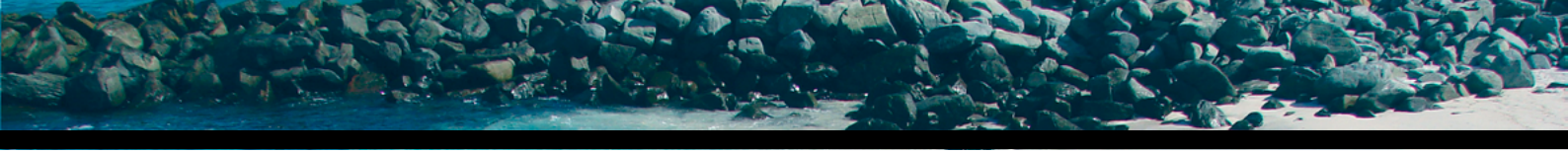
Bastias, Cristian[1]., Blanco, Elías[1].,Gysling, Katia[1].,[1]Departamento Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile.

Corticotrophin Releasing Factor Binding Protein (CRFBP) is a 37 kDa glycoprotein with high affinity for both CRF and urocortin-1 peptides. Traditionally, it has been accepted that CRFBP has an inhibitory role by binding CRF and/or urocortin-1 impeding their binding to CRF receptors. However, recently it is has been shown that CRFBP also facilitate CRF receptor actions. Previously we have shown that CRF-BP is secreted through the regulated secretory pathway, like a neuropeptide. However, the sorting determinants to the regulated secretion pathway are presently unknown. The purpose of the present study was to identify the sorting determinants of CRFBP to the secretory pathway. We used *in silico* tools to determine putative sorting domains in CRFBP. The analysis using secondary structure prediction programs (GORV and NPS@ Consensus Secondary Structure Prediction) and structural modelling (ROBETTA 3D modelling web server) showed putative alpha helical domains in CRFBP. In addition, the helical wheel projection program (PEPWHEEL) showed that the (50-74)CRFBP alpha helical domain has an amphipathic configuration, signature of other conserved sorting domains. The predicted (50-74)CRFBP amphipathic alpha-helical domain was capable of sorting to the regulated secretory pathway a mutant form of cocaine and amphetamine related transcript pro-peptide (proCART) without its sorting domain. The (1-53)ProCart/(50-74)CRFBP-EGFPm chimera signficiantly colocalized with secretogranin II and presented a subcellular localization associated to the trans golgi network and secretory granules in PC-12 Cells. Preliminary data showed that this chimera is readily secreted upon depolarization. Thus, CRFBP has an amphipathic alpha helix determining its sorting to the secretory pathway.

72) **The lack of a functional allele for Methyl CpG Binding Protein-2 alters reproductive lifespan and fertility in female mice.**

Alarcon, Gloria[1]., Camila , Navia[1].,Kerr, Bredford[2].,[1]Laboratorio de Biología Centro de Estudios Científicos-CECs, Universidad Austral De Chile.[2]Laboratorio de Biología Centro de Estudios Científicos. (Sponsored by Fondecyt 1140162, PFB 01/2007.)

Methyl CpG Binding Protein-2 (Mecp2) is a transcription factor that binds methylated CpG dinucleotides in the promoter of its target genes, regulating gene expression through the interaction with histone deacetylase complex or Creb1, and therefore either silencing or activating gene expression. Previous studies show that Rett syndrome patients, a severe neurological and progressive disorder mainly caused by mutations in MeCP2, exhibit alterations in the age of puberty onset and first menarche. Moreover, observations from our mouse facility suggest a decreased fertility in heterozygous female mice carrying a *Mecp2*-null allele (*Mecp2*$^{+/-}$). However, the mechanism underlying the attenuated fertility observed in the absence of a fully functional *Mecp2* allele has not yet been completely elucidated. The aim of this study was to characterize the reproductive function of *Mecp2*$^{+/-}$ mice and understand the mechanism by which the absence of a fully functional *Mecp2* allele alters female reproductive lifespan. To accomplish our aim, we compared the reproductive phenotype exhibited by *Mecp2*$^{+/-}$ and wild type females assessing fertility, ovarian morphometry, estrous cycle evaluations and qRT-PCR for key genes required for the proper control of the reproductive axis. Our results show a decreased number of litters and pups per dam in *Mecp2*$^{+/-}$ in comparison with wild type females. In addition, *Mecp2*$^{+/-}$ exhibited longer estrous cycles in comparison with wild type females. The estrous cycle is orchestrated by the expression of several genes at different levels of the reproductive axis. In order to evaluate whether the absence of a functional allele for *Mecp2* alters the expression of some of these key genes, we performed qRT-PCR. Our results show that the expression of the GnRH receptor mRNA is increased in ovarian tissue, while the Kisspeptin receptor GPR54 mRNA is decreased in pituitary. It is worthy to note that no litters were observed from 6 month old *Mecp2*$^{+/-}$ females in mating, time at which wild type females are still fertile. To determine whether an alteration in follicular development underlies the premature ending of reproductive lifespan observed in *Mecp2*$^{+/-}$, we assessed a morphometric analysis of ovaries from 8 day-old *Mecp2*$^{+/-}$ and wild type females. We observed a significant decrease in primordial, primary and secondary follicles; which indicates an alteration in follicular development and may be associated to a reduction in the number of follicles in adulthood. In summary, our data indicates that Mecp2 is an epigenetic modification-associated transcription factor essential for the proper reproductive lifespan and fertility in females.



73) **Exposure to a high fat diet during pregnancy and nursing increases serum estradiol in the offspring through a decrease in its metabolism**

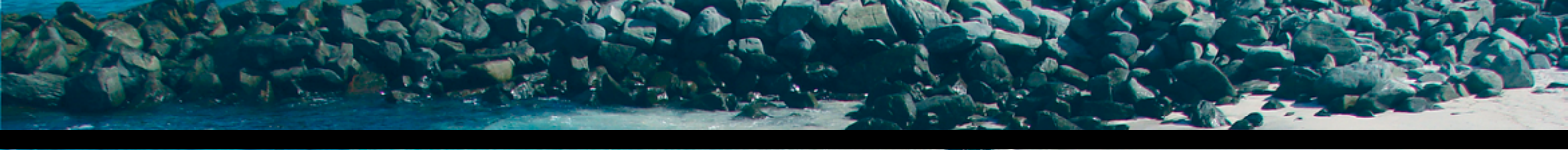
Alvarez, Daniela[1]., Reyes, Aldo[1].,Ramírez, Luisa [1].,Olguín, Sofía[1].,Ambrosetti , Valery[1].,Guerra, Marcelo[1].,Fernandois, Daniela[1].,Cerda, Tania[1].,Cruz, Gonzalo[1].,[1]Laboratorio de Alteraciones Reproductivas y Metabólicas. Instituto de Fisiología, Facultad de Ciencias, Universidad De Valparaíso. (Sponsored by FONDECYT INICIACION 11130707 (GC) And Centro De Neurobiología Y Plasticidad Cerebral (CNPC) Of Universidad De Valparaíso.)

In Chile 2 out of 3 people is either overweight or obese, and obesity is more prevalent in women than in males. This leads to the fact that 50% of women have malnutrition by excess of nutrients during pregnancy. Maternal obesity has an impact on the normal development of pregnancy and also could contribute to the development of reproductive and metabolic diseases in the offspring. Since exposure to estrogenic compounds could produce some similar alterations than maternal obesity we aimed to study the metabolism of estradiol and its plasmatic concentrations in the offspring of obese mothers. Sprague dawley rats were fed with a high fat diet (60%Kcal fat) from 1 month previous to pregnancy until weaning of the offspring. Control rats received a control diet (12%Kcal fat). Hepatic expression of CYP3A2 was determined by western blot and plasmatic estradiol and estriol by ELISA in the offspring. Analyses were performed at 1, 7, 14, 30 y 60 posnatal days. Estradiol levels were increased in rat offspring of obese mothers at all ages. Hepatic *CYP3A2* expression was decreased from posnatal day 1 until posnatal day 60. Estriol plasmatic levels were reduced at posnatal day. In conclusion, maternal obesity causes an increase in serum estradiol from childhood to adulthood, probably due to a decrease in the expression of hepatic CYP3A2. The fact that there is a reduced level of estriol, estradiol metabolite, confirms a lower hepatic metabolism of this hormone.

74) **Cryopreservation induces alterations in the mitochondrial function of Atlantic salmon spermatozoa (*Salmo salar*).**

Figueroa, E[3]., Valdebenito , Iván [1].,Risopatrón, Jennie [2].,Short, S.E[3].,Zepeda, A.B[3].,Figueroa, C.A[3].,Farias, J.G[3].,[1]School of Aquaculture Catholic University of Temuco, Temuco, Chile.[2]BIOREN Center for Biotechnology in Reproduction La Frontera University, Temuco, Chile.[3]Departamento de Ingeniería, Facultad de Ingeniería y Ciencias, Universidad De La Frontera. (Sponsored by FONDECYT 1151315 (F.JG); FONDEF D10I1064 (I.V). CONICYT Doctorate Grant (F.E; S.SE))

To date there are few data on the effect of cryopreservation on the mitochondrial dynamic in fish spermatozoa. The objective of this work is to assess the effect of cryopreservation on the mitochondrial dynamic in Atlantic salmon spermatozoa. The sperm were frozen in Cortland® medium + 1.3M DMSO + 0.3M glucose + 2% BSA for the treatment (T); fresh semen was used for the control (C). We determined [ATP] with the CellTiter-Glo® kit and [O_2] with the MitoXpress® Xtra kit. In these analyses we used electron transport chain inhibitors and uncouplers, namely: rotenone (R, 10µM), antimycin A (A, 10 µM), cyanide (C, 0.5 µM) and 2,4 dinitrophenol (D, 10µM). In the cryopreserved spermatozoa (T), the base [ATP] was 5.7±1.2 nmoles/10^9sp presenting significant differences from the control (7.4±0.64 nmoles/10^9sp, $p<0.05$); likewise the cells incubated with R (2.9±0,78 nmoles/10^9sp), A (3.98±0.92 nmoles/10^9sp), C (1.37±0.66 nmoles/10^9sp) and D (1.59±0.48 nmoles/10^9sp) presented statistically significant differences during the first 10 seconds of incubation as compared to the control (5.5±0.84 nmoles/10^9sp; 6.1±0.56 nmoles/10^9sp; 4.1±0.99 nmoles/10^9sp and 4.9±0.79 nmoles/10^9sp respectively, $p<0.05$). The base [O_2] in control spermatozoa was 4230±520 RFU/10^9sp, presenting significant differences from T (3040 RFU/10^9sp); the treatments incubated with R (3508±320 RFU/10^9sp), A (3627±480 RFU/10^9sp) and D (4290±429 RFU/10^9sp) presented significant differences from the control (R: 2704±298 RFU/10^9sp; A:2852±570 RFU/10^9sp and D:3442±612 RFU/10^9sp respectively, $p<0.05$). The changes in the [O_2] rate in spermatozoa in the presence of inhibitors and uncouplers occurred after 10 seconds of incubation. Preliminary results suggest that cryopreservation induces alterations in the mitochondrial function of Atlantic salmon spermatozoa.



75) **The environmental toxicants induce sperm acrosome reaction (AR) through a protein Kinase A (PKA) pathway**

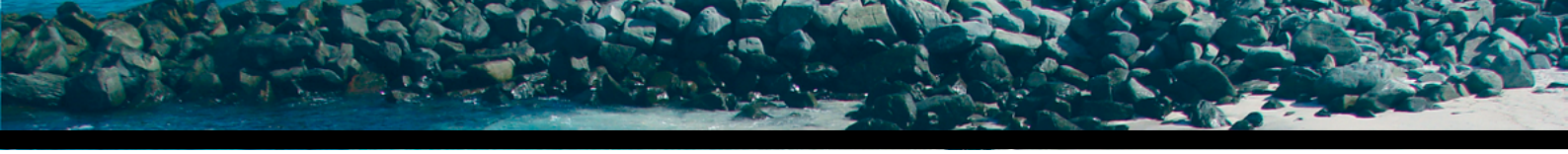
Gallardo, Luz[1].,**Moreno, Ricardo[1]**., [1]Fisiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 1150352 (RD.M), LMG: Becaria Doctorado CONICYT)

Environmental toxicants, could affect the reproductive outcome at different levels, but there is no study about if they affect directly the different steps of the fertilization prosseses. In this work, we select two environment contaminants,Endothall, a wildely used pesticide, which inhibits the phosphatase PP2A and nonylphenol, a xenoestrogen that mimic the estrogen. The aim of our work was to determine whether Endothall and nonylphenol induce the AR, by regulating activation of PKA. To this end, mouse epididymal caudal spermatozoa were recovered with or without the PKA inhibitor H89 and, incubated with Endothall, nonylphenol, or progesterone as a positive control, in capacitating or non capacitating conditions. The role of PKA was determinated by studying its phosphorylated (pT197) form by Westerblot. The AR percentage was quantified by Comassie-G Blue or LysoTraker dyes. Our immunofluorescents results showed that PP2A, was located in sperm tail and acrosome regions. Endothall induces the AR up to 31% in a range of concentrations allowed by the US EPA and nonylphenol induce the AR up to 19% at concentrations found in human fluid, depending on sperm capacitation status. Moreover, Endothall and nonylphenol potentiate the AR inductor effect of progesterone. In addition, we found that H89 prevented the AR induced by Endothall and nonylphenol. Finally, Endothall and nonylphenol increase the level of pT197 PKA 4 and 2.5 times, respectively, regardless the capacitation satus of the spermatozoa. In conclusion these environmental contaminants induce the AR in mice spermatozoa in a PKA-dependent way and they could disturbed mammalian fertilization.

76) **Organotypic culture as an *in vitro* spermatogenesis model: comparison between rat and mouse**

Carmona-Rojas, E[1]., Berrios-Amaro, C[1].,Moreno, R[2].,Reyes, J[1].,[1]Chemistry, Science, Pontificia Universidad Católica de Valparaíso.[2]Endocrinology and Reproduction, Biological Sciences, Pontificia Universidad Católica De Chile.

Spermatogenesis is a complex process that occurs inside of seminiferous tubules (ST) in the testis. Germ cells present in the ST include spermatogonia, which can proliferate or differentiate, meiotic cells known as primary and secondary spermatocytes, and haploid cells, spermatids and sperm. Additionally, Sertoli cells (SC) are somatic epithelial cells located inside the ST, and Leydig cells (LC) located in the interstitium. LC synthesize testosterone, and SC are in contact with all germ cells, supporting them. There is a blood testis barrier formed by tight junctions between SC. In this manner, SC are capable of controlling the internal microenvironment of the ST. The organotypic culture maintains the structural integrity of the ST, so it is able to produce *in vitro* spermatogenesis in mouse. The lipid rich albumin, AlbuMAX, seems to be the determinant factor in the culture media for completion of the process *in vitro*. The goal of this work was to characterize and to compare this type of culture in mouse and rat using media supplemented with Knockout Serum Replacement (KSR), fetal bovine serum (FBS) with AlbuMAX (AlbM), and FBS with bovine serum albumin fatty acid free (AlbFF). We cultured 7 days BALB/c mice and Sprague-Dawley rats ST for 30 and 40 days, respectively. The progression of the first wave of spermatogenesis in each condition was studied analyzing (i) the histology of slides of cultures by bright field and transmission electron microscopy, (ii) presence and location of specific cell markers by immunohistochemistry, and (iii) cell DNA content by flow cytometry. Spermatogenesis progression in rat cultures was minimum, showing a few primary spermatocytes at the end in KSR and AlbM. On the other hand, mouse cultures in KSR showed formation of elongated spermatids cells, and some round spermatids cells were seen in AlbM. In both, rat and mouse, there was a little or no spermatogenesis progression in AlbFF. We never observed spermatozoa when cultures were disaggregated in any condition. Cell DNA content analysis indicated the presence of a haploid population only in cultures of mouse in KSR and AlbM. According with it, some haploid antigens were present in these cultures. Our data suggest that there are differences in the regulation of spermatogenesis in mouse and rat. Rat cultures did not proceed *in vitro* beyond primary spermatocyte, probably due to the absence of some regulatory molecule(s) in the media, which is not necessary in the mouse. This work was supported by FONDECYT N° 1140758.



77) **Novel LC-MS/MS method for simultaneous determination of serum corticosteroids and the role of 11β-HSD enzymes in essential hypertension**

Allende, Fidel[1]., Benitez, Agustin[1].,Silva, Patricio[1].,Campino, Carmen[2,3].,Vecchiola, Andrea[2,3].,Valdivia-Pizarro, Carolina[2].,Rojas, Maria[4].,Lagos, Carlos[2,3].,Baudrand, Rene[2].,Carvajal, Cristian[2,3].,Solari, Sandra[1].,Fardella, Carlos[2,3].,[1]Department of Clinical Laboratories, School of Medicine, Pontificia Universidad Catolica de Chile.[2]Department of Endocrinology, School of Medicine, Pontificia Universidad Catolica de Chile.[3]IMII Millennium Institute on Immunology and Immunotherapy.[4]Department of Family Medicine, School of Medicine, Pontificia Universidad Catolica de Chile. (Sponsored by Supported By FONDECYT 1130427, FONDECYT 1150437, CORFO 13CTI-21526-P1 & IMII P09/016-F Grants.)

Background Cortisol (F) homeostasis is important in maintaining blood pressure and its deregulation is implied in hypertension (HT) and metabolic syndrome (MetS). The availability of F is regulated by the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) which preferentially converts the inactive cortisone (E) to active F and 11β-HSD2, which transforms F to E, protecting the mineralocorticoid receptor from erroneous activation by F. Accordingly, the F to E ratio in serum is potentially useful in evaluating the activity of 11β-HSD in patients with HTA and/or MetS. Therefore, the detection of F and E in serum by liquid chromatography tandem mass spectrometry (LC-MS/MS) is aimed to be a reliable, highly sensitive and selective method. **Aim** To develop and validate of a LC-MS/MS method for the simultaneous determination of F and E in serum to be use in a clinical laboratory for contributing to explain the pathogenesis of hypertension and/or metabolic syndrome through 11β-HSD activity. **Methods** Steroid-free serum enriched with known concentrations of each analyte were used for method optimization and validation. Steroids were extracted from 300 µL of serum with solid phase extraction, using D4-Cortisol and D2-Cortisone as internal standards, being afterwards analyzed in LC/MSMS, with a chromatographic column Intersil® ODS-3 and the ionization source in mode ESI+. For clinical validation of the methodology, serum from hypertensive patients (HT) and controls (NT) were analyzed. **Results** The method quantification range was 1-200 ng/mL with a average correlation coefficient of 0.998 for both analytes, an 85% average recovery, matrix effect between 85-115% for F and E. LOD and LLOQ were 0.2 and 1.0 ng/mL respectively. The coefficient of variation intra and inter assay were and the accuracy between 96-105%. The methodology allowed quantifying F and E in clinical samples, finding average values for F and E in serum of 150 and 23 ng/mL respectively and a significant difference ($p<0.05$) in F/E ratios between the HT and NT evaluated. **Conclusion** The developed LC-MS/MS method is accurate and precise according to FDA and suggested acceptance criteria for method validation. It also showed a good sensitivity, recovery and matrix effect for the simultaneous measurement of F and E. The method provides an innovative tool to be used as a guide by clinical endocrinologist to determine the etiology of essential hypertension and/or metabolic syndrome through 11β-HSD activity.

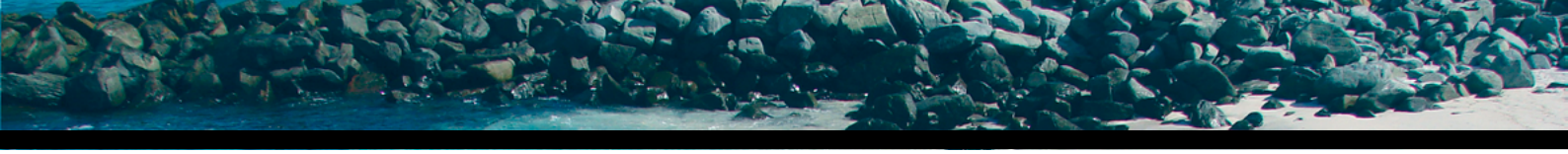
78) **Evaluation of functional connectivity between the prefrontal cortex and hippocampus in freely moving mice**

Negron, Ignacio[1]., Aguilar, Marcelo[1].,Espinosa, Nelson[1].,Aboitiz, Francisco[1].,Fuentealba, Pablo[1].,[1]Departamento de Psiquiatria Pontificia Universidad Catolica De Chile. (Sponsored by We Were Supported By Millenium Center For The Neuroscience Of Memory, NC10-001-F, From The Ministry For Economics, Fomentation And Tourism, Chile, FONDECYT For Postdoctoral Grant Nº 3140370 To I.N-O.)

We have recently shown that prenatal stress induced persistence of spatial memory in an aversive environment in adult mice. This was paralleled with a reduction of firing rate in the prefrontal cortex (PFC), and alterations of functional connectivity between the PFC and hippocampus. This was measured with LFP recordings *in vivo* in anesthetized mice after memory evaluation. Hence, if these neurophysiological alterations are related to neural processes associated to the encoding of space, goal, strategy or another behavioral relevant parameter remains unknown.

To evaluate this possibility, we propose to assess the effect of prenatal stress on neural activity in the PFC and hippocampus in freely moving mice. Due to the lack of commercial devices to record activity simultaneously in these two different brain structures in freely moving mice, we have designed and constructed a microdrive for tetrode recording.

With our microdrive, we have obtained stable recordings of LFP and units for up to 2 months, both in the PFC and hippocampus. Recordings allowed off-line analysis of spectral power both in the PFC and hippocampus, spectral coherence between these structures, and spike sorting in the PFC. We recorded the implanted mice in the Barnes maze for evaluation of spatial memory in an aversive environment. Preliminary results showed an increase of spectral coherence between PFC and hippocampus at the beta-frequency band (20-30 Hz) at the moment when the animal found the escape box, suggesting the encoding of goal location. Spectral coherence increased as the animal progressed through the trials, suggesting that neural representation is dependent on or related to learning. Future studies will allow us to evaluate the effect of prenatal stress on goal representation, or other neurophysiological parameters. Finally, our microdrive will allow us to evaluate neural activity in freely moving transgenic mice, including for the implementation of optogenetic manipulations.



79) **Behavioral and electrophysiological indices of a modified error monitoring in meditators.**

Andreu, Catherine I.[1]., Slagter, Heleen A. [2].,Franken, Ingmar H.A.[3].,López, Vladimir[1].,Cosmelli, Diego[1].,[1]Escuela de Psicología Pontificia Universidad Católica De Chile.[2]Department of Psychology University of Amsterdam.[3]Institute of Psychology Erasmus University Rotterdam. (Sponsored by This Work Was Supported By The National Committe Of Science And Technology Of Chile (CONICYT), Through A Doctoral Scholarship Given To Catherine Andreu (number 21140175).)

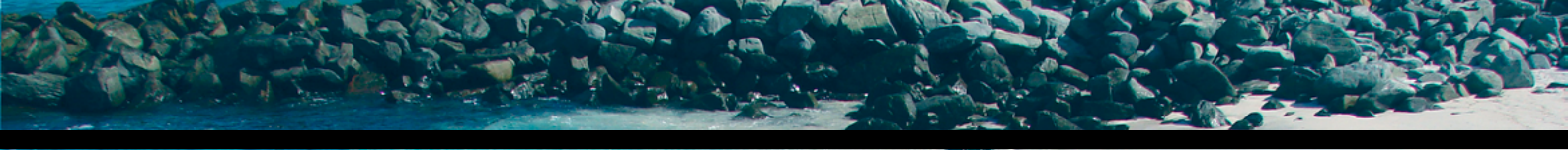
Error monitoring is defined as the ability to monitor ongoing performance in order to detect and correct errors. Impairments in cognitive control processes, such as error monitoring, have been associated with several psychiatric disorders, including ADHD and substance abuse. Recent research indicates that the practice of meditation as a mental training technique, may improve cognitive control. Yet, if and to what extent meditation may enhance error monitoring is currently unknown. The present study addressed this gap in knowledge and examined effects of meditation practice on behavioral and electrophysiological indices of error processing and performance monitoring, specifically the error-related negativity (ERN). Two groups (meditators and non-meditators controls) performed an Eriksen-Flanker task while their brain activity was recorded using electroencephalography (EEG). Behaviorally, meditators showed a significant decrease in the number of errors compared to controls. EEG analyses revealed an increase in the amplitude of the ERN component in meditators compared to controls. These findings, which are indicative of enhanced error monitoring in meditators, suggest that meditation could be a recommendable practice to train and improve error monitoring.

80) **Gestational stress induces resilience to depressive-like behaviors in the post-weaning**

Arriagada, M[1]., Dagnino, A.[1].,[1]Laboratory of Behavioral Neurobiology, Center for Neurobiology and Brain Plasticity, Institute of Physiology, Faculty of Sciences, Universidad De Valparaíso.

Gestational stress can induce depressive-like behaviors in rats during early postpartum. However, it's unknown the effects of gestational stress on post-weaning. The aim of this study was to determine the effects of gestational stress, applied at last third of rat pregnancy, on depressive-like behaviors in the post-weaning. *Sprague-Dawley* rats were pregnant and afterward subjected to daily restraint stress (45 minutes/ 3 times per day). Pregnant rats undisturbed and one group of virgin rats were used as controls. After weaning, depressive-like behaviors were evaluated in the mothers by forced swimming and sucrose preference tests, respectively. Additionally, anxiety-like behaviors and locomotor activity were analyzed by open field and elevated plus maze tests, respectively. Dams that were subjected to gestational stress spent more time in climbing during forced swimming test compared to control animals. Gestational stress did not affect locomotor activity and dams with gestational stress showed more number of entries into the open arms in the elevated plus-maze. We have developed an animal model of postpartum resilience, which could be compared with well stablished animals model of postpartum depression. Thus, we can contribute to understanding of the neurobiological basis of postpartum depression and resilience.

Acknowledgements: This work was supported by FONDECYT 1141276 grant (Alexies Dagnino). Labsite: www.stress.cl.



81) **Characterization of feedback error-related negativity for the study of adaptive behavior and the reward system.**

Astudillo, Aland[1]., Vinales, Laura[2].,Orio, Patricio[1].,Quilodran, Rene[2].,[1]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad De Valparaíso.[2]Medicina, Facultad de Medicina, Universidad De Valparaíso. (Sponsored by FB0008 From Conicyt (PO). The CINV Is Supported By The Millenium Science Inititative (Ministerio De Economía, Chile))

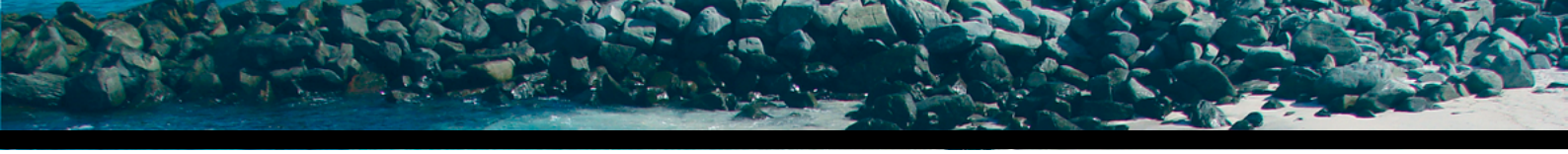
Prefrontal cortex (PFC) activity is related to cognitive control (e.g. planning, problem resolution, performance monitoring), with an important function in optimal behavioral adaptation (Miller & Cohen, 2001). PFC activity is an important target of dopaminergic afferences from the midbrain, and in neurodegenerative diseases such as Parkinson Disease (PD), this network shows altered activity secondary to dopamine depletion in the brain (Rodriguez-Oroz et al., 2009). Therefore, it is important to understand how these alterations affect the pattern of the electrical activity. However, the first step is to understand the relationship between the electrical pattern and the adaptive behavior and the reward system. Here we propose to evaluate cognitive impairments and measure related electrophysiological markers in control subjects by using electroencephalography (EEG) while they perform a trial-and-error problem solving task. This protocol has been designed to evaluate executive functions and modified from event related potentials (ERP) analized in monkey (Procyk & Joseph, 1996; Quilodran, Rothé, & Procyk, 2008). From the characterization of the pattern of a mid-frontal evoked potential, the Feedback Error-Related Negativity (fERN), which is observed mainly in erroneously performances in various cognitive tasks, in association to sensory feedback signaling an error, we can obtain a viable biomarker to characterize the PFC activity (Debener et al., 2005). Current research investigates this pattern for its use in early diagnosis of neurodegenerative diseases (Willemssen, Müller, Schwarz, Falkenstein, & Beste, 2009). In this study we characterize the fERN patterns obtained from subjects, by using temporal and frequency analysis and linking this characterization with the adaptive pattern.

82) **Determining the role of insular cortex in anxiety: a study of anxiety behavior in different zones of the Insula**

Escorza, Tomás[1]., Tamburini, Giovanni[1].,Méndez, Luis[1].,Rojas, Sebastián[1].,Díaz-Galarce, Raul[1].,Moraga-Amaro, Rodrigo[1].,Stehberg, Jimmy[1].,[1]Laboratorio de Neurobiología, Centro de Investigaciones Biomédicas, Universidad Andrés Bello.

Anxiety is a common symptom associated to disease, and stress in the general population, which together with anxiety disorders, have become a huge unresolved problem in public health of emergent economies. Inspite of the enormous prevalence of stress, anxiety and anxiety disorders, our current knowledge on the brain areas and mechanisms associated to anxiety itself is only beginning to be unveiled. Understanding the mechanisms and brain pathways associated to anxiety may lead to the development of novel treatment strategies and drug targets. The Insula or Insular cortex is a complex cortical structure buried deep with the temporal lobe, which has been associated to many brain functions, including anxiety disorders. We set out to determine if the Insula is involved in anxiety and to identify the area within the insula that mediates anxiety. To this end, we performed intra-insular microinjection of AMPA antagonist CNQX in different areas with the insular cortex in rats (rostral anterior (RAIC), medio rostral (MRIC), gustatory (GIC) and the Somatosensory (SIC)) either without previous stress or after 30 min of acute immobilization stress. Anxiety was measured using the Elevated Plus Maze paradigm. Results suggest differential role of the more rostral areas compared to the caudal ones. Our results suggest that different insular cortex areas may have different roles on anxiety and postulate the insula as a critical regulator of anxiety.

This work was funded by Proyecto FONDECYT N°1130724



83) **Effect of humor on decision making: a behavioral and electrophysiological report**

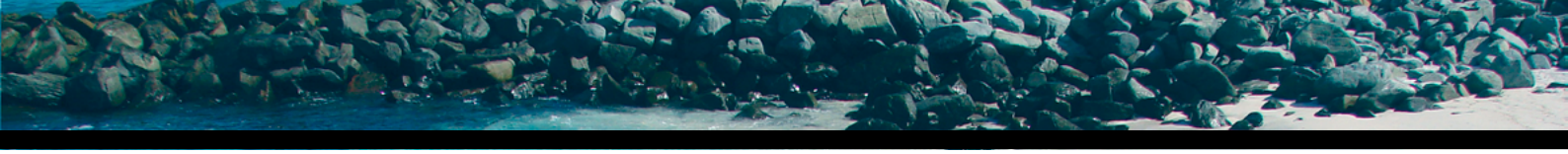
Flores, Jorge[1]., Rodríguez, Eugenio[1].,Campos, German[1].,[1]Psychology, Social Sciences , pontificia universidad de chile. (Sponsored by Beca Doctorado Nacional 2014)

It has been shown that emotional regulation, and especially loss sensitivity (negative emotional reaction after bad decisions) could explain differences in decision making performance in women, when comparing with men during the Iowa Gambling Task (IGT) (van den Bos, Homberg & Visser, 2013). This “loss sensitivity effect” after disadvantageous decisions could be modulated, in fact behavioral results suggests down regulation of negative emotions by humor (Samson & Gross, 2012). So we propose humor to affect IGT performance especially during its first stages which are supposed be guided by emotions (Bechara, 2005). In order to substantiate this idea the participants will be shown humorous or not humorous short films previous to each decision making trial, and after decision is taken fRN potential will be measured. We expect a tendency toward significant statistical differences in IGT performance during the first 2 blocks (trials 1-41) obtaining more long term advantage decisions during the humorous condition, especially in women compared with men. On the other hand, we expect a tendency toward a significant statistical difference in fRN amplitude during the humorous condition, specifically a decreasing in fRN amplitude during the first 2 blocks in women compared with men.

84) **Effects of n-3 PUFAs supplementation on auditory attention of chronically stressed rats.**

Gárate-Pérez, Macarena[1]., Dagnino-Subiabre, Alexies[1].,[1]Laboratory of Behavioral Neurobiology, Center for Neurobiology and Brain Plasticity, Institute of Physiology, Faculty of Sciences, Universidad De Valparaíso. (Sponsored by This Work Was Supported By FONDECYT 1141276 Grant (Alexies Dagnino). Labsite: Www.stress.cl)

Chronic stress impairs auditory attention in rats, while omega-3 polyunsaturated fatty acids (n-3 PUFAs) supplementation induces anti-stress effects. In this context, the aim of this study was to analyze the effect of n-3 PUFAs supplementation on auditory attention of chronically stressed rats. *Sprague-Dawley* rats were trained in a two-alternative choice task (2-ACT), a behavioral paradigm to study auditory attention in rats. Trained animals that reached a performance over 80% of correct trials in the 2-ACT were randomly assigned to control and stress (chronic restraint stress) experimental groups. Afterward, adult animals were supplemented with n-3 PUFAs (DHA and EPA mix) or water. To analyze the effects of chronic stress and n-3 PUFAs supplementation on the auditory attention, trained rats of both groups were subjected to 50 2-ACT trials one day before and one day after of the stress period. A difference score was determined by subtracting the number of correct trials after from those before the stress protocol. Stressed rats that were supplemented with n-3 PUFAs showed an increases of correct trials during the 2-ACT than that of stressed animals treated with water. We speculate that n-3 PUFAs supplementation could be used in the treatment of stress-related psychiatric disorders to improve cognitive functions like attention.



85) **Characterization of the role of Octopamine and Tyramine on locomotor, olfactory and anxiety-related behaviors in *Drosophila melanogaster*.**

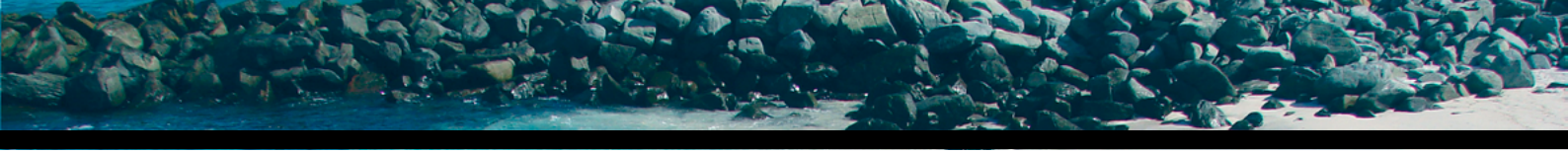
Herreros, Claudia[1]., Fuenzalida-Uribe, Nicolás[1].,Campusano, Jorge[1].,[1]Biologia celular y molecular, Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by Supported By Fondecyt 1141233)

It has been shown that biogenic amines (BAs) play an important role in the generation of complex behaviors, ranging from locomotor control to olfactory learning. In recent years it has been evident the evolutionary conservation of tasks played by Dopamine in the generation and modulation of several behaviors in vertebrates and invertebrates, including the fly *Drosophila melanogaster*.Less is known on the contribution of other aminergic systems to behaviors. Here we studied the role of Octopamine (OA) and Tyramine (TYR) neural systems in innate behaviors in *Drosophila*. Using video recordings, we analyzed several behaviors in single male flies, in absence and presence of an aversive odorant (Benzaldehyde, Bz). We blocked OA/TY neurotransmission by expression of tetanus toxin peptide (Tetx) using the GAL4/UAS system, and assessed the effect of this manipulation in animal behavior. This genetic manipulation induced a decrease in several locomotor parameters and also in Centrophobism, an anxiety-related parameter. On other hand, blocking OA and/or TY release have opposite effects on olfactory acuity to the odorant. Furthermore, our data show that different OArgic clusters have opposite roles in the aforementioned parameters. In sum, these results suggest that OA and TY play complex roles on olfaction, locomotor and anxiety-related behaviors.

86) **Can you control your attention when you are stressed?**

Palacios-Garcia, Ismael[1]., Villena-Gonzalez, Mario[1].,Artigas, Claudio[1].,Jaramillo, Karina[2].,Campos, German[1].,Silva, Jaime[2].,Rodriguez, Eugenio[1].,[1]Facultad de Psicologia Pontificia Universidad Católica De Chile.[2]Facultad de Gobierno Universidad Del Desarrollo. (Sponsored by This Work Was Supported By The National Committe Of Science And Technology Of Chile (CONICYT), Through A Doctoral Scholarship Given To Ismael Palacios (number 21140884) And Funded By Fondecyt 1130810)

Every day we have to divide our limited attentional resources into different external and internal demands, in a process which we could define as attentional control. The Psychosocial stress is a state that promotes the allocation of attentional resources internally, especially to threat-related stimuli such as the social evaluation, the aim of the study is (1) to investigate if psychosocial stress affects the behavioral performance in an attentional shifting task and (2) to search, under an exploratory approach, some of its neural correlates. 40 healthy participants were exposed to either an electroencephalogram-compatible version of the Trier Social Stress Test (TSST) or a control protocol. Additionally, immediately before and after these protocols, subject participated in the attentional shifting task. Manipulation checks were verified through the changes of the heart rate, salivary concentration of cortisol and the score in the anxiety scale in the “stress” condition respect the control. When we compared the behavioral performance in the attentional task prior and after both conditions, we found that the control group showed a clear improvement in performance, characterized by a relative increase of correct trials and a decrease of omissions. Analogously, after the TSST, participants did not showed the same significant increase of performance, moreover, we showed that as far as the scores in the STAI-State increased after the TSST, the number of corrects trials decreased and the number of omissions increased. In addition to behavioral results, we found that the oscillatory activity in alpha (8-12Hz) and gamma bands (30-50 Hz) were different in both conditions. Behavioral and electrophysiological results, suggest that psychosocial stress directs the attention internally, affecting the attentional control and limiting the attentional resources for attending the external demands, which leads to cognitive failures.



87) **Effects of prenatal stress on the development of depressive-like behaviors in infant rats.**

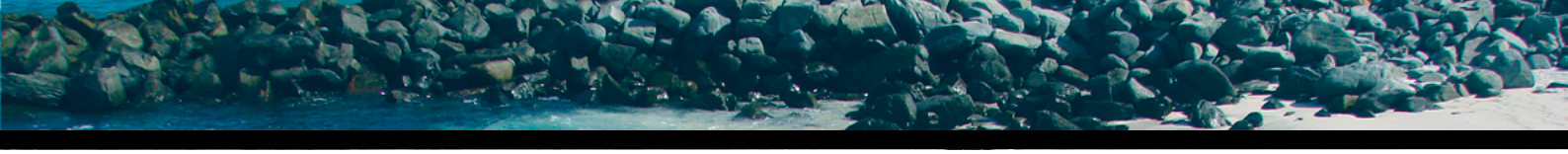
Iturra-Mena, Ann [1]., Dagnino-Subiabre, Alexies[1].,[1]Laboratory of Behavioral Neurobiology, Center for Neurobiology and Brain Plasticity, Institute of Physiology , Facultad de Ciencias, Universidad De Valparaíso. (Sponsored by This Work Was Funded By FONDECYT Grant N° 1141276 To AD-S. Labsite Www.stress.cl)

Depression is a mental disorder that has been studied primarily in adulthood. However, it is currently known that this disease also affects children. Recent research has pointed to prenatal stress as a risk factor to develop depression and anxiety disorders in adulthood, but it is unknown whether it produces the same effect on children. The aim of this study was to determine if prenatal stress induces depression and anxiety-like behaviors in prepuberal rats. For this, female Sprague-Dawley rats were subjected to a restraint stress protocol between gestational days 14 and 21. Control group included pregnant rats that remained undisturbed. Behavioral tests were applied to all male and female pups at postnatal day 24. Locomotor activity, anxiety- and depression-like behaviors (anhedonia and learned hopelessness), social interaction and social play were measured. While the following stress markers were measured: body weight gain, adrenal weight, basal and acute-stress (swim in water at 20°C during 60 seconds) evoked levels of serum corticosterone. Prenatal stress increased the physiological stress markers in prepuberal rats, as well as depression-like behaviors in both males and females, although they showed some differences according gender. In conclusion, we developed a new animal model of childhood depression that can be useful to study the neurobiological basis of this disease at a pre-clinical level.

88) **Low and high-level visual features modulate saccade-related EEG signals in humans.**

Devia, Christ[1,2].,José , Egaña[4,1].,Montefusco-Siegmund, Rodrigo[4,3].,**Maldonado, Pedro[4]**., [1]Departamento de Anestesiología y Reanimación, Facultad de Medicina , Universidad de Chile.[2]Department of Brain and Cognitive Sciences, The Picower Institute for Learning and Memory, Massachusetts Institute of Technology..[3]Department of Psychology, Centre for Vision Research , York University.[4]PDFB, BNI , Facultad de Medicina , Universidad de Chile. (Sponsored by This Work Was Made Possible In Part By A Grant From CONICYT, FONDECYT/Postdoctorado 3140306 To CD And By ICM-P09-015F.)

Our nervous system is adapted to explore the environment triggering a self-paced stimuli input. In visual processing, this active sensing of visual scenes occurs through saccadic eye-movements. On each saccade the eye reaches a visual location, engaging the brain in processing new information. Details of this process come from work on animal models, but little is known about how this occurs in humans. Here, we show that during self-paced image exploration, saccade event-related potentials (sac-RP) are modulated by scenes features and by top-down effects. We recorded electroencephalographic brain activity and eye movements while human subjects freely explored natural and artificial scenes. We found that the sac-RP is the largest brain activity related to self-paced exploration, it is restricted to occipital sites, and is modulated by low-level image features. In addition, our evidence demonstrates that under equal low-level image conditions, high-level image features also modulate the amplitude of sac-RP. Our results suggest that self-paced visual perception in humans is dependent on both low-level and high-level features of the visual scene, arguing in favor of bottom-up and top-down modulations of the activity in the primary visual areas.



89) **Studying the neural correlates of Conscious Perception with a Low-Features visual stimulation: P3b as the earliest ERP NCC.**

Boncompte, Gonzalo[1]., Cosmelli, Diego[1].,[1]Psicología Pontificia Universidad Católica De Chile. (Sponsored by We Would Like To Acknowledge CONICYT For Its Financial Help In The Form Of A PhD Grant To GB)

Conscious perception is an very frequent process in our everyday life, yet the neural substrates necessary for its occurrence are far from being elucidated. Most of the work on this topic focuses on what has been called the Neural Correlates of Consciousness (NCC), neural activity that directly correlates with conscious experience, yet there is a considerable diversity in what has been proposed as NCCs. Influential theories of consciousness like Global Neuronal Workspace and Integrated information Theory emphasize the importance of neuronal integration as a key aspect of perceptual consciousness. In this line a wide spread brain activation of associative cortices, like the P3 component of the Event Related Potential (ERP), has been a consistent candidate for the earliest ERP NCC. However, several studies report differences in the magnitude of early ERPs like P1 as NCC, yet early ERP components like C1 or P1 are related to the processing of visual features of what is presented to subjects, like color, shape and texture. This is why we believe that they are not necessarily linked to conscious perception, thus we hypothesized that if we reduced the amount of feature processing required of what subjects perceive, but we maintain the fact that subjects consciously perceive it, early ERP components should cease to correlate with conscious perception. To test this we designed a visual detection experimental paradigm to compare seen and unseen trials, e.i. those in which our reduced-features target stimulus was consciously perceived and when it was missed. In this paradigm the target stimulus complexity, in terms of the amount of early neuronal processing needed, was maximized. Our results show no ERP component before 200ms in the general ERP waveform, independently of whether subjects detected the target. This is coherent with our low-feature stimulation. Secondly we see a robust P3b, and only when subjects consciously perceived the target stimulus. It appeared consistently for every subject. Moreover, this P3b was not only amplitude-modulated, but was completely absent when subjects missed the target, and with a typical magnitude as compared to results obtained with complex stimulus. Our results support the idea of P3b as the earliest ERP NCC, which is in line with two major integrative theories of consciousness.

90) **Dendritic cells are necessary for the upregulation of the intrarenal RAS and renal sodium transporters in Angiotensin II and high salt**

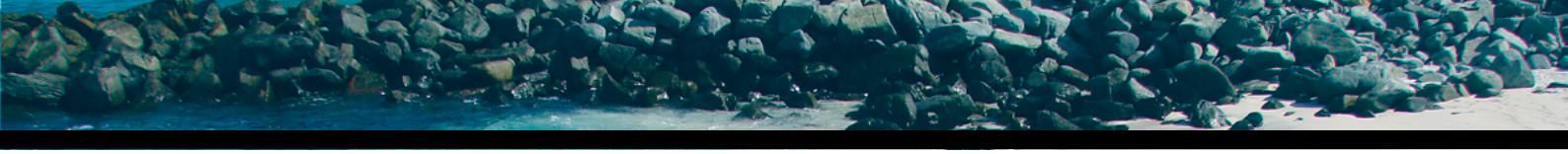
Araos, Patricio[1]., Hevia, Daniel[1].,Fuentes, Eugenia[1].,Prado, Carolina[2].,Pacheco, Rodrigo[2].,Michea, Luis[1].,[1]Fisiología, Medicina, Millennium Institute on Immunology and Immunotherapy, CEMC, ICBM.[2]Laboratory of Neuroimmunology Fundación Ciencia y Vida. (Sponsored by FONDECYT1130550, IMII P09-016-F, BECA CONICYT 21130482)

The development of hypertension caused by Angiotensin II (AngII) and a high salt diet is mediated by a rapid increase in the expression sodium tubule transporters, which leads to a positive sodium balance. In a normal conditions, the intrarenal levels of AngII are ≈200 times de plasma levels due to the presence of the intrarenal renin–angiotensin system (iRAS), the tissular expression of all the classical components of the Renin-Angiotensin –System. The modulation of renal sodium transport depends on the activity of the iRAS. Moreover, recent studies have shown that the AngII plus high salt diet (HS) treatment cause the upregulation of the iRAS, suggesting that this effect could be a major factor causing hypertension and/or renal damage. Our previous studies showed that the ablation of Dendritic Cells (DC) in mice prevented the development of HT in response to AngII+HS. In the present study, we evaluated if the ablation of DCs alters the upregulation of the iRAS and tubular sodium transporters by AngII+HS.

CD11c.DOG mice, for selective loss of DCs ($CD11c^{Hi}$) cells after Diphteria Toxin (DT) injection, received vehicle, AngII+HS (AngII, osmotic minipump 450 µg Kg/day+1% NaCl in drinking water) or AngII+HS+DT (DT, 8ng/g) during 14 days; Paired WT mice received vehicle, AngII+HS or AngII+HS+DT. We measured blood pressure (days 0, 4, 8, 14), and at day 14 we harvested tissues to measure the abundance of renal DCs ($MHC\text{-}II^{+}$ and $CD11c^{+}$ by inmunofluoresce), the iRAS, the sodium-proton exchanger 3 (NHE3), the sodium-chloride cotransporter (NCC) and the Epithelial Sodium Channel (αENaC; qRT-PCR and Western blot).

The injection of DT prevented the development of HT in response to Ang II+HS only in CD11c.DOG mice. CD11c.DOG and WT mice showed increased abundance of DCs in the cortex (peritubular); Only the CD11c.DOG mice showed a sharp reduction of renal DCs after DT injection (n=3). Both, in WT as in CD11c.DOG mice the administration of AngII+HS increased the iRAS (in fold of induction: AGT, 1.5; ACE, 1.9; and AT1R, 5), NHE3, NCC and αENaC (in fold of induction: 4.2; 6; 2), respectively vs vehicle–treated mice ($p<0.05$; n=5-9). The injection of DT concomitant to AngII+HS prevented the changes in sodium transporters and iRAS in CD11c.DOG mice ($p<0.05$ compared to AngII+HS; n=5-9).

We conclude that DCs are required for the modulation of iRAS and tubular sodium transporters by AngII+HS.



91) **Aldosterone downregulates the expression of Sodium Potassium ATPase β3 subunit in kidney and renal collecting duct cells.**

Diaz, Pablo[1]., De Gregorio, Cristián[2].,Cutiño, Andrea[2].,González, Magdalena[2].,Michea, Luis[2].,[1]Fisiología, Medicina, Universidad de Chile.[2]Fisiología Universidad de Chile. (Sponsored by CONICYT, FONDECYT Regular N°1130550; IMIIP09-016-F And BECA CONICYT N°21120658)

Introduction: Sodium homeostasis is regulated by aldosterone, in part by modulating activity of the Na^+-K^+-ATPase (NKA) present in the basolateral membrane of principal cells (PC) of kidney collecting ducts (CD). The NKA is a heterodimer with a catalytic α subunit and a regulatory β subunit. The β subunits may be determinant of subcellular localization and trafficking of the α-β heterodimers, and also may have a role in the cell-cell adhesion that may be relevant in paracellular permeability. The α_1, β_1 and β_3 are the only subunit isoforms expressed in the kidney. Previous studies have focused on the modulation of α_1 expression in response to aldosterone via the mineralocorticoid receptor (MR). However, the role of aldosterone in the isoform-specific regulation of β subunits in the kidney remains poorly understood and the potential modulation of the β_3 subunit by aldosterone has not been analyzed.

Hypothesis: Activation of the MR downregulates the expression of NKA β_3 subunit in CD increasing NKA activity.

Material and methods: C57Bl/6 mice underwent adrenalectomy (ADX) or sham surgery (SHAM). The ADX mice received high salt diet or hormone replacement therapy with deoxycorticosterone (ADX+DOCA, 20 mg/mL). In a second set of experiment mice were administered with spironolactone (Spi, 50 mg/Kg/day) or vehicle (Control) treatment. After 3 days kidneys were harvested (cortex and medulla) for the analysis of NKA α_1, β_1 and β_3 subunits (mRNA and protein abundance by qRT-PCR and Western blot). Finally, we studied the effect of aldosterone in primary culture of inner medullary collecting ducts cells (IMCD, 24 hours).

Results: Adrenolectomy and spironolactone treatment increased the NKA β_3-subunit expression in mouse renal medulla but not in kidney cortex (n=9 $P<0,001$ increase of 50%, SD 0,6; and n=5 $P<0,05$ increase 120%, SD=1). In contrast, neither the ADX nor Spi modified the renal abundance of α_1 or β_1 transcripts and proteins in mice.

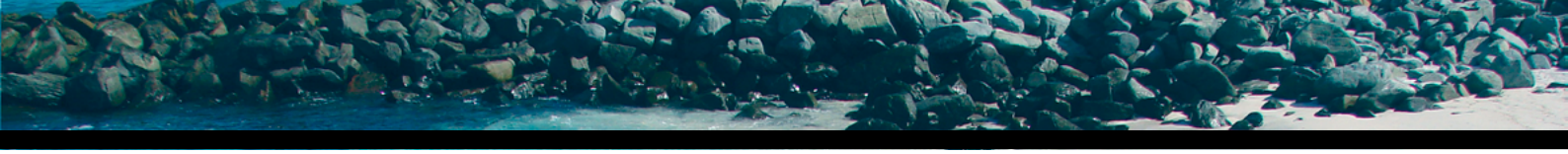
The incubation of IMCD cells in the presence of Aldosterone 10 nM for 24 hours decreased NKA β_3-subunit expression (n=3, $p<0,05$, mean 45%, SD 0,01) and the over expression of NKA β_3 subunit decreased NKA activity.

Conclusions: The results indicate that the NKA β_3 subunit decreases the NKA activity and that increment in NKA activity caused by aldosterone may involve the downregulation the NKA β_3 subunit expression.

92) **Role of Angiotensin II and Vasopressin on the expression of Renin in renal collecting duct cells**

Gonzalez-Vergara, Alex[1]., Salinas-Parra, Nicolás[1].,Henríquez, Ricardo [2].,Gonzalez , Alexis A[2].,[1]Instituto de Quimica, Facultad de Ciencias, Pontificia Universidad Católica De Valparaíso.[2]Instituto de Biología , Facultad de Kinesiología, Pontificia Universidad Católica De Valparaíso. (Sponsored by FONDECYT 11121217)

The renin angiotensin system (RAS) plays a key role on the regulation of arterial blood pressure and salt and water homeostasis. One of the final effectors of the RAS is vasopressin hormone released in response to an increase in plasma osmolality and angiotensin II (Ang II) in the hypothalamus. Recently, it has been shown that all the components of the RAS are expressed in the renal collecting ducts (CD), a segment with a primary role in the reabsorption of salt and water. Despite the suppressed renin expression in juxtaglomerular (JG) cells mediated by Ang II, renin expression is augmented in response to Ang II in the CD, which may promote sodium and water reabsorption. We hypothesize that activation of V2R increases renin expression independent of AT1 receptor in CD cells. In water deprived mice (48 h) pro-renin and renin protein abundance was augmented in renal medullary tissues (free from JG cells). To see if this effect was independent of RAS activation and only due to osmolality, we used the AT1 receptor blocker losartan (30 mg/kg) and angiotensin-converting-enzyme inhibitor captopril (40 mg/kg). Pro-renin band was upregulated, even in the presence of RAS inhibition (~1,5 fold; $P<0.05$) suggesting a vasopressin direct effect. Using intraperitoneal injections of mannitol (20%) as a strategy to increase plasma osmolality we observed an increase in pro-renin and renin protein abundances in medullary tissues (pro-renin~1,4 fold; renin~1,5 fold; $P<0.05$). These results demonstrate that vasopressin stimulates renin synthesis independent of RAS activation.



93) **Angiotensin-(1-7) prevents the skeletal muscle atrophy induced by myostatin decreasing the Smad signaling pathway.**

Aravena, J[1]., Simon, Felipe[2].,Cabello, Claudio[1].,[1]Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad Andrés Bello.[2]Departamento de Ciencias Biológicas, Facultad Ciencias Biológicas, Universidad Andrés Bello. (Sponsored by Association-Francaise Contre Les Myopathies AFM #16670; FONDECYT #1120380, 1121078; IMII #P09-016-F; UNAB DI-741-15/N.)

Skeletal muscle atrophy is characterized by the loss of muscle mass and strength mainly due to an increase in the degradation of myofibrillar proteins such as myosin heavy chain (MHC). Myostatin (MSTN) is a soluble factor that regulates muscle mass. Its levels are augmented in several types of muscle atrophy. Among the effects of myostatin is the increase of the ubiquitin-proteasome system (UPS) activity, specially an increase of Atrogin-1 and MuRF-1, two E3 ubiquitin ligases which result in muscle protein breakdown. MSTN promotes skeletal muscle atrophy through Smad signaling pathway when coupled specifically to activin receptor.

Ang (1-7) is a peptide from non-classic axis of renin-angiotensin system (RAS). We have previously demonstrated that Ang-(1-7) has anti-atrophic activity in skeletal muscle in models dependent on angiotensin II and lipopolysaccharide, two models in which we have found high levels of circulating MSTN. We evaluated the effect of Ang (1-7) on the muscle atrophy and Smad signaling induced by MSTN.

For that, C_2C_{12} myotubes were treated with MSTN in absence or presence of Ang (1-7) and several parameters of atrophy were measured: myotube diameters, proteins levels of MHC, Atrogin-1 and MuRF-1. Smad signaling pathway was evaluated by the phosphorylation of Smad2 (pSmad2).

Our results indicate that Ang-(1-7) has a preventive effect upon atrophy parameters induced by MSTN. Myotube diameters and MHC protein levels were diminished in presence of MSTN, whereas Atrogin-1, MuRF-1 and pSmad2 were increased. Interestingly, Ang (1-7) prevented the atrophic effects induced by myostatin restoring its value to a similar levels observed in the untreated myotubes.

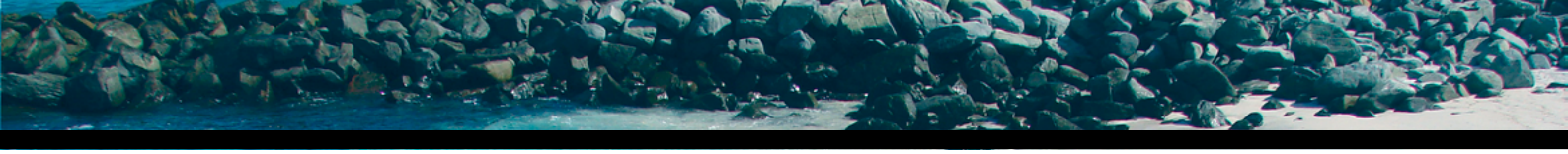
In summary, our results suggest that Ang-(1-7) is a peptide capable to prevent the induction of atrophic parameters and Smad signaling pathway induced by MSTN.

Funding: Association-Francaise Contre Les Myopathies AFM #16670; FONDECYT #1120380, 1121078; IMII #P09-016-F; UNAB DI-741-15/N.

94) **Hydrogen peroxide and nitrite increase in exhaled breath condensate after low-intensity aerobic exercise in non-trained active subjects**

Tuesta, Marcelo[1,2]., Araneda, Oscar [3].,[1]UDA Ciencias de la Salud, Carrera de Kinesiología, Facultad de Medicina, Pontificia Universidad Católica de Chile.[2]Physiology and Immunology Laboratory, Faculty of Biology, Universidad de Barcelona.[3]Integrative Laboratory of Biomechanics and Physiology of Effort (LIBFE), Kinesiology School , Faculty of Medicine, Universidad de los Andes.
(Sponsored by Funded By Fondo De Ayuda A La Investigación (FAI), Universidad De Los Andes Project INOGTO2013, And The National Fund For Scientific & Technological Development (FONDECYT), Project Number 11130082.)

It is a well-established fact that exercise increases pro-oxidants and promotes oxidative stress; however, this phenomenon has been poorly studied at lung level. In non trained subjects, it has been observed an increase on pulmonary pro-oxidants after high intensity aerobic exercise for nearly an hour, but it has not been studied in low-intensity exercise and less in under-trained subjects. Pro-oxidative generation (H_2O_2, NO_2^-), lipid peroxidation markers (MDA) and inflammation (pH) in exhaled breath condensate (EBC) were obtained from 11 active under-trained subjects. All subjects completed two sessions of cycloergometric exercise at low intensity (30-40% Heart Rate Reserve) and equal lung ventilation during 30 and 90 minutes respectively. Samples from both protocols were obtained immediately before, at 20 and 80 minutes post exertion. There were no differences in lung ventilation between both exercise tests, moreover heart rate remained within the established ranges for both protocols. On $[H_2O_2]_{EBC}$ an increasement was observed at 80 post in the 30 min protocol (Pre: 0.13±0.13 µmol·l^{-1} and $Post_{80}$: 0.24±0.17 µmol·l^{-1};p<0.05). This same finding was observed on the 90 min protocol (Pre: 0.08±0.08 µmol·l^{-1} and $Post_{80}$: 0.15±0.01 µmol·l^{-1}; p<0.05). $[NO_2^-]_{EBC}$ showed a tendency towards an increase at 80 post in the 30 min protocol, while there was an increase in the 80 post on the 90 min protocol (Pre: 0.35±0.49 µmol·l^{-1}and 0.92±1.66µmol·l^{-1};p<0.05). There were no differences in $[MDA]_{EBC}$ on both protocols. pH_{EBC} values showed no variations in the 30 min protocol (p=0.35), while there was a tendency towards increase in the 90 minute protocol (p=0.086). In conclusion, low intensity exercise increases lung originated pro-oxidatives in under-trained subjects. There was no evidence of changes on lipid peroxidation or early inflammation.



95) **DIFFERENTIAL EXPRESSION OF CHOP AND GADD34 IN HUMAN FETAL ENDOTHELIUM FROM GESTATIONAL DIABETES.**

Valdivia, Luz[1]., Susana, Rojas[1].,Andrea, Saavedra[1].,Astrid, Haensgen[1].,Marcela, Cid[2].,Marcelo, Farías[3].,Marcelo, Gonzalez[1].,[1]Department of Physiology Universidad De Concepción.[2]Obstetrics & Childcare Universidad De Concepción.[3]Division of Obstetrics and Gynaecology Pontificia Universidad Católica De Chile. (Sponsored by Supported By VRID-Associative 213.A84.014-1.0 And FONDECYT 1121145/11100192.)

Gestational diabetes mellitus (GDM) has been associated with fetoplacental vascular dysfunction, a condition associated with changes in vascular responses to different agonist like U46619 (thromboxane A2 analog) and insulin, which modulate the vascular tone in normal placenta in different ways.The C/EBP homologous protein 10, CHOP, and DNA-damage inducible protein, GADD34, are associated with endoplasmic reticulum (ER) stress. The mechanism of GADD34 induction during cellular stress is not well understood.The ER stress is a condition linked to maternal obesity and GDM, both pathologies associated with insulin resistance (IR).

Objectives: To determine the expression of CHOP and GADD34 in fetal endothelium and vascular reactivity of placental circulation from GDM in the presence and absence of U46619 and insulin.

Methods: Normal and GDM samples were obtained from Hospital Guillermo Grant Benavente, Concepción (ethics committee approval and informed patient consent were obtained). Human umbilical vein endothelial cells (HUVEC) were isolated (collagenase digestion) and maintained in medium 199 (M199) with sera (20%). Total RNA was isolated and CHOP, GADD34 and 28S expression was determined by RT-PCR. A suitable fetal vein and artery pair on the surface of the chorionic plate of normal and GDM, leading to a peripheral cotyledon, was cannulated and continuously perfused with a Krebs solution (95% O_2/5% CO_2, pH 7.4, 37°C). After stabilization, the effects of U46619 (5 nM) and/or insulin (0.1 nM) on perfusion pressure were determined.

Results: In GDM-HUVEC, CHOP and GADD35 mRNA were increased (p<0.05) 2.8-fold and 2.5-fold respect to control, respectively. The high level of CHOP mRNA was reverted by insulin (0.1 nM) but GADD34 mRNA levels was not altered by the hormone in GDM. U46619 (10 nM) increased (5-fold) perfusion pressure of isolated cotyledon of placenta and pre-incubation with insulin attenuated (53%) this response. In GDM, U466619 increases the pressure (3-fold) but there is no effect of pre-incubation with insulin.

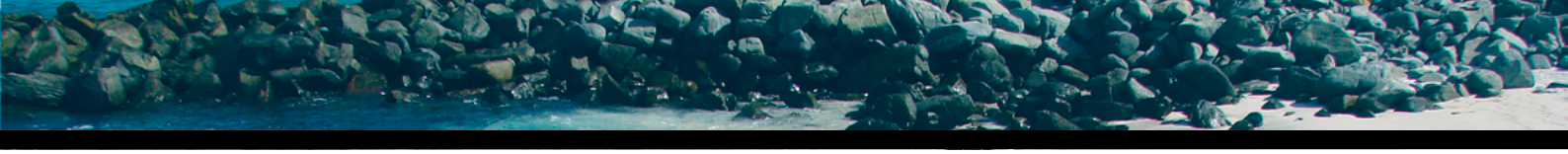
Conclusions: There is an increase of contractile response in GDM placenta associated with high expression of CHOP and GADD34 in fetal endothelium. Insulin relaxation is impaired in GDM, linked to null regulation of GADD34 expression by the hormone.

Supported by VRID-Associative 213.A84.014-1.0 and FONDECYT 1121145/11100192.

96) **Inducción de estrés oxidativo en el hígado graso experimental y suproyección sobre la funcionalidad hepática en ratones machos.**

Lopez-Ortega, Aura[1].,Marquez, Ysabel[1].,Sanabria, Mariana[2].,Plaza, Miguel[3].,Murillo, María Divina[3].,[1]Ciencias Básicas, Ciencias Veterinarias, Centroccidental Lisandro Alvarado.[2]Medicina y Cirugía, Ciencias Veterinarias, Centroccidental Lisandro Alvarado.[3]Farmacología y Fisiología, Ciencias Veterinarias, Zaragoza.

El daño celular debido al estrés oxidativo (EO) desempeña una función vital en la patogénesis de las alteraciones hepáticas, entre ellas el hígado graso (HG). En medicina veterinaria, el rol del EO en la producción y reproducción animal ha adquirido relevancia debido al deterioro de ambas funciones en animales con hepatoesteatosis. El objetivo de este estudio fue determinar si el HG experimental inducido por etionina (7,5 mg/20 g peso corporal), en ratones NMRI machos adultos, provocaba EO y afectaba la función hepática. Se utilizaron dos grupos de 10 animales: uno control y otro tratado con DL-etionina. El HG se evaluó por métodos histológicos y por cuantificación de los triglicéridos hepáticos, que indicaron hepatoesteatosis en los machos inyectados con etionina. Como parámetros de EO, se determinó por espectrofotometría la concentración hepática de malondialdehído (MDA) y de dienos conjugados (DC). Para evaluar la funcionalidad hepática se cuantificó la concentración plasmática de las aminotransferasas ALT y AST a través de kits comerciales. La inducción de HG causó una elevación significativa del MDA: de 364,91±17,73 nmoles/mg proteínas a 852,91±55,26 nmoles/mg proteínas ($P<0,001$), así como de los DC: de 231,18±15,53 mmoles/mg proteínas a 297,45±23,10 mmoles/mg proteínas ($P<0,027$). En el HG, la actividad plasmática de las aminotransferasas aumentó significativamente: ALT de 59,40±5,16 U/l a 169,86±18,78 U/l ($P<0,001$) y AST de 158,35±13,54 U/l a 241,93±10,14 U/l ($P<0,05$). Estos resultados muestran que en el HG inducido por etionina en ratones machos se produce un EO que podría ser responsable de la alteración en la funcionalidad hepática.



97) **Cannabinoid receptor type 1 modulates the effects of polyunsaturated fatty acids on memory consolidation of stressed rats**

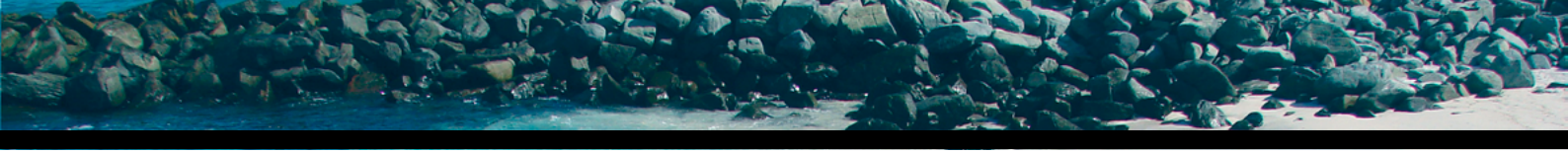
Jujihara, Germán[1]., Dagnino-Subiabre, Alexies[1].,Peñaloza Sancho, Valentín [1].,[1]Laboratory of Behavioral Neurobiology, Center for Neurobiology and Brain Plasticity, Institute of Physiology, Sciences, Universidad De Valparaíso. (Sponsored by Support:This Work Was Funded By FONDECYT Grant N° 1141276 To AD-S. Labsite: Www.stress.cl.)

It has been shown that omega-3 polyunsaturated fatty acids (n-3 PUFAs) have anti-stress effects in rats, while endocannabinoids regulate learning and memory. Thus, relationship between PUFAs and endocannabinoid system on stress responses remain unknown. The aim of this study was to evaluate whether cannabinoid receptor type 1 (CB_1) regulates the effects of PUFAs on memory of stressed rats. Male Sprague-Dawley rats were subjected to chronic restraint stress. In the course of the stress period, animals were supplemented with n-3 PUFAs (fish oil) or n-6 PUFAs (primrose oil) as well as vehicle (i.p., physiologic serum) or AM251 (CB_1 antagonist; i.p., 0.3 mg/kg/day) or WIN55,212-2 (CB_1 agonist; i.p., 0.5 mg/kg/day). Afterward, CB_1 levels were determined by immunofluorescence and memory consolidation was evaluated in the Morris water maze. CB_1 levels were up-regulated in CA1 and CA3 of stressed compared to that of controls, while AM251 and n-3 PUFAs improved the stress-induced memory impairments. Moreover, n-6 PUFAs impaired memory and this effect was prevented by AM251, but not by WIN55,212-2. These results suggest that chronic stress and n-6 PUFAs increases the endocannabinoid system activity, which in turn decreases memory of rats. Conversely, AM251 and n-3 PUFAs would decline the effects of stress-induced endocannabinoid system overactivity in the hippocampus. This study opens a new approach to understand the interactions between stress and PUFAs by endocannabinoid system.

98) **Structural insights on the rP2X4 receptor channel allosteric activation by alfaxolone from electrophysiology to molecular dynamics simulations**

Alveal, Natalia[2]., García-Huidobro Toro, Juan Pablo[1].,Navarrete, Camilo[2].,Barrera, Nelson P.[2].,[1]Biología, Química y Biología, Universidad De Santiago De Chile.[2]Physiology, Biological Sciences, Pontificia Universidad Católica De Chile. (Sponsored by Funded By ICM P10-035F, Fondecyt 1141132 And 1120169, And Anillo ACT-1108 Grants.)

Danio rerio P2X4 receptor crystals (zfP2X4R) with (holo) and without ATP (apo) in the orthosteric site offered the possibility of understand at the structural/atomic level the function of this receptor channel. Alfaxolone, a prototype neurosteroid, activates Ca^{2+} currents in *Rattus novergicus* P2X4 receptor (rP2X4R) through a positive allosteric modulation. Larger steroid concentrations, through an interaction occurring likely in the transmembrane (TM) rP2X4R domain, elicited *per se* an ionic current suggesting pore opening in the absence of ATP (Codocedo et al., 2009). Our aim is to characterize the steroid binding site and understand how the structure of the pore changes in response to alfaxolone binding to the TM domain of rP2X4R in the absence of ATP. Based on the crystallized zfP2X4R in the apo and holo states, corresponding rP2X4R models of the extracellular and TM domains for both states were built, including the N and C-terminus cytoplasmic tails which are absent in the crystallized zfP2X4R. These structures were used for the construction of three models: apo, apo with 3 docked alfaxolone molecules (apo-alfax), and holo rP2X4R. All-atom molecular dynamics (MD) simulations of the three models embedded in a lipid membrane environment and solvated were run for 100 nanoseconds. MD analysis of the apo-alfax trajectory maps the steroid binding site to the TM domain and show how the neurosteroid interaction triggers initial conformational changes in the TM domain that permit water pass through the gate. Results reveal that in the apo state, 3 alfaxolone molecules interact with the TM domain throughout the simulation; however, only a single steroid forms hydrogen bonds across the subunit interface of the TM, while the other two steroid molecules only bind to the TM domain of the binding subunit. It is already known that P2X4R activation involves the separation between TM helices of neighbouring subunits in the pore area (Hattori & Gouaux, 2012). Single alfaxolone binding partially opens the rP2X4R pore, revealed by the increases in the solvent accessible surface area for TM residues when comparing the apo-alfax and apo states. This feature is also observed between the apo and holo structures of both rP2X4R and zfP2X4R. Alfaxolone binding further produces a strong effect on the greater number of hydrogen bonds between the TM1 and TM2 of the subunit that interacts with it, reminiscent of the TM conformation in the holo state. These studies provide insights of the mechanism for positive allosteric activation of rP2X4R, helping to understand the motion of the TM domain during rP2X4R channel gating.



99) **Molecular dynamics simulations of the dynamin-2 mutation R465W: impact on dynamin-2 monomer structure and dimer interactions**

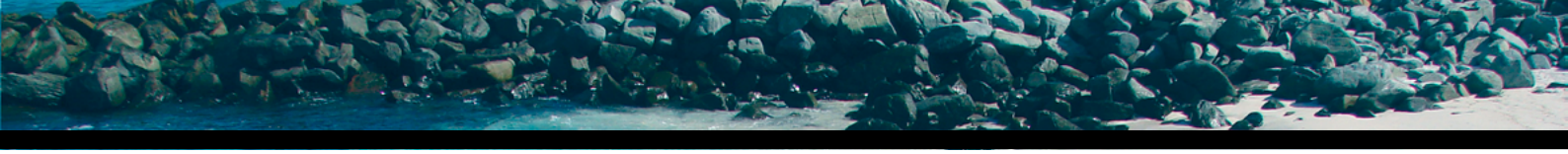
Hinostroza, Fernando[1]., Maraboli, Vanessa[2].,Cardenas, Ana[3].,Gonzalez, Danilo[2].,[1]Centro Interdisciplinario de Neurociencia de Valparaíso, Ciencias, Universidad De Valparaíso.[2]Centre for Bioinformatics and Integrative Biology Universidad Andrés Bello.[3]Centro Interdisciplinario de Neurociencia de Valparaíso Universidad De Valparaíso. (Sponsored by This Work Has Been Supported By Proyecto Anillo ACT-1121 (CONICYT) And Centro Interdisciplinario De Neurociencia De Valparaíso (CINV).)

Dynamin-2 is a cytosolic GTPase that plays a key role in several cellular processes like endocytosis. Specifically, it catalyzes membrane fission during endocytosis. This protein has five different domains: a GTPase domain (G domain) that hydrolize GTP, a middle domain that interacts with other dynamin, a pleckstrin homology domain (PH) that binds phospholipids, a GTPase effector domain, and a proline-arginine rich domain (PRD) that interacts with SH3 protein containing domain. Mutations in this enzyme produce centronuclear myopathy (CNM), a congenital disease characterized by a progressive muscular weakness. The most common mutation is R465W in the middle domain. *In vitro,* this mutation forms more stable oligomers and hydrolizes more GTP than wild type dynamin. In the absence of crystallographic structure, a structural model of dynamin-2 has been built, using homology modeling techniques. Using this new model and Molecular Dynamics (MD) simulations, we can elucidate structural features produced by R465W mutation in Dynamin-2 monomer and dimer. We observed preliminarily that exchanging an arginine for a tryptophan in the position 465 increase the number of interactions between dynamin monomers. Our results explain at molecular level why this mutation forms more stable oligomers *in vitro*.

100) **The endogenous agonist dopamine at the D_1 dopaminergic receptor. A molecular dynamics study**

Hugo, Estefanía[1]., Fierro, Angelica[2].,Cassels, Bruce[1].,[1]de quimica, de ciencias, Universidad De Chile.[2]de organica, química y farmacia, Pontificia Universidad Católica De Chile. (Sponsored by Grants From FONDECYT, N° 1110146, And CONICYT, N° 24121296.)

Dopamine (DA) is chemical messenger in blood vessels and different organs. In the central nervous system (CNS) it modulates neuronal activity related to many disorders such as addictions, schizophrenia, depression and Parkinson's disease. However, the mechanism involved in dopamine receptor (DR) function is still unknown. Some authors have proposed, on the basis of mutagenesis studies and molecular simulations, the ways in which various residues in transmembrane (TM) segments III, V and VII might participate in complexes between agonists and the D_1 receptor type (D_1R),[1,2] but the specific relationships among them and DA has not been explored yet. Such structural studies have given us some insights about the general architecture and conformational states of G-protein coupled receptors (GPCR), although the molecular models used have relied on the crystal structures of non-DA receptors as templates. Using the closely related D_3R crystal structure as a template, we have built a 3-D structure of the D_1R by homology modeling in order to describe its transition from the inactive to the active state on binding DA. Our molecular simulation results show that DA stays in the binding pocket proposed for catecholamine receptors, forming a salt bridge between the protonated amine group and aspartate residue Asp3.32. Less predictable on the basis of current knowledge is an evolving network of hydrogen bonds between the catechol moiety and TMV, TM III and TM VI which seems to be crucial for receptor activation and a key to the design of new D_1R agonist drugs. Importantly, the salt bridge near the cytoplasmic ends of TM III and TM IV breaks as a consequence of DA binding, as had been proposed as part of the GPCR activation mechanism. **1. J. P. Cueva, A. Gallardo-Godoy, J. I. Juncosa, P. A. Vidi, M. A. Lill, V. J. Watts, D. E. Nichols, Journal of Medicinal Chemistry. 54, 5508 (2011) 2. B. R. Chemel, L. A. Bonner, V. J. Watts, D. E. Nichols, Molecular Pharmacology, 2012, 81, 729 (2012)**



101) **Pharmacophore and shape-based virtual screening identification of selective 11β-HSD1 inhibitors**

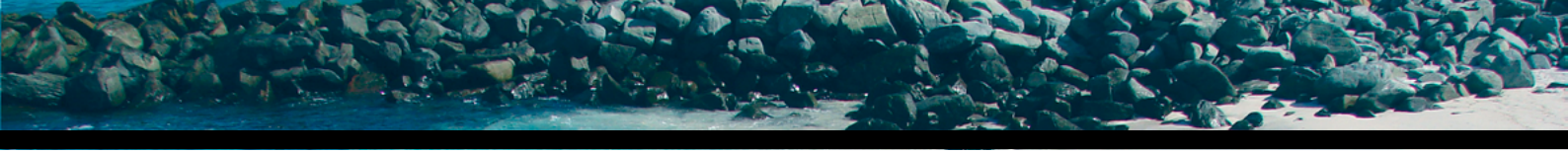
Lagos, Carlos F.[3,1]., Vecchiola, Andrea [3,1].,Ortiz-Canales, David[3].,Allende, Fidel[2].,Fuentes-Ibacache, Nataly[3].,Fuentes, Cristobal[3].,Gonzalez-Gomez, Luis Martin[3].,Solari, Sandra[2].,Baudrand, Rene[3].,Campino, Carmen[3,1].,Cifuentes, Mariana[4].,Carvajal, Cristian[3,1].,Fardella, Carlos[3,1].,[1]IMII Millennium Institute on Immunology and Immunotherapy.[2]Dept. of Clinical Laboratories, School of Medicine, Pontificia Universidad Catolica de Chile.[3]Department of Endocrinology, School of Medicine, Pontificia Universidad Catolica de Chile.[4]Institute of Nutrition and Food Technology (INTA) Universidad de Chile. (Sponsored by Supported By CORFO 13CTI-21526-P1, FONDECYT 1150437 & 1130427, And IMII P09/016-F Grants. CFL Acknowledges OpenEye Scientific Software And Inteligand:GmbH For Academic License Of Their Products, And The DTP/NCI For Providing The Compounds Screened In Thi)

Background 11-beta hydroxysteroid dehydrogenase type 1 (11β-HSD1), regenerates cortisol (F) from inactive cortisone in key metabolic tissues, and is a therapeutic target for central obesity, and metabolic syndrome. Herein we report the identification of novel selective non-steroidal 11β-HSD1 inhibitors using pharmacophore and shape-based virtual screening and *in vitro* assays in human adipose cell-line. **Methods** Crystal structures of human 11β-HSD1 were retrieved from the Protein Data Bank database. Protein preparation, structural alignment, and ligand clustering were performed using Discovery Studio v2.1 (Accelrys Inc). Structure-based pharmacophore models were generated with LigandScout v3.1 (Inteligand), and ligand three-dimensional alignment for each cluster was used to generate the shape-based queries. The OpenNCI database (~260,000 compounds) conformers were generated by OMEGA v2.5.1.4 (OpenEye). Virtual screening of the database was performed with ROCS v3.2 (shape-queries) and further scored and ranked by accomplishment with the corresponding pharmacophore model. Compounds were obtained from the Developmental Therapeutic Program (DTP/NCI), and 11β-HSD1 activity assays performed in differentiated LS-14 adipose cell line. Cortisone (E) and Cortisol (F) production were quantified by LC-MS/MS. The citotoxicity of the compounds was determined using the MTT assay (Promega). **Results** A set of 25 11β-HSD1-ligand complexes was structurally aligned, and the Cα-RMSD for the superimposition of all proteins was less than 2Å. Enrichment rates for the shape-query and pharmacophore models were higher than 75%. The top 1000 hits from shape-based query virtual screening filtered with the pharmacophore models render the best 100 hits. A final selection of 40 compounds was obtained from the DTP/NCI and biologically assayed, showing no effect over cellular viability. Inhibition assays identified 2 novel hit compounds displaying enzyme inhibitory activities in the low micromolar range in cell-based assay. **Conclusion** Virtual screening and subsequent *in vitro* evaluation of promising hits revealed several selective inhibitors. Efficient inhibition of human 11β-HSD1-mediated cortisol production in LS-14 adipocytes was demonstrated for 2 compounds, which display selectivity for the reductase activity of 11β-HSD1 and over 11β-HSD2 isoform.

102) **New N-Arylsulfonylindoles based serotonin 5-HT6 antagonists. Synthesis and binding evaluation studies**

Lagos, Carlos F.[1,2]., Vera, Gonzalo[3].,Almendras, Sebastian[3].,Hebel, Dan[3].,Flores, Francisco[3].,Valle-Corvalan, Gissella [4].,Pessoa-Mahana, Carlos David[3].,Mella-Raipan, Jaime[4].,Recabarren-Gajardo, Gonzalo[3].,[1]Department of Endocrinology, School of Medicine, Pontificia Universidad Catolica de Chile.[2]Facultad de Ciencia Universidad San Sebastian.[3]Department of Pharmacy, Faculty of Chemistry, Pontificia Universidad Catolica de Chile.[4]Department of Chemistry and Biochemistry, Faculty of Sciences, Universidad de Valparaiso. (Sponsored by Supported By Proyecto FONDECYT INICIACION 11121418.)

Background The 5-HT_6 receptor is the most recently identified and cloned member of the serotonin (5-hydroxytriptamine, **5-HT**) receptors family. Previous studies demonstrated that 5-HT_6 receptor has a major role in obesity, thus boosting the search for novel selective 5-HT_6 receptor antagonists. All these reported antagonists share a common pharmacophore consisting of a sulfonamide moiety separated from basic ionizable amine functionality by an aryl group and linker. Based on this known pharmacophore model for 5-HT_6 receptor antagonists, a series of novel derivatives based on the indole scaffold were designed and identified as a new class of 5-HT_6 receptor ligands. **Goals** To design, synthezise, determine the pharmacological profile and identify structure-activity relationships for a novel series of *N*-arylsulfonylindole compounds, targeted to 5-HT_6 receptor. **Methods** The pharmacophore model for *N*-arylsulfonylindole class of 5-HT_6 ligands was explored. The designed compounds were synthesized by classic organic chemistry and structures confirmed through spectroscopic methods. Radioligand competition binding assays were performed against [^{125}I]-SB-258585, in HEK293 cells expressing human 5-HT_6 receptors with Clozapine as control.The pharmacological profile was assessed by intracellular calcium mobilization assay using 2-Me-5HT as control. **Results** All compounds tested displayed inhibition of [^{125}I]-SB-258585 binding to 5-HT_6 receptors. Compounds **4b**, **4f**, **4g**, **4i**, and **4j** were the most potent compounds with K_i values of 13.6 nM, 369 nM, 18.4 nM, 149 nM and 14.6 nM respectively, and Clozapine displayed a K_i value of 11.9 nM (IC_{50} value of 12.4 nM). All ligands evaluated showed antagonist profile, and reduce the effects of addition of 2-Me-5-HT. In this assay the most potent compound was **4j** which IC_{50} value was 32 nM. CoMFA contour maps analysisindicates that major contribution to activity is given by the steric properties of the compounds rather than the electrostatic potentials**. Conclusion** We present the design, chemical synthesis, biological evaluation and CoMFA based QSAR studies of novel antagonists of the 5-HT_6 receptor. A convenient synthesis of the extended arylpiperazine derivatives was achieved to readily access diversely substituted analogues. Several of the tested compounds exhibited nanomolar affinity for the 5-HT_6 receptor. Finally, two compounds (**4g** and **4j**) showed strong inhibition of 2-Me-5HT induced Ca^{2+} mobilization in a cell-based assay, suggesting the potent cellular activity may be induced through antagonism of 5-HT_6 receptor.



103) **Kappa opioid control on dopamine basal levels in dorsal striatum: study of no-net flux microdialysis**

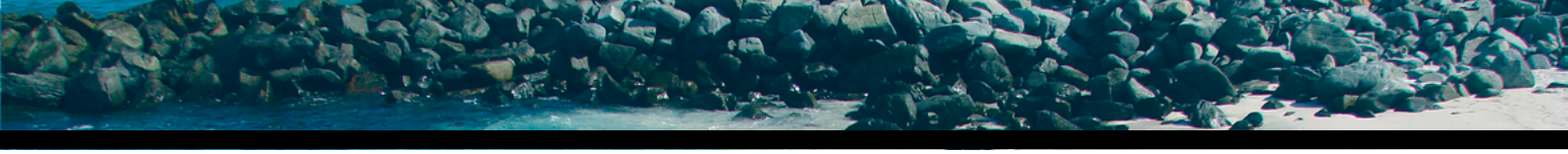
Azócar, V.H.[1]., Aguilera, C.[1].,Fuentealba, J.A.[1].,[1]Laboratorio de Neuroquímica, Departamento Farmacia, Facultad de Química, Pontificia Universidad Católica De Chile. (Sponsored by Sponsored By FONDECYT N° 1141088)

The dopaminergic neurotransmission in the ventral and dorsal striatum is involved in several functions like motor control, motivation, learning and reinforcement. The extracellular levels of dopamine (DA) in the ventral striatum are regulated by a complex interaction between dopaminergic D2 auto-receptors (D2R), kappa opioid receptors (KOR) and dopamine transporter (DAT) (Meiergerd et a., 1993; Chefer et al., 2005). However, there is a lack of evidence regarding how these systems control DA extracellular levels in the dorsal striatum. The aim of the present study is to assess the effect of KOR and D2 receptor blockade on both DA extracellular levels as DAT activity in the rat dorsolateral striatum (DLS). Conventional microdialysis experiments were carried out to study the effect raclopride (D2 antagonist) and norBNI (KOR antagonist) on DA extracellular basal levels. The DAT activity was study using the no-net flux microdialysis technique (Smith and Justice, 1994). The results show a dose-dependent increase in DA extracellular basal levels after norBNI perfusion in DLS (169.7±19.08% relative to DA baseline, p=0.0412). Also nor-BNI perfusion significantly increase the extraction fraction (Ed), an indirect measure of DA uptake (0.243±0.016 versus 0.311±0.005, p=0.0235). Surprisingly, the DA basal levels remain constant after the perfusion of several concentration of raclopride in DLS. Moreover, a non-significant decreased in Ed was observed after raclopride perfusion in DLS (0.243±0.016 v/s 0.196±0.016, p=0.091). These evidences demonstrate that KOR exerts a tonic inhibitory control on DA extracellular levels in DLS and suggest a presynaptic control of dynorphin on DA release. Our results also demonstrate that KOR activation decrease Ed and suggest that dynorphin decreases DA uptake in DLS. On the other hand, a tonic D2R inhibitory control on DLS DA extracellular levels was not observed with raclopride doses used in this work. Regarding to Ed, our results suggest that D2R activation increases the DA uptake. In conclusion, in the DLS the KOR controls both DA extracellular basal levels as DAT activity. However, in DLS D2R appears to control only DAT activity. These results show that dopaminergic neurotransmission in the dorsolateral striatum differs from that observed in the ventral striatum, where an important D2R mediated presynaptic control is observed.

104) **GR2 and α_1-receptor expression in the PVN and effects of their activation on the hypothalamic-pituitary-adrenal axis in fetal malnourished rats.**

Pérez, Hernán[1].,Urrutia, Patricia[1].,Sánchez, Sussan[1].,Aravena, Marcela[1].,García, Carolina[1].,Morgan, Carlos[2].,Barra, Rafael[3].,**Hernández, Alejandro[4].**, [1]Laboratorio de Fisiología Patológica, Facultad de Medicina, Universidad Pedro De Valdivia.[2]Laboratorio de Nutrición y Regulación Metabólica, Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad De Chile.[3]Unidad de Farmacología, Facultad de Ciencias Médicas, Universidad De Santiago De Chile.[4]Departamento de Biología, Facultad de Química y Biología, Universidad De Santiago De Chile. (Sponsored by Funded By Fondecyt 1080684, Fondecyt Postdoctorado RB3130573 And A Grant From Pedro De Valdivia University.)

In utero calorie-malnourished rats develop hypertension when adults. In previous studies we showed that, in malnourished rats (i) basal neuronal activity in the paraventricular nucleus (PVN) of the hypothalamus is increased, (ii) excitatory noradrenergic input to the PVN from the locus coeruleus is enhanced, and (iii) corticosterone feedback control of the hypothalamic-pituitary-adrenal axis is decreased. To study whether hyperactivity of PVN neurons in prenatal malnutrition-induced hypertension is the result of increased noradrenergic input and/or decreased corticosterone feedback control to the PVN, we studied the effect of the intra-PVN microinjection of the α_1-adrenergic agonist phenylephrine or the GR2 agonist dexamethasone on the multiunit neuronal activity of the PVN, systolic pressure and heart rate, and plasma levels of corticosterone in rats undernourished during fetal life and in control eutrophic animals. Fetal malnutrition was induced by restricting the diet of pregnant mothers to 10 g daily. In addition, the Bmax (Scatchard analysis) and the expression levels (immunohistochemical assay) of GR2 and α_1-adrenoceptors were measured in the whole hypothalamus and the PVN, respectively. At day 40 of postnatal life: (i) one day after administration, dexamethasone intra-PVN induced significantly lower effects on PVN neurons, systolic pressure, heart rate and plasma levels of corticosterone in malnourished animals, as compared to controls; (ii) thirty min after injection, phenylephrine intra-PVN produced similar increases of PVN neuronal activity, systolic pressure and heart rate in malnourished and control animals; and (iii) Bmax and expression level for GR2 were enhanced in the hypothalamus and PVN of malnourished rats, while there were no differences for the α_1-adrenoceptor. Data suggest that the increased activity of PVN neurons and the subsequent hypertension mainly results from a decreased sensitivity of PVN neurons to corticosterone negative feedback found in malnourished animals, rather than from increased sensitivity to coerulear noradrenergic input.



105) **Purinergic Signaling differentially regulates the proliferation of Normal and Gastric Cancer cells through $P2Y_2$ and P2X4 receptors**

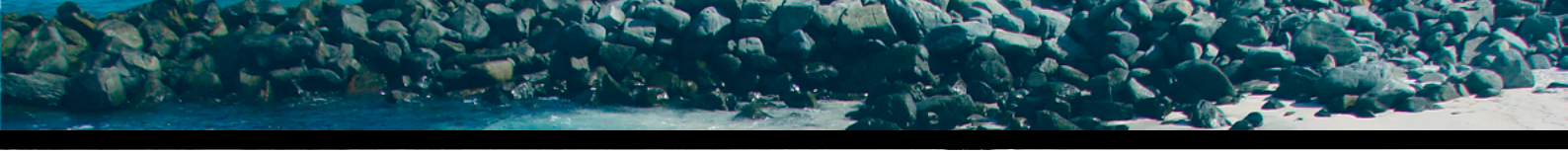
Hevia, María José[1].,Ramírez, Sebastían[1].,Bernal, Giuliano[1].,**Coddou, C[1]**., [1]Departamento de Ciencias Biomédicas, Facultad de Medicina, Universidad Católica Del Norte. (Sponsored by FONDECYT 11121302)

Gastric Cancer (GC) is the leading cause of cancer-induced deaths in our country with an estimated mortality of 20/100000 inhabitants over the last ten years. Previously, we have found that the mRNA for the purinergic receptor $P2Y_2$ is significantly increased in GC samples as compared to adjacent healthy mucosa taken from patients diagnosed with GC. The expression of the $P2Y_2$ receptor is increased in other types of cancer and its activation promotes cell proliferation through the G_q-IP_3/DAG signaling pathway. In order to study the role of purinergic signaling in GC, we used AGS cells, a cell line derived from a gastric tumor, and performed proliferation studies with MTT. ATP regulated AGS cell proliferation in a biphasic manner, increasing cell proliferation at 10 and 100 µM, but at 300 µM ATP cell proliferation was significantly inhibited. On the other hand 1-300 µM UTP, a selective $P2Y_2$ agonist, increased cell proliferation in a concentration-dependent manner. The effects of UTP were prevented by the addition of the wide range purinergic antagonist suramin. Moreover, we found that ATP and UTP can elicit increases in intracellular calcium in AGS cells, confirming the functional expression of purinergic receptors. These differences between ATP and UTP suggests that there are other purinergic receptors expressed in AGS cells. To search for other P2 receptors we performed real-time PCR and found that besides $P2Y_2$, AGS cells also express the P2X4 receptor, a ligand-gated ionic channel. Western-blot analysis confirmed the presence of both $P2Y_2$ and P2X4. Finally, we studied the expression of these receptors in both tumoral and healthy tissues derived from patients diagnosed with GC and found that whereas in tumor-derived tissue the expression of $P2Y_2$ is significantly increased, the expression of P2X4 is significantly decreased, as compared to healthy tissues. Taken together, these results demonstrate the involvement of different purinergic receptors and signaling in GC, and the pattern of expression changes in tumoral cells, and this change probably directs ATP and nucleotide signaling from an anti-proliferative effect in healthy cells to a proliferative effect in tumoral cells.

106) **THYROID HORMONE INDUCES LIVER PROTEIN DISULFIDE ISOMERASE AND ENDOPLASMIC RETICULUM OXIDO REDUCTIN-1α BY A REDOX-SENSITIVE MECHANISM.**

Cornejo, P[1]., Fernández, Virginia[2].,Vargas, Romina[3].,Carrasco, Juan[4].,Fernández, Javier[4].,Videla, Luis[4].,[1]Escuela de Tecnología Médica, Facultad de Salud y Odontología, Universidad Diego Portales.[2]ICBM, Programa Farmacología, Medicina, Universidad De Chile.[3]ICBM; Programa farmacología, Medicina, Universidad De Chile.[4]ICBM, Programa de Farmacología, Medicina, Universidad De Chile. (Sponsored by Acknowledgements: Proyecto FONDECYT 1150104)

T_3 triggers oxidative stress (OxS) in the liver, linked to its calorigenic effect with concomitant endoplasmic reticulum stress and PERK activation, in response to protein oxidation (UPR). The effect of T_3 and the antioxidant N-acetylcysteine (NAC) on the PDI/ERO1α system, catalyzing disulfide bonds generation in UPR, was evaluated. Following the administration to Sprague Dawley rats of 0.1 mg T_3/kg and/or 0.5 g NAC/kg for 3 consecutive days, oxidative stress parameters and the expression of PDI and ERO1α (qPCR and Western-blot) were assessed. T_3 induced significant enhancements in the rectal temperature ($p<0.05$) and in the liver content of 8-isoprostanes and oxidized proteins, with concomitantly elevated mRNA and protein levels of PDI and ERO1α. As these effects were suppressed by NAC, it is concluded that OxS has a causal role in PDI/ERO1α induction, essential for UPR and its role in protein homeostasis in liver OxE injury.



107) **Glycine Receptor β Subunit: A Critical Target for Pain Sensitization**

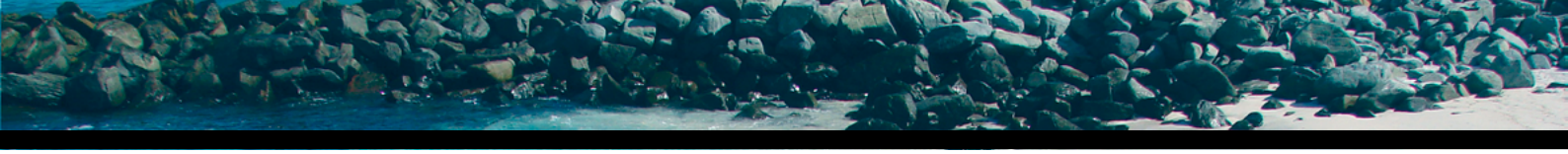
Galaz, Pablo[1]., Cespedes, Nicole[1].,Jorquera, Manuel[4].,Treuer, Adriana[2].,Ponce, María José[4].,Utreras, Jonathan[4].,Coronado, Cesar[3].,Mariqueo, Trinidad[4].,[1]Departamento de Bioquímica y Biología Molecular Universidad De Chile.[2]Instituto de Química de los recursos naturales Universidad De Talca.[3]Departamento de Morfo-función, Escuela de medicina, Universidad Diego Portales.[4]Escuela de medicina Universidad De Talca. (Sponsored by Programa De Excelencia De Investigación En Química Y Bioorgánica De Recursos Naturales (PIEI-QUIM-BIO), Universidad De Talca)

Glycine receptors (GlyRs) are the main inhibitory chloride channels that control the excitability of spinal cord. Plastic changes in the excitability of peripheral and central nociceptive pathways accounts for development of chronic pain. Previous studies have shown the relevance of α3 homomeric GlyRs in pain sensitization induced by spinal PGE_2 inflammation. A limited number of works have investigated the allosteric modulation of β subunit in αβ heteropentameric GlyRs. Recently, we reported that homomeric GlyRs are more sensitive to the effects of ethanol and G protein; therefore, β subunit may be negatively modulating α1β pharmacological profile in response to ethanol (Mariqueo et al., 2014). Considering the pharmacologic potential of this modulation, the present study examines the expression of β subunit in a rat neuropathic chronic pain model (CCI). Western blot analysis of CCI spinal cord samples revealed an increase in β subunit expression in comparison with the control (Sham), after 3 days of pain development (mean ± SEM relative abundance 18±1 and 28±1 A.U. in the Sham and CCI slices, respectively, $P<0.05$, n=6). Similar results were observed by immunofluorescence of β subunit in spinal slices (mean ± SEM fluorescence intensity 15.6 ± 1 and 47.6 ± 2 A.U. in the Sham and CCI slices, respectively, $P<0.001$, n=4). RT-qPCR analysis showed increased level of β gene expression at 10 days after CCI surgery (2.2-fold, $P<0.001$, n=5). These results highlight the importance of determining the role of β subunit in allosteric modulation of glycine receptors as a strategy against chronic pain establishment.

108) **Cx43 hemichannels play a critical role in neuroinflammatory responses promoted by prenatal stress or epilepsy**

Maturana, Carola[1]., Lagos, Carlos[2].,Sáez, Juan[1].,[1]Fisiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile.[2]Endocrinología, Medicina, Pontificia Universidad Católica De Chile. (Sponsored by Acelerador UC PMIPUC1206 And P09-022-F From ICM-ECONOMIA Grants)

Upon inflammasome activation cells release inflammatory mediators (e.g., IL-1β) that may play a crucial role in the development of neuroinflammation present in neurodegenerative disorders such as epilepsy and different psychiatric disorders. The possible role of connexin (Cxs) and pannexin 1 (Panx1) hemichannel as well as $P2X_7$ receptor ($P2X_7R$) in the ATP-induced inflammasome activation has been suggested. Now, we hypothesize that Cx43 hemichannels play a relevant role in cell sensors of the CNS microenvironment, allowing a strong response to stress or an epileptogenic condition, while Panx1 hemichannels and $P2X_7R$ might be critical for maintaining the neuroinflammation. To this end, we used offspring (1 day old) of control and dexamethasone treated (last 5 day of gestation) mice. The hemichannel activity was determined using the ethidium (Etd, "snapshot") uptake assay in acute hippocampal slices. The hemichannel activity increased in microglia, astrocytes and oligodendrocytes from offspring of stressed mothers and was inhibited by hemichannel (^{10}panx1 and Gap26 peptides) and $P2X_7R$ (A740003) blockers. This response persisted for at least four postnatal weeks. Therefore, the inflammasome of glial cells is activated by stress via Panx1/Cx43 hemichannels and $P2X_7Rs$ and since is maintained for a long postnatal time it might alter perinatal neurogenesis and connectomic. We also hypothesize that increased neuronal activity promotes activation of glial cell Cx hemichannels, which might perpetuate a condition that favors recurrence of neuronal activity in epilepsy. We used D4, a selective Cx hemichannel blocker identified by virtual screening of the NCI database towards the Cx26 crystal structure. Adult male mice were treated with pentylenetetrazol (PTZ), an epileptogenic agent that acts as nonselective agonist of $GABA_A$ receptors. Seizures were evident at ~7 min after PTZ administration followed by a period of several hours, in which mice presented very low motor activity and sporadic contractions with ~60% survival. However, D4-pretreated animals showed only a brief convulsion period at ~7 min post-PTZ administration and then, behaved as control animals with 100% survival. In addition, D4 inhibited completely the PTZ-induced glial cell HC activity. Thus, we propose the glial cell Cx HCs as novel anticonvulsant targets.



109) **Effect of supraphysiological aldosterone level on adipogenesis of human liposarcoma cell line SW872**

Fuentes-Zuñiga, Cristobal[1]., Gonzalez-Gomez, Luis Martin[1].,Allende, Fidel[2].,Fuentes-Ibacache, Nataly[1].,Ortiz-Canales, David[1].,Campino, Carmen[1,5].,Carvajal, Cristian[1,5].,Cifuentes, Mariana[3].,Solari, Sandra[2].,Owen, Gareth[4].,Kalergis, Alexis[5].,Lagos, Carlos[1,5].,Vecchiola, Andrea[1,5].,Fardella, Carlos[1,5].,[1]Department of Endocrinology, School of Medicine, Pontificia Universidad Catolica de Chile.[2]Department of Clinical Laboratories, School of Medicine, Pontificia Universidad Catolica de Chile.[3]Institute of Nutrition and Food Technology (INTA) Universidad de Chile.[4]Department of Physiology, Faculty of Biological Sciences, Pontificia Universidad Catolica de Chile.[5]IMII Millennium Institute on Immunology and Immunotherapy. (Sponsored by Supported By Proyecto SOCHED 13-6, IMII P09/016-F, CORFO 13CTI-21526-P1 And FONDECYT 1150437 & 1130427 Grants.)

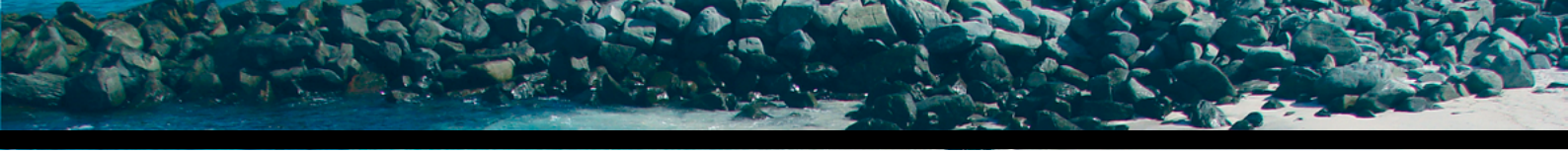
Background Excessive adipose tissue growth has been correlated with cardiovascular risk factors included in the metabolic syndrome. The adipose tissue differentiation is characterized by increased expression of gene markers (C/EBPβ, PPARγ and HSD11B1) and triglyceride accumulation. Cortisol is a known promoter of differentiation commitment by signaling through the glucocorticoid and mineralocorticoid receptors (GR &MR) in the adipocyte. Recent reports show that Aldosterone (ALD), the natural ligand of the MR, can be synthesized in fat. In primary aldosteronism, ALD levels can increase up to 1000-fold (10 nM), but the effect of supraphysiological levels of ALD in the proliferation and differentiation of human preadipocytes has not been well established. **Aim** To study the effect of a supraphysiological concentration of ALD in proliferation and differentiation of a human liposarcoma cell line SW872 in vitro. **Methods** The effect of ALD (0.1 and 10nM) in proliferation of preadipocytes, was studied in SW872 cell cultures maintained in growing medium plus 1% corticoid-free fetal bovine serum for 12 and 24 hr. DNA was marked with propidium iodide and quantified by flow-cytometry. The population in proliferation was defined as the sum of S, G2 and M cellular cycle (% of total cell population). The differentiation process was induced with an adipogenic MIX for 48 hr. The effect of ALD (0.1 and 10nM) added in the MIX was evaluated for 7 days. Differentiation markers expression was studied for 48 hr by qRT-PCR. Lipid accumulation was measured by Oil Red O staining and quantified by spectrophotometry on days 3 and 7 of differentiation. **Results** Untreated preadipocytes population in proliferation at initial time was 36%, reducing to 23% at 12 hr and an increase of 27% at 24 hrs. At 0.1nM of ALD showed a 25% proliferation at 12hr (2% less respect to time control) and a 26% at 24 hr. 10nM ALD generates no changes at 12 hr respect to the control, but showed a decrease to 24% at 24hr (3% less respect to time control). The levels of differentiation markers C/EBPβ, PPARγ and HSD11B1 mRNA showed an increase when induced with MIX respect to untreated. When MIX plus ALD 0.1nM was added, HSD11B1 showed a shift (24 hours) in its maximum peak. MIX with 10nM ALD did not affect markers expression, and lipid accumulation experiments showed that MIX with 10nM ALD increase triglycerides accumulation at day 7 respect to the MIX condition. **Conclusion** Supraphysiological aldosterone treatments tend to reduce proliferation and promote differentiation of preadipocytes.

110) **Environmental enrichment alters the expression of hypothalamic genes associated with food intake**

Hernández, Sergio[1]., Guzmán, Luis[1].,Kerr, Bredford[2].,[1]Laboratorio de Biología Centro de Estudios Científicos-CECs, Universidad Austral de Chile.[2]Laboratorio de Biología Centro de Estudios Científicos-CECs. (Sponsored by Fondecyt 1140162, PFB 01/2007)

The proper control of body weight requires the integration of metabolic signals by the arcuate nucleus of the hypothalamus (Arc), which through the projection of neuronal axons toward the paraventricular nucleus (PVN) drives feeding behavior. *Pomc* and *Agrp* expressing neurons are first order neurons located in the Arc and through the sensing of peripheral signal release endogenous agonist or antagonist of melanocortin receptor 4 (MC4R) in the PVN, drive satiety or appetite, respectively. Previous evidence exists demonstrating that the sensitivity of hypothalamic neurons to metabolic signals can be modulated by environmental factors. Moreover, the hypothalamus maintains high levels of plasticity even during adulthood, which strongly suggests a role for neuronal plasticity in body weight control. To test our hypothesis, we housed 129/B6 wild type male mice, since weaning at 3 weeks of age, in environmental enrichment conditions (EE), which is a widely used paradigm to induce synaptic plasticity. To determine the effect of the EE on mouse body weight-related phenotype, mice were weighed weekly and locomotor activity and food intake were assessed in metabolic cages at 7 weeks of age. To determine changes in the expression of feeding behavior-associated genes, we evaluated the expression of hypothalamic genes from 7-week old mice housed in EE or standard conditions by qRT-PCR.

Our results show that the exposure to EE increased mouse food intake, however it did not alter body weight. We also observed that EE induced an increase in locomotor activity, which may contribute, at least in part, to maintaining body weight in spite of the increased food intake. To determine the mechanism underlying the increased food intake observed in mice exposed to EE, we assessed hypothalamic gene expression. We found an increase in the expression of *Agrp*, which is a pivotal gene that controls food intake and we did not detect any change in the expression of either *Pomc* or *MC4R.* Body weight maintenance in spite of increased food intake observed in mice exposed to EE, strongly suggests the remodeling of neuronal circuits driving feeding behavior and energy expenditure. To assess this hypothesis, we are currently performing morphological analysis in hypothalami from mice exposed to EE or standard conditions to evaluate the synaptic plasticity-associated parameter in Arc and PVN neurons. In summary, our results show that EE exposure alters gene expression and feeding behavior without modifying body weight, which strongly suggests remodeling of hypothalamic neuronal circuits driving feeding behavior and energy expenditure.



111) **Cold stress decreases serotonin release in rat paraventricular nucleus**

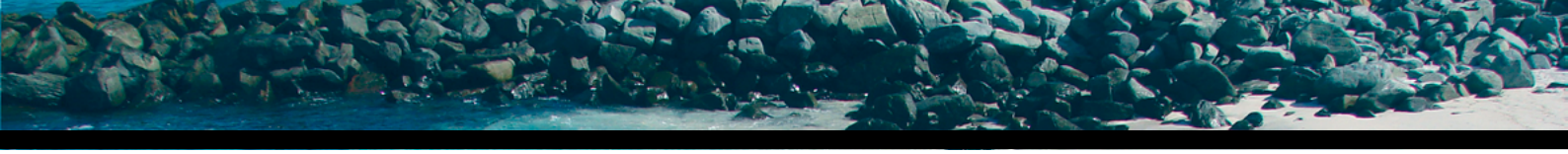
Jara, Pablo[1]., Lara, Hernán[2].,Espinosa, Pedro[3].,Sotomayor-Zárate, Ramón[3].,[1]Laboratorio de Farmacología, Facultad de Química y Biología, Universidad de Santiago De Chile.[2]Laboratorio de Neurobioquímica, Facultad de Ciencias Químicas y Farmacéuticas , Universidad de Chile.[3]Laboratorio de Neuroquímica y Neurofarmacología , Facultad de Ciencias, Universidad de Valparaíso.

Hypothalamic Paraventricular Nucleus (PVN) is a brain structure that synthesize and release neuroendocrine hormones controlling endocrine functions and is responsible for integrate stress signals and modulate their response. Cold stress is a physiologic stressor modulated by PVN; in this context, rats exposed to different cold stress protocols showed increased peripheric sympathetic nervous activity and activating central thyrotropin-releasing hormone (TRH) neurons in the PVN; this activation is mediated by multiple brain regions related to noradrenergic, glutamatergic and GABAergic transmission. The present study explore the effect of cold stress on the serotonin (5-HT) and 5-Hydroxyindoleacetic acid (5-HIAA, its major metabolite) release by measuring these molecules in the PVN, in an animal model of cold-stress. For this purpose, we measured 5-HT and 5-HIAA levels in hypothalamic PVN tissue section in control (room temperature) and stressed female rat (4ºC for 64 hours) using an HPLC coupled to a electrochemical detector. In these experiments, we observed a decrease in 5-HT and 5-HIAA levels (50% and 65%, respectively) in cold stressed rats. Furthermore, the ratio of 5-HIAA/5-HT showed a tendency of increase, which could indicates an augmented 5HT metabolization. In summary our results shows that cold stress induces a decrease in serotonin and 5-HIAA release, probably due to an increase in the serotoninergic activity, supporting the role of serotonin in the activation of the TRH-neurons but not discarding other PVN neurons. Grant support: DICYT 021443JP, Universidad de Santiago de Chile to P. Jara, FONDECYT 1020581, 1050765 and 1090036 to H.E. Lara.

112) **Individual susceptibility to obesity and the role of the orexin and dynorphin peptides.**

Perez-Leighton, Claudio[2,1]., Gac, Lily[2].,Mella, Ricardo[2].,Ramirez, Beatriz[2].,[1]Department of Food Science And Nutrition University of Minnesota.[2]Facultad de Medicina Universidad Andres Bello. (Sponsored by Proyecto CONICYT PAI 8230017, Proyecto FONDECYT REGULAR 1150274, Proyecto Interno UNAB DI-523-14/R)

Understanding the neuronal and behavioral mechanisms mediating energy expenditure is key to developing successful anti-obesity therapies. Our laboratory focuses on how the hypothalamic orexin/dynorphin (ox/dyn) modulate energy balance in animal models of obesity. As an obesity model, we fed mice a cafeteria (CAF) diet. Our data shows that mice fed CAF diet can be classified as either obesity prone (OP) or obesity resistant (OR) based on whether their increase in fat mass percent was higher (OP) or lower (OR) compared to maximum value of control fed mice. Our data shows that increased spontaneous physical activity (SPA), but not differences in caloric intake, is key for expression of the OR phenotype and that OP, but not OR mice show decreased sucrose preference. Interestingly, OR mice fed with a CAF diet personalized for individual susceptibility to obesity showed hyperphagia compared to OP mice, but failed to develop obesity. These data has validated a model of differential susceptibility to obesity to explore the function of the orexin and dynorphin neuropeptides. The hypothalamic paraventricular nucleus (PVN) is an important brain site for regulation of feeding behavior and physical activity. We recently demonstrated the non-opioid DYN peptide DYN-A_{2-17} in PVN increases food intake, SPA and interacts with orexin-A (OXA) to enhance their individual effects in food intake. Currently, we are exploring the effects of orexin and dynorphin peptides on food intake. In these experiments, we measured responses to orexin and dynorphin peptides in PVN before an after feeding with either control CAF diet. Our current data indicate that, prior to the dietary interventions, DYN-A_{1-13} and OXA both significantly increased short-term chow intake. In OP and control diet-fed mice, we observed no change in the ability of OXA or DYN-A_{1-13} to increase short-term chow intake relative to the pre-dietary treatment. In contrast, OR mice increase their food intake after OXA injection and no changes in the effectiveness of DYN-A_{1-13}. We are repeating these experiments and extending these results testing if voluntary physical activity (wheel running) alters gene expression of the orexin and dynorphin genes in the lateral hypothalamus and behavioral effects of these peptides injected into PVN. These experiments will improve our understanding of the behavioral and neuronal mechanisms mediating individual susceptibility to obesity.



113) **Amyloid-β peptide increases P2X2 receptor levels, modifying the intracellular distribution of Fe65 which affects the amyloidogenic pathway**

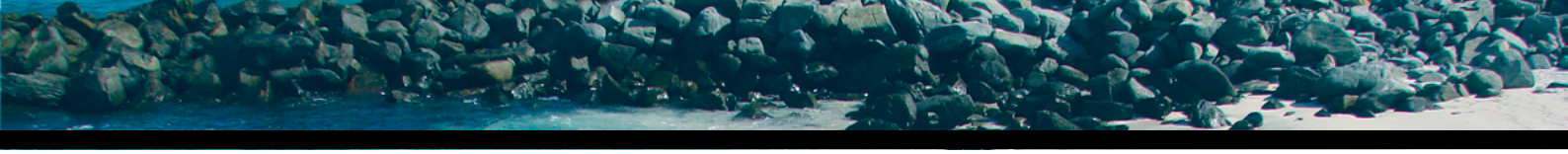
Godoy, Pamela[1]., Barra, Karen[1].,Silva-Grecchi, Tiare[1].,Fuentealba, Jorge[1].,[1]Physiology, Biological Sciences, Universidad De Concepción. (Sponsored by This Work Has Been Funded By The FONDECYT Project 1130747.)

Alzheimer's disease (AD) is a neurodegenerative disease, mainly caused by an imbalance in the processing of the Amyloid Precursor Protein (APP), in which the production of the amyloid-β peptide (Aβ) is increased. Recent studies have suggested that the purinergic receptors P2X may have a role in the Aβ toxicity mechanisms. On the other hand, Fe65 is a multidomain adaptor protein which interacts with the YEMPTY sequence present in the amyloid intracellular domain (AICD) of APP; this sequence is also known as an endocytosis signal for APP and it enables the amyloidogenic pathway which takes place mostly at endocytic compartments. It is widely known that Fe65 interacts with AICD and Tip60 -an acetyltransferase histone- and this complex translocate to the nucleus to participate in the transcription of different genes. But, there is contradictory evidence for the role that Fe65 has in the APP processing; nonetheless, the interaction with the YEMPTY sequence could prevent the endocytosis of APP and Aβ production. Besides, it has been described that Fe65 interacts with the P2X2 receptor, interaction that we corroborated. Furthermore, we have seen an increase in the P2X2 expression after treatment with Aβ. The aim of this work was to study how the increase on the expression levels of the P2X2 receptor affected its interaction with Fe65, the APP processing and Aβ formation. To achieve this, we studied the P2X2 and Fe65 presence and distribution in PC12 cells after treatment with Aβ (0.5µM, 24h) by immunofluorescence, and also we overexpressed P2X2 by transfection to quantify possible Aβ changes in the cellular lysates. We observed an increase in the P2X2 presence after treatment with Aβ (C: 100 ± 3%, Aβ: 130 ± 7%), while Fe65 did not show any global changes (C: 100 ± 3%, Aβ: 97 ± 4%); however, a decrease of its presence in the nucleus was observed (C: 100 ± 5%, Aβ: 84 ± 5%). At last, we observed an increase in Aβ levels from cellular lysates after transfection with P2X2 (C: 100%; P2X2: 216 ± 48%). In conclusion, these data show that Aβ peptide induces an increment of P2X2 expression levels, while Fe65 expression remains stable but it shows a diminishment on its nuclear localization. This can suggest that the increase in the receptor could generate a sequestration of Fe65, which would explain that this protein is less available to interact with AICD and therefore to translocate to the nucleus. These results along with the increase that we observed in the Aβ production after transfection with P2X2, could mean that the increase in P2X2R sequestrates Fe65, reducing availability of this protein to interact with APP, which could lead to an increase in endocytosis of APP and the subsequent production of Aβ peptide.

114) **Effect of dichlorvos in spatial learning and memory during the ontogeny of Sprague-Dawley rats**

Pancetti, Floria[1].,**Gámiz, Fernando[1]**., [1]Ciencias Biomédicas, Medicina, Universidad Católica Del Norte. (Sponsored by This Research Was Supported By The FONDECYT Postdoctoral Grant Nº 3140437 And FONDECYT Regular Grant N° 1140856)

Dichlorvos (DDVP) is an organophosphate (OP) that has been mainly used as a pesticide and also as a cognitive enhancer due to its inhibitory effect on acetylcholinesterase (AChE) enzyme. However recent studies have shown that this drug may act through alternative mechanisms to AChE inhibition. Previous studies have shown that when the enzyme acylpeptide hydrolase (APEH) is selectively inhibited by low doses of DDVP positive changes in neural plasticity and cognitive performance are observed. Nevertheless we have found that the same DDVP dosage given during the ontogeny of rats produces biphasic response triggering beneficial pharmacological effect in young rats through the enhancement of learning and memory and a toxicological effect in old rats impairing such processes. In order to determine the DDVP doses displaying nootrophic or toxic effects in learning and memory we have chronically injected during 28 days Sprague-Dawley rats of 1 and 3 month-old and 1 year-old with a range of DDVP doses: 0.03, 0.1, and 2 mg/kg per day. After the treatment, rats were tested in the Morris water maze to assess the spatial learning during five training days and memory 24h after the training. After this, rats were sacrificed and the AChE and APEH activities were assessed in homogenized hippocampus. Besides this, synaptic plasticity parameters were also measured ex vivo. Data obtained at this moment have showed that chronic treatment of DDVP 2 mg/kg have no effect in 1 month-old rats, a deteriorating effect in 3 months-old rats and a toxic effect in 12 months-old rats in learning and memory. Treatment with DDVP 0.1 mg/kg produces an improvement in learning and memory in 1 month-old rats. However no effect and a deteriorating effect is observed in 3 and 12 months-old rats respectively. Finally the treatment with DDVP 0.03 mg/kg showed no effect in 12 months rats. At this moment we are testing DDVP 0.03 mg/kg in 1 and 3 months old rats and DDVP 0.01 mg/kg in 12 months old rats. At all these DDVP doses, only hippocampal APEH activity was specifically inhibited, remaining AChE activity unaffected. Taken together, these results indicate that the magnitude of APEH inhibition could be used as a predictor of pharmacological or toxicological effects when learning and memory are used as endpoints.



115) **Vulnerability of dopaminergic neurons following recurrent metabolic insults: effects of perinatal asphyxia in organotypic cultures.**

Sehrt, M[1]., [1]Tecnología Médica, Medicina, Universidad de Chile.

Vulnerability of dopaminergic neurons following recurrent metabolic insults: effects of perinatal asphyxia in organotypic cultures.

Sehrt-Uribe, M.,Pérez-Lobos R., Palacios, E., Bustamante, D., Morales, P., Herrera-Marschitz, M.

Millenium Institute BNI-Chile; Programme of Molecular & Molecular Pharmacology, ICBM, Medical Faculty, University of Chile, Santiago, Chile.

Perinatal asphyxia (PA) features apoptotic and neuroinflamation mechanisms, leading to short- and long-term neuronal death. PA affects neuronal networks dependending on the insult timing and metabolic requirements, being basal ganglia neurocircuitry particularly prone to vulnerability, inducing functional impairments of dopaminergic systems.

We investigated whether PA primes the vulnerability of dopaminergic neurons to recurrent metabolic insults, using basal ganglia organotypic cultures from asphyxia-exposed neonate rats.

Cultures from asphyxia-exposed and control animals were subjected to H_2O_2 (45 mM) and glucose deprivation for 18h at day *in vitro* 18 (DIV18). Two days after the insult, cultures were treated for cell viability *in vitro*, or formalin fixed to analyse neuronal (MAP-2), neurochemical (tyrosine hydroxylase, TH) phenotype, caspase-3 (CASP-3) as apoptotic marker, and DAPIto analyse cell number and morphological nuclear changes.

After a second metabolic insult, there was: (i) a decreased cell viability in organotypic cultures, (ii) Sn recurrent insult cause a decrease in cell number and asphyxia exposed control decreases significantly about its non-exposed control. Str recurrent insult cause a decrease in cell number only for its asphyxia exposed group. Cx decrease in cell number only for its control group treated for a recurrent insult. Further, asphyxia exposed group cell number decreased about its control, (iii) increase in apoptotic cell death; (iv) increase in activation of CASP-3-dependent apoptotic pathways in neurons, and (v) increased number of TH^+/$CASP\text{-}3^+$ neurons of organotypic cultures from asphyxia-exposed, compared to non-asphyctic tissue. Furthermore, morphological analysis revealed a (vi) decreased number of apoptotic bodies in tissue submitted to a recurrent metabolic insult, suggesting the activation of different death pathways in dopaminergic neurons, depending on the priming mechanisms induced by PA.

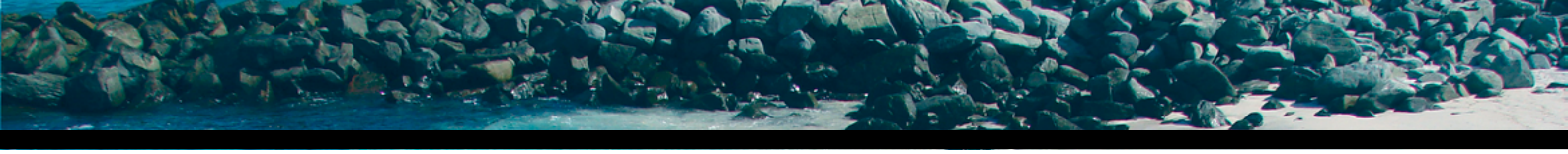
The present work describes differential vulnerability affecting neurons from basal ganglia, confirming dopaminergic neurons as a main target for the impairing effects of recurrent metabolic insults following PA in CNS.

Sponsor: Herrera-Marschitz, M.

116) **REGULATION OF VOLTAGE SENSING STRUCTURES OF $Ca_V1.2$ CALCIUMCHANNEL BY THE AUXILIARY b-SUBUNIT (β_3)**

De Giorgis, Daniela[2]., Contreras, Gustavo[2].,Savalli, Nicoletta[1].,Navarro-Quezada, Nieves[2].,Gonzalez, Carlos[2].,Olcese, Riccardo[1].,Neely, Alan[2].,[1]Department of Anesthesiology, Division of Molecular Medicine, David Geffen School of Medicine, University of California.[2]Centro Interdisciplinario de Neurociencias de Valparaíso, Ciencias, Universidad De Valparaíso. (Sponsored by FONDECYT 1120802 And ACT1104 (GC), NIH.R01GM110276 (RO). The Centro Interdisciplinario De Neurociencia De Valparaíso Is A Millennium Institute Supported By The Millennium Scientific Initiative Of The Chilean Ministry Of Economy, Development And Tourism.)

High Voltage-Activated channels (HVA) translate membrane depolarizations to local increases in intracellular Ca^{2+}. HVAs are composed of four similar but non-identical repeats, each one with its own voltage-sensing domain. The cardiac HVA$Ca_V1.2$ is co-expressed with two auxiliary subunits, $\alpha_2\delta$ and β, that regulate channel function. To track the movement of individual voltage sensors we introduced cysteine residues at strategic locations in one voltage sensor at a time, attached a tetramethylrhodamine based fluorophore and monitored fluorescence changes produced by membrane depolarizations (Cut-open oocyte voltage clamp fluorometry). Using this approach we have shown that individual voltage-sensors contribute to channel opening and gating currents to a different degree (Pantazis et al. 2014, PNAS.). Here we show that β-subunit (β3) co-expression regulates the equilibrium between the relaxed and active configurations of the voltage-sensor. Voltage-sensors of all members of the voltage-gated ion channel family have three main conformations: resting, active and relaxed. Normal channel operation involves transit from the resting to active conformation, and this transition promotes channel opening. The more stable relaxed configuration that is populated by prolonged depolarizations does not contribute to channel opening. Once in the relaxed state, strong hyperpolarizations (~ -100 mV) are necessary to restore the resting state. In contrast, during normal function the resting/active ratio at +40 mV approaches 0.5. When $Ca_V1.2$ channels are co-expressed with β3, membrane hyperpolarization is more effective in driving the voltage sensor of the first repeat out of the relaxed state, so that at voltages near the resting potential the majority of the voltage sensors will be available for activation. In the absence of β3, ~50% of the voltage-sensors from the first repeat remain in the relaxed state at the resting potential, explaining, at least in part, why $Ca_V1.2$ channels lacking the β-subunit do not open at physiological depolarizations.



117) **Role of cytoskeleton and RhoA in regulation of Gap Junction Channels and Hemichannels**

Jara, Oscar[1]., Maripillán, Jaime[1].,Momboisse, Fanny[1].,García, Isaac[1].,Pinto, Bernardo[1].,Cárdenas, Ana María[1].,González, Carlos[1].,Martínez, Agustin[1].,[1]Centro Interdisciplinario de Neurociencias de Valparaíso, Facultad de Ciencias, Universidad De Valparaíso.

The role of the actin cytoskeleton in the functional regulation of Gap Junction channel (GJC) has been well established; however less is known about the molecular mechanisms underlying its action on Hemichannels (HC). To this end, we treated HeLa cells expressing connexins (Cx) with the actin-depolarizing agent Cytochalasin B and the effect on HCs were functional addressed performing dye uptake, patch clamp recordings and single-cell TIRF microscopy. We found that in cells stably expressing Cx43, the treatment significantly reduced the size of GJC plaques as well as the dye and electrical coupling. In contrast, the relative amount and activity of HCs in non-appositional plasma membranes were significantly increased. On the other hand, in cells stably expressing Cx26, actin depolimerization also reduced the size of GJ plaques, but did not affect the functional state of GJCs and HCs. Since members of the RhoA GTPase family have been shown to regulate many aspects of intracellular actin dynamics, and they have been implicated in the regulation of HCs and GJCs, we studied to the contribution of RhoA signaling to this mechanism. We found that cells transfected with a dominant negative form of RhoA or with a siRNA against RhoA exhibit a decreased of GJC plaques in cells expressing either, Cx26 or Cx43. These constructs also promotes a gain of function of Cx43 HCs but not on Cx26 HCs. These results suggest that GJCs and HCs composed by Cx26 or Cx43 are differential regulated by the actin cytoskeleton, and RhoA regulates the traffic but not the function of Cx26 HCs.

This work supported by MECESUP UVA0603 to OJ, FONDECYT 1130855 and ANILLO-ACT1104 to ADM. The Centro Interdisciplinario de Neurociencias de Valparaíso is a Chilean Millennium Institute (P09-022-F).

118) **KCNN4 Attenuates Chronic Allergic Asthma Features in an Ovalbumin Mouse Model**

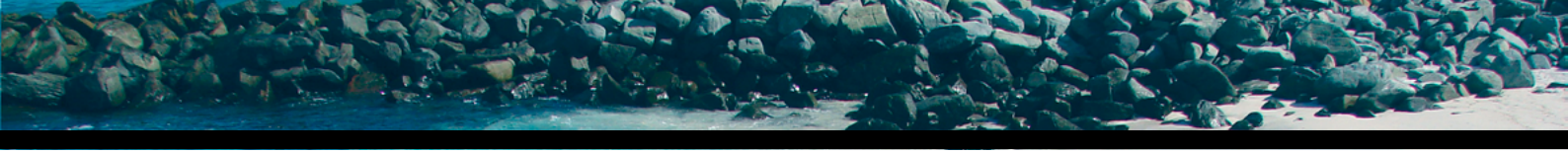
Philp, Amber[1,2]., Flores, Carlos[2].,[1]Bioquimica, Facultad de Ciencia, Universidad Austral De Chile.[2]Biología Centro de Estudios Cientificos.

Asthma is a complex disease characterized by chronic inflammation, hyperreactivity, increased production of mucus and airway remodelling. Many features of this disease are thought to reflect consequences of a Th2-dominated immune response, where the mast cell is one of the principal effectors. Previous reports demonstrated that the potassium ion channel KCNN4 is important for certain mast cell functions, such as degranulation and migration. The aim of this work was to study the potential use of KCNN4 to inhibit the function of the mast cell in the development of a mouse model of chronic allergic asthma.

Chronic allergic asthma was induced through immunization with ovalbumin for 10 weeks in control animals (WT) and animals which lack expression of KCNN4. A group of animals with deficiency of mast cells (*Kit*$^{W\text{-}sh/W\text{-}sh}$) was also included. The results showed that WT mice immunized with ovalbumin produced thickening of airway epithelium, increased collagen deposits in distal airways and increased goblet cell numbers in the trachea. *Kccn4*$^{-/-}$ showed significant reductions compared with WT animals in the development of all asthma characteristics analysed in this work.

This work shows the importance of KCNN4 in the development of chronic allergic asthma, nevertheless the protective effect delivered through KCNN4 inhibition is only partially due to the inhibition of mast cells and it is probable that the inhibition of other immune cells and airway epithelium function also contributes to the observed attenuations.

Funded by FONDECYT 1151142



119) **Molecular determinants involved in cold and menthol sensitivity of the TRPM8 channel**

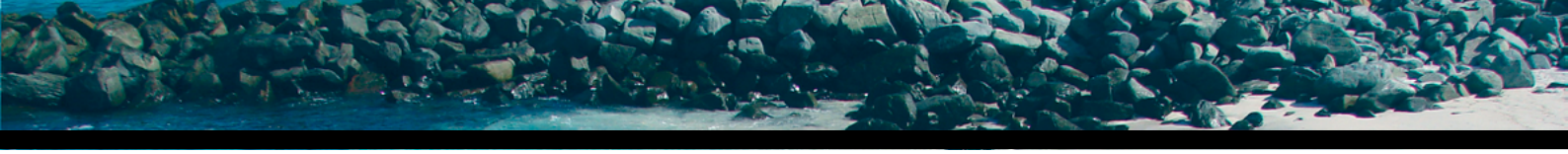
Rivera, Bastián[1]., González , Alejandro[1].,Tralma, Karina[1].,Salas, Jeremy[1].,Madrid, Rodolfo[1].,Pertusa, María[1].,[1]Biología, Química y Biología, Universidad De Santiago De Chile. (Sponsored by Supported By FONDECYT 11130144 (MP), 1131064 (RM), And CONICYT ACT-1113 (RM, MP).)

TRPM8 is the main ion channel responsible for cold detection in the somatosensory system. This thermoTRP channel is activated by cold, cooling compounds such as menthol and by voltage. Among the different TRPM8 orthologs, chicken TRPM8 (cTRPM8) displays different sensitivity to cold and menthol compared to mouse TRPM8 (mTRPM8), suggesting that non-conserved regions could be able to tune the chemical and thermal sensitivity of this polymodal ion channel. Nevertheless, the molecular bases underlying the differences among these orthologs remain poorly understood. In order to identify structural domains involved in TRPM8 sensitivity to cold and menthol, we performed sets of chimeras using the orthologs mTRPM8 and cTRPM8. We evaluated the responses to cold and menthol of these mutants, using calcium imaging and patch clamp techniques in transfected HEK293 cells. We identify one region of 30 residues in the proximal N-terminus that is involved in the sensitivity to cold and menthol of this channel. We found that the transference of this domain from cTRPM8 to mTRPM8 is sufficient to obtain a chimera that include increased responses to cold and menthol, a 3°C shift in the temperature threshold to warmer temperatures, and a reduced EC_{50} to menthol. Electrophysiological analysis revealed that the enhanced responses to agonists are due to a shift in the voltage-dependence of activation, increasing the probability of channel openings at physiologically relevant negative membrane potentials. This change occurs along with an increase in the Gmax value. Our results suggest a key role of the proximal region of the N-terminus in the fine tune of cold and menthol sensitivity of this polymodal thermoTRP channel.

120) **Characterization of chaos in a bursting neuronal model and its interaction with noise.**

Caviedes, Mauricio[1]., Maidana, Jean Paul[1].,Quero, Daniel[2].,Aguirre, Pablo[2].,Orio, Patricio[1].,[1]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso.[2]Departamento de Matemática Universidad Técnica Federico Santa María. (Sponsored by Supported By Grants Fondecyt 1130862(PO), Fondecyt 3130497(PA), ACT-1103(PA), ACT-1104(PO), ACT-1113(PO), FB0008(PO), PFB03(PA). The Centro Interdisciplinario De Neurociencia De Valparaíso (CINV) Is A Millennium Institute Supported By The Millennium Scien)

Many neurons in the central and peripheral nervous system exhibit a bursting firing pattern. Bursting appears as a consequence of ion channels that gate with slow time constants (such as I_M or I_h) or that respond to slow intracellular mechanisms (such as calcium-activated potassium channels). We are studying the behavior of a neuronal model with parabolic bursting inspired in thermoreceptors. A persistant sodium current, a calcium activated potassium channel and a hyperpolarization-activated current provide a slow membrane oscillation that elicits regular firing of action potentials. Depending on model parameters, the periodic firing can take the form of burst firing, tonic firing or an irregular multimodal firing of chaotic nature. Generally speaking, chaotic behavior is characterized by an irregular, non-periodic behavior in the absence of any random or noisy influence. It also presents a short-term predictability that vanishes exponentially with time, a feature quantified in the Lyapunov exponent. Bifurcation analysis of the model reveals a homoclinic bifurcation in the transition from tonic firing to silence, causing a chaotic behavior known as Shilnikov chaos. Numerical analysis of inter-spike interval (ISI) sequences shows a positive Lyapunov exponent in many regions of the parameter space. We also show that the chaotic behavior is disrupted by noise, both internal (stochastic opening of ion channels) and external (random current added), converting a deterministic chaotic behavior into a random behavior. Ongoing work is focused on complementing the numerical chaotic characterization with entropy measures and on the functional consequences of chaos to the neural coding.



121) **Comparative study in porcine model anesthesia isofluorane/oxygen with and without acute alcohol administration**

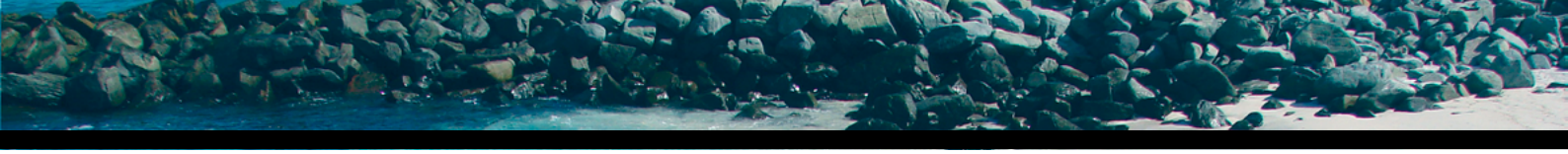
MONTERO, EDSON[1]., ARANGUIZ, MACARENA[1].,AVENDAÑO, CINTIA[2].,CONTRERAS, ENRIQUE[3].,SEPULVEDA, MARIA[4].,[1]FARMACOLOGIA, FACULTAD DE MEDICINA VETERINARIA, Universidad San Sebastián.[2]LABORATORIO CLINICO, FACULTAD DE CIENCIAS DE LA SALUD, Universidad San Sebastián.[3]FARMACOLOGIA, FACULTAD DE MEDICINA, Universidad Católica De La Santísima Concepción.[4]FARMACOLOGIA, FACULTAD DE CIENCIAS BIOLOGICAS, Universidad De Concepción. (Sponsored by USS2011-00007-R)

In Chile, the high vehicular accidentability because the combination ethanol and driving, a study to evaluate the effects of ethanol administration in a porcine model with Volatile Induction and Maintenance Anesthesia (VIMA) isoflurane / oxygen (I / O), was designed. There are no studies to recommend safe anesthetic protocols. We controlled in a porcine model in order to determine whether plasma electrolytes and EKG parameters are modified by action of ethanol clinical study. Nine pigs, Landrace-Largewhite, ASA 1, average weight of 13 kg were used. These are pre-medicated with Ketamine-Diazepam. The study was conducted in two similar anesthetics periods (40 min.) with spontaneous inhalation anesthesia. In the first period he underwent anesthetic induction using a VIMA mask I / O with a maintenance dose of I / O 2%/2L.min^{-1}. In the second period, after a wash-out of three days, the same anesthetic protocol of the 1st half of the study with administration of ethanol 96 diluted to 50% and 250 mg.kg^{-1} dose was administered. EKG was obtained: Heart rate (HR), PR interval, QT and QTc, QRS complex and ST segment, with plasma electrolytes: Na +, K +, Cl- and bicarbonate of a sample of arterial blood 0, 20 and 40 minutes. Statistical analysis Student's t test was used. Our results indicated that during the administration of VIMA I / O with ethanol slows HR and plasma concentrations K+ and Cl-, compared to control.Declining HR is accentuated by the synergistic enhancement between ethanol and isoflurane at central level. While transient low plasma concentration of K + and Cl- is presented as an effect renal plasma ADH decreased by the administration of alcohol induced, thereby decreasing the reabsorption of K + and Cl- with increased diuresis. Due to the principle of electronegativity plasma Na + and plasma bicarbonate suffer no significant changes. There were no significant differences between the two anesthetic periods for the rest of the parameters studied. Induction time and anesthetic recovery experienced no significant differences between the periods analyzed. We conclude that VIMA I / O produces a stable anesthetic in the presence of ethanol.

122) **Triphenylphosphonium alkyl derivatives of gallic acid decrease tumor growth *in vivo*: potentiation with doxycycline.**

Peredo-Silva, Liliana[1]., Castro-Castillo, Vicente[2].,Saavedra-Olavarría, Jorge[2].,Pavani, Mario[1].,Ferreira-Parker, Jorge[1].,[1]Departamento de Farmacología, Facultad de Medicina, Universidad De Chile.[2]Departamento de Química, Facultad de Ciencias Básicas, Universidad Metropolitana De Ciencias De La Educación.

It has been found that *n*-alkyl gallates are able to induce apoptosis in various tumor cell lines, primarily by activating the mitochondrial dependent apoptotic pathway due to an association with mitochondrial oxidative metabolism, altered in these cells. It has been reported that increasing concentrations of esters of gallic acid, generate an uncoupling effect of oxidative phosphorylation system, followed by inhibition of electron flow through the mitochondrial respiratory chain (at higher concentrations), mainly at NADH-CoQ oxidoreductase. These effects, prevent the synthesis of ATP that ultimately led to cell death. Besides, it is also known that the structure and lipophilicity of the alkyl side chain (alkyl length), is relevant to the antitumor activity of this compounds. Mitochondria play a role in regulating energy metabolism, cytosolic calcium concentration, ROS production and apoptosis. Importantly, between tumor and non-tumor cells, the mitochondria present significant differences in terms of oxidative phosphorylation. Additionally, tumor cells exhibited increased mitochondrial membrane potential ($\Delta\Psi_m$). The inner mitochondrial membrane of tumor cells, have a $\Delta\Psi_m$ about 150-180 mV, more negative on the inside. This potential is much higher than any other cell organelle, and greater than that of other tissue and non-tumor cell. To enhance the cytotoxic effect of gallic acid esters, we synthesized various delocalized lipophilic cations, where gallic acid with different alkyl chain lengths, were conjugated with triphenylphosphonium group (TPP^+). These compounds accumulate selectively in mitochondria of tumor cells, guided by their higher membrane potential. In this work, we evaluated the antitumor activity and selectivity *in vivo* of this derivatives, in a syngeneic mouse model. Specifically, the derivative of gallic acid with 10 carbon atoms conjugated with TTP^+ (TPP^+C_{10}) inhibits tumor growth *in vivo* after 30 days treatment, and its combination with the antibiotic doxycycline, achieves an elimination of 80% of tumors in tumor-bearing mice. Moreover, the survival rate of animals following treatment compared to the control group is 90% without experiencing relapse after 60 days of treatment ends. Furthermore, treatment with TPP^+C_{10} and its combination with doxycycline produces no systemic toxicity. In conclusion, the treatment with TPP^+C_{10} in combination with doxycycline, is safe to administered in animals producing a selective effect in reduce tumor size versus control groups, without producing systemic toxicity. This work was funded by FONDECYT Grant N° 1130772 and CONICYT scholarship N° 21110084.

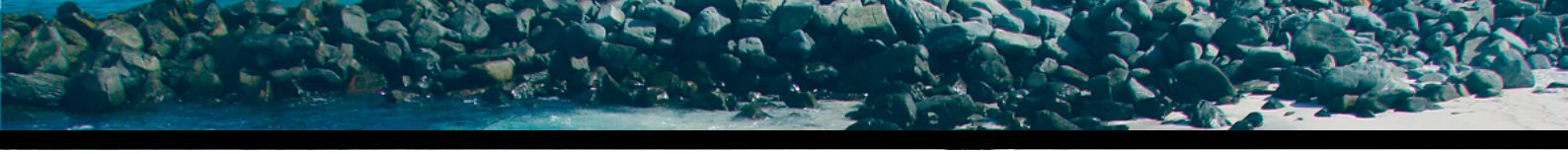


POSTERS II

1) **A novel competitive antagonist nAChR α4β2, ((S)-1-methylpyrrolidin-2-yl) methyl benzoate, reduces ethanol intake in UChB bibulous rats**

Quiroz, Gabriel[1].,Sotomayor-Zarate, Ramon[2].,Quintanilla, Maria Elena[3].,Reyes-Parada, Miguel[4].,**Iturriaga-Vasquez, Patricio[5]**.,
[1]Programa de Doctorado en Farmacología, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile.[2]Centro de Neurobiología y Plasticidad Cerebral, Facultad de Ciencias, Universidad De Valparaíso.[3]Instituto de Ciencias Biomedicas, Facultad de Medicina, Universidad De Chile.[4]Escuela de Medicina, Faculatd de Ciencias Medicas, Universidad De Santiago De Chile.[5]Ciencias Quimicas y Recusos Naturales, Facultad de Ingenieria y Ciencias, Universidad De La Frontera. (Sponsored by FONDECYT N° 1150615 (PIV), 1130185 (MRP), 1130012 (MEQ), CONICYT National PhD Scholar Fellowship (GQ))

Alcohol (ethanol) abuse is related as main cause of preventable diseases. Ethanol and their metabolites (such as acetaldehyde and salsolinol) acts over many pharmacological targets in Central Nervous System (CNS) modulating the biological effects of ethanol. Similarly to other drugs of abuse, like cocaine, amphetamine, nicotine, etc., ethanol (and their metabolites) could modulates the mesolimbic pathway, inducing an increment of dopamine in nucleus accumbens, considered the main key of drug abuse reinforcement properties. Electrophysiological evidences showed that ethanol increase acetylcholine currents of nicotinic acetylcholine receptors (nAChR) subtype α4β2, the most abundant nAChR of CNS and mesolimbic pathway. Pharmacological evidences indicate that mecamylamine (non selective nAChRs antagonist) and dihydro-β-erythroidine (selective nAChR α4β2 competitive antagonist) reduce ethanol intake in restrictive ethanol access paradigm rat model. Furthermore, has been reported that varenicline and cytisine (selective nAChR α4β2 parcial agonists) reduced the ethanol intake in free choice paradigm in UChB rats, a potent high-drinking rat model for ethanol intake. Herein, we showed the effect of novel competitive antagonist nAChR α4β2, ((S)-1-methylpyrrolidin-2-yl) methyl benzoate (named FPy), in ethanol intake conduct in free choice paradigm in UChB rats. Briefly, UChB rats were exposed to ethanol free access for 20 days to reach the plateau ingesting. From day 21, rats were separated in three groups for the administration of saline solution, 5 mg/Kg or 10 mg/Kg of FPy i.p. daily for seventeen days. And in the course of twenty days the ethanol, water consumption and body weight was recorded daily. Our results showed that both FPy doses (5 and 10 mg/Kg i.p daily) reduced the voluntary ethanol intake around 50% of plateau consumption. Our results indicate that, selective and competitive nicotinic antagonist could be a useful tool for to help in pharmacotherapy of alcohol abuse.



2) **Behavioral characterization of the acute effects in rats of 2,4-DMA (2,4-dimethoxyamphetamine) as precursor of atypical psychotropic derivatives**

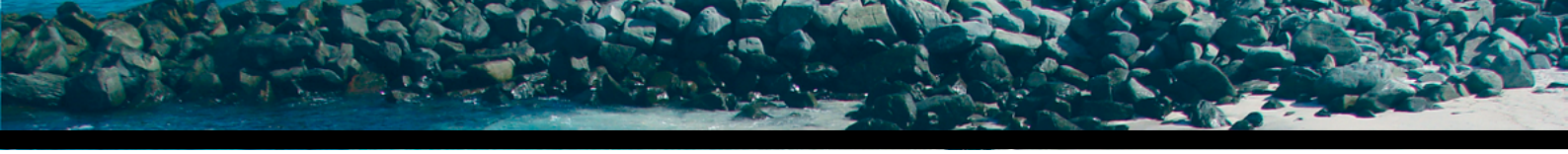
Klagges-Troncoso, Jorge[1,2]., Burgos-Villaseca, Jorge[1,3].,Benavente-Schonhaut, Sofia[1,2].,Malhue-Olmos, Valeska[1,2].,Hernández, Alejandro[4].,Burgos, Hector[5].,Castro-Castillo, Vicente[6].,Sáez-Briones, Patricio[1,2].,[1]Laboratory of Neuropharmacology and Behavior, Faculty of Medical Sciences, Universidad De Santiago De Chile.[2]School of Medicine, Faculty of Medical Sciences, Universidad De Santiago De Chile.[3]Department of Biology, Faculty of Basic Sciences, Universidad Metropolitana De Ciencias De La Educación.[4]Laboratory of Neurobiology, Faculty of Chemistry and Biology, Universidad De Santiago De Chile.[5]School of Psychology, Faculty of Social Sciences, Universidad Central.[6]Department of Chemistry, Faculty of Basic Sciences, Universidad Metropolitana De Ciencias De La Educación. (Sponsored by DICYT-USACH Grant 021401SB And DICYT-USACH Grant For Undergraduate Students 2014-JKT)

2,4-dimethoxyamphetamine (2,4-DMA) is a synthetic psychotropic phenylalkylamine structurally related to the hallucinogen mescaline but has been described to possess weak hallucinogenic properties. Nevertheless, the subjective effects reported for 2,4-DMA in humans suggest a more complex pharmacological profile, including not only standard stimulant-like properties but also entactogenic-like effects as well. This peculiar condition as “mixed” drug could be considered as a start point to develop novel derivatives exhibiting single psychotropic properties. Unfortunately, the available in vivo data to support the latter assumptions are scarce and incomplete. In the present work, the acute behavioral effects of 2,4-DMA have been studied in male Sprague Dawley rats after i.p. administration of single doses of the drug as a water-soluble salt (dose range 1 – 20 mg/kg). The pharmacological characterization included measurements of spontaneous psychomotor activity (e.g. motor activity, locomotion, grooming and rearing behaviors, head-shakes responses, stereotypy-inducing responses), the evaluation of anxiolytic/anxiogenic effects at the elevated plus maze and the effects on acquisition using the active avoidance conditioning paradigm. The results obtained indicate that 2,4-DMA exhibit a complex profile that do not follow a strict dose-response correlation, including weak anxiolytic-like effects and a decrease in locomotion at different doses (10 mg/kg and 1 mg/kg respectively), with no significant increase in the number of head shakes. In contrast, rearing behavior was consistently decreased and acquisition was almost abolished in a dose independent manner ($p < 0.01$). Interestingly, a decrease in the stereotypy-inducing response was also observed at 1 mg/kg and 20 mg/kg. None of the effects elicited by 2,4-DMA were consistent with an entactogenic-like profile. Taken together, the results obtained are in agreement with the notion that 2,4-DMA may possess mixed psychotropic effects, but they appear to include hallucinogenic-like and stimulant-like properties only.

3) **Rats exposed prenatally to valproate display decreased colonic permeability to macromolecules**

Olavarría-Ramírez, Loreto[1]., Moyano-Porcile, Valentina[1].,González-Arancibia, Camila[1].,Díaz-Zepeda, Camilo[1].,Valencia, Martina[2].,Aliaga, Esteban[2].,Bravo, Javier[1].,Julio-Pieper, Marcela[1].,[1]Grupo de NeuroGastroBioquímica, Laboratorio de Química Biológica. Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica De Valparaíso.[2]Laboratorio de Neurociencias, Escuela de Kinesiología, Facultad de Ciencias, Pontificia Universidad Católica De Valparaíso. (Sponsored by Funding: PUCV DI 037.302/2013, Conicyt 79112017 And Fondecyt 1130213)

The existence of a rich gut-to-brain communication has led to suggest that alterations in the intestinal barrier may take part in the pathophysiology of mental disorders. Particularly in autism spectrum disorder (ASD) patients, gastrointestinal alterations including abdominal pain, diarrhea, constipation and bloating are frequently reported, although part of the expert medical community feels that the prevalence of these conditions is not yet completely understood. The “leaky gut” hypothesis suggests that food compounds which are able to cross through a hyper-permeable intestinal mucosa could induce the behavioral symptomatology of autism. However, the evidence for increased intestinal permeability in individuals with ASD is controversial and limited, with most reports to date showing methodological caveats including inadequate controls and small subject populations. The aim of this investigation was to evaluate whether intestinal permeability is increased in an animal model of autism. For the ASD model, pregnant Sprague Dawley rats received valproate (VPA) at gestational day 12.5 (450 mg/kg intraperitoneal). This treatment is known to induce alterations in behaviour and social interaction in the offspring, resembling that of ASD in humans. Controls were treated with saline at the same gestational time. Samples of colon and ileum were taken from male pups between postnatal days 30 and 33. Permeability evaluation was performed ex vivo by measuring the traffic of different size fluorescent dextrans (FITC-40 kD and TRITC-4.4 kD) from the mucosal to the serosal side of intestinal tissue. Tissues were incubated for 120 minutes and measurements were made every 30 minutes. Also a section of the tissue was fixed in 4% PFA for morphological evaluation. The results show that in VPA exposed rats, the permeability of the ileum to macromolecules is not affected. In contrast, the colon of VPA rats presents a significant decrease in permeability compared to the control group, for both macromolecules after 120 minutes of incubation. As for morphological evaluation of colon, the mucosal thickness was not significantly different from control. Although these findings are opposed to what is proposed by some clinical evidence, a decrease in colon permeability may be related to an altered gut brain axis communication, having a potential effect on the central nervous system (CNS). It is still necessary to investigate if such intestinal changes are a cause or a consequence of CNS alterations described in this animal model.



4) **Early-life dysbiosis in infant sprague-dawley rats: effect on anxiety-like behaviors and plasma corticosterone levels**

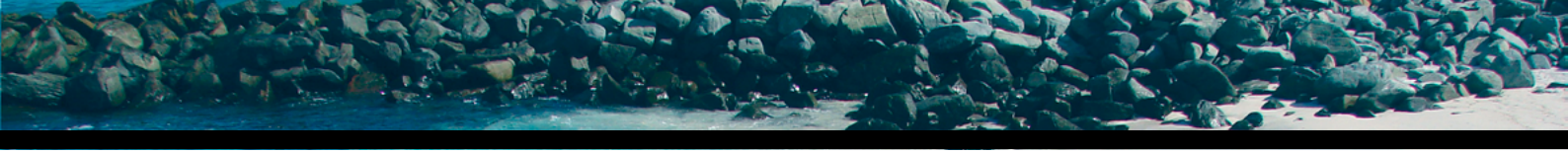
Ponce- Guequen, Excequel[1]., Barrera-Bugeño, Camila[1].,Eyzaguirre-Velasquez, Johana[1].,Escobar-Luna, Jorge [1].,Olavarría-Ramirez, Loreto[1].,Gotteland, Martín[2].,Julio-Pieper , Marcela[1].,Bravo, Javier[1].,[1]Grupo de Neurogastrobioquímica, laboratorio de Química Biológica. , Instituto de Química, Facultad de Ciencias., Pontificia Universidad Católica De Valparaíso.[2]Departamento de nutrición. , Facultad de Medicina, Universidad De Chile. (Sponsored by Funding: FONDECYT #1140776)

Intestinal colonization in neonates begins at birth by bacterial acquisition through the birth canal. Later, gut microbiota composition varies throughout postnatal development by many others factors, such as type of feeding or exposure to antibiotics. On the other hand, these gut symbionts are recognized to affect key aspects of host physiology, e.g. embrionary development of the blood brain barrier and the communication between the gut and the central nervous system. Evidence shows that germ-free mice have reduced anxiety-like behaviors, while having an exaggerated hypothalamic-pituitary-adrenal (HPA) axis response. In addition, non-absorbable wide-spectrum antibiotics have anxiolytic effects in adult mice. All these observations have been made in adult subjects. However, very little is known about the effects of acquiring an altered gut microbiota early in life on stress-related behavior and HPA function in infant rats. Therefore, we hypothesize that intervention in maternal gut microbiota, like exposure to wide-spectrum non-absorbable antibiotics in the perinatal period modifies the infants gut microbiota, impacting on stress-related behavior and HPA axis function. To test this we administered a combination of neomycin (100mg/kg), bacitracin (100mg/kg), pimaricin (5mg/kg) and vancomycin (100mg/kg) orally to pregnant Sprague-Dawley dams, starting three days before parturition and maintained it until post-natal day (PD) 7. On PD 21 pups were weaned and behavioral testing begun on PD 22. Open field test showed that male but not female pups exposed to antibiotics, spent more time in the central area of the apparatus in comparison to control rats. Also, pups exposed to antibiotics had reduced number of fecal boli during this test. On PD 23, elevated plus maze test revealed that both female and male pups exposed to antibiotics had higher number of entries and spent more time in the open arms than control rats. On PD 24 rats were subjected to the forced swim test, however there were no differences between groups in depression-like behaviors. Then, 30 min after the last behavioral test plasma corticosterone concentration was determined. Animals exposed to early-life antibiotics had more variability in plasma corticosterone than the control group. No difference in body weight was found between groups. Together these data suggests that acquisition of a conventional gut microbiota in early-life is important for an adequate coupling between HPA axis function and behavioral responses to stress.

5) **Betamethasone treatment effect in patients with spastic paraparesis associated with HTLV-1 retrovirus**

Valenzuela, Maria[1]., Alberti, Carolina[1].,Puente, Javier[1].,Quintremil, Sebastián[1].,Medina, Fernando[1].,Barriga, Andres[1].,Ramírez, Eugenio[2].,Cartier, Luis[3].,[1]Departamento de Bioquimica y Biologia Molecular, Ciencias Químicas y Farmacéuticas, Universidad De Chile.[2]Virología, Facultad de Medicina, Universidad de Chile.[3]Departamento de Ciencias Neurologicas, Facultad de Medicina, Universidad de Chile. (Sponsored by Fondecyt 108 0396)

Progressive HTLV-1 associated Tropical Spastic Paraparesis or HAM/TSP is considered a central axonopathy caused by an axoplasmic transport dysregulation. Up to 3.0% of infected people develop this disease, remaining the rest as asymptomatic carriers. In all patients, T-CD4+ cells are the main target of HTLV-1, *in vivo*. The viral protein Tax has been associated to alterations observed in HAM/TSP. Currently, there is no specific treatment for HAM/TSP. However, therapy based on corticosteroids has been successful in decreasing patient symptomatology. Taking into account the above information, the effect of a treatment using systemic betamethasone (a monthly injection) in HAM/TSP patients was investigated, evaluating clinical and molecular aspects related with immunological markers. To understand the effects of betamethasone we determined in PBMCs from patients mRNA and protein of FoxP3 and Tax, using real time PCR and flow cytometry. After treatment, an improvement of motor disability was observed together with a decrease in T-CD4+Tax+ and an increase in T-CD4+FoxP3+ populations at both protein and mRNA levels. In order to determine a relationship between decreased mRNA Tax and CD4+Tax+ population with regard to treatment with betamethasone, we searched in the base Transcription Element Search System for the presence of response elements inside and outside the promoter of HTLV-1, finding a response element in the viral promoter at position 79 corresponding to the 5 \'LTR U3 region. betamethasone increased the amplification of this region of HTLV-1 virus followed by chromatin immunoprecipitation. The decrease in viral promoter amplification in the presence of glucocorticoids suggests the binding to a repressor of the viral transcriptional activity, represented by the decrease in mRNA levels of Tax. These results suggest a relationship between the HTLV-1 promoter and glucocorticoid receptor, indicating that betamethasone treatment might contribute to decrease viral load and raise Treg population.



6) **Neonatal programming with Estradiol Valerate does not produce conditioned place preference to amphetamine in adult female rats**

Sanguinetti, N[1]., Venegas, Francisca[1].,Espinosa, Pedro[1].,Renard, Georgina[1].,Sotomayor-Zárate, Ramón[1].,[1]Institute of Physiology, Faculty of Sciences, Universidad de Valparaíso. (Sponsored by This Work Was Supported By FONDECYT Project No. 11121205 For RS-Z)

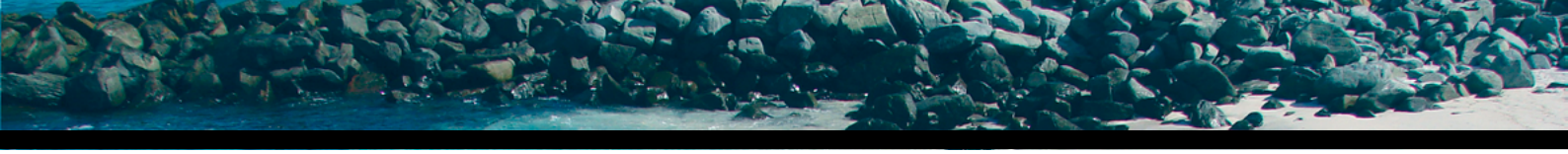
The programming concept is defined as the physiological redirection of an organ or tissue due to an early insult in sensitive developmental periods. In this context, our laboratory has shown that neonatal exposure to estradiol valerate (EV) increases the amount of dopamine (DA) in brain circuits associated with reward and locomotion in adult female rats. However, amphetamine-induced DA release (systemic and intra-nucleus accumbens) is significantly lower in EV treated-females than control female rats.

So, the main objective of this study was to evaluate the effects of repeated administration of amphetamine in male and female rats exposed to EV at postnatal day (PND) 1 on the expression of conditioned place preference (CPP) to amphetamine in adulthood. In this work, Sprague-Dawley rats of both sexes were used. At PND1, they received a dose of EV (0.1 mg/50 µL s.c. in sesame oil). Control male and female rats were injected with 50 µL sesame oil s.c. at PND 1. At PND60 a seven days CPP protocol was performed. This protocol consisted in a pretest day, five days of conditioning with a daily dose of amphetamine (1 mg/Kg i.p.), and a test day. Time spend in the compartment associated with amphetamine is an index of the reinforcing value of the drug. Our results showed that control females, control males and EV treated-males expressed CPP to amphetamine (measured as the increases in time spend in the compartment associated with the amphetamine at the test day). However, consistent with our neurochemical results, we found that EV treated-female rats did not express CPP to amphetamine. These results suggest that neonatal administration of EV does not produce CPP behavior in adult female rats, possibly by altered expression of the dopamine transporter (amphetamine molecular target). However, this will be evaluated in subsequent work by Q-RT-PCR and WB.

7) **Amphetamine conditioned place preference and the vasopressinergic system: a study on male and female rats**

Bahamondes, Carolina[1]., Ahumada, Catalina[1].,Silva, Roxana[1].,Cruz, Gonzalo[1].,Sotomayor-Zárate, Ramón[1].,Renard, Georgina[1].,[1]Centro de Neurobiología y Plasticidad Cerebral - Instituto de Fisiología, Facultad de Ciencias, Universidad De Valparaíso. (Sponsored by Financial Support: FONDECYT N° 11140065 To GMR And Committee For Aid And Education In Neurochemistry (CAEN), International Society For Neurochemistry (ISN).)

Research in neurobiology of drug addiction has been focused on the effects of drugs of abuse at reward circuit and the role of Lateral Septum (LS) has regained importance in this field. This nucleus is involved in integrated different areas of the brain and has been shown that vasopressinergic neurotransmission in the LS is involved in regulation of anxious-like behavior. However the regulation of drugs of abuse over this peptide has not been widely studied. The aim of this research was to study the effect of amphetamine (AMPH) administration during a conditioned place preference (CPP) protocol over extrahypotalamic vasopressin (AVP) system of adult females and males rats. Female and male Sprague Dawley rats (55-60 days old) were used. The stage of the estrus cycle was daily determined by vaginal smears examination. We measured the rewarding effect of AMPH by CPP protocol. CPP apparatus consist in a white compartment, corridor and a black compartment. First, a pre-test was performed on day 1, in which animal were allowed to freely explore all compartments for 15 minutes. Time spent in each compartment was recorded. Then a conditioning phase was performed, where animals received a daily AMPH dose (4 days) and then they were confined for 60 min in the white compartment. At day 6, animals were again allowed to freely explore all the compartments (test). Time spend in the compartment associated with AMPH is an index of the reinforcing value of the drug. We considered that an animal was conditioned if it spends > 60% of the time in the AMPH-associated compartment the test day compared to the pre-test day. Besides, we measured AVP content in LS by ELISA kit and AVP mRNA expression in MeA by RT-q-PCR. Our results showed that 50% of female rats (6 conditioned females from 12 AMPH-treated females) and 78% of male rats (7 conditioned males from 9 AMPH treated) expressed CPP to AMPH. In female case this behavior was independent of the stage of estrous cycle they were. AMPH treatment did not produce difference in AVP content on LS, but in AMPH conditioned animals the AVP mRNA expression on MeA was lower than control animals.Therefore, AMPH treatments produce sexual differences in acquisition of CPP to AMPH and produce alteration in the vasopressinergic system.



8) **Long-term effects of prenatal Fluoxetine on memory and motivation in adult male rat offspring**

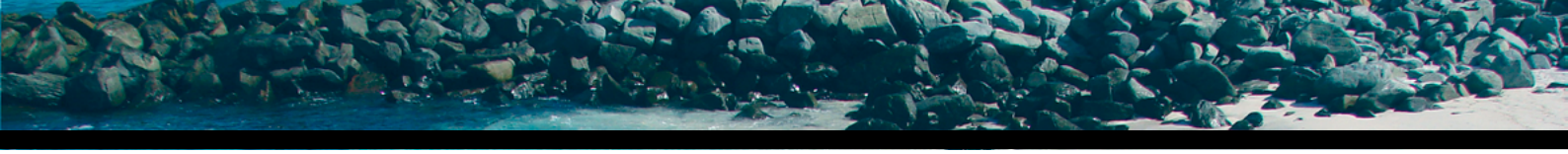
Tamburini, G[1]., Rubio, Francisco[2].,Moraga-Amaro, Rodrigo[1].,Diaz-Galarce, Raul[1].,Ampuero, Estibaliz[3].,Wyneken, Ursula[4].,Stehberg, Jimmy[5].,[1]Laboratorio de Neurobiología Universidad Andrés Bello.[2]Behavioral Neuroscience Research Branch, Intramural Research Program National Institute on Drug Abuse, National Institutes of Health, U.S..[3]Center for Biomedical Research, Faculty of Biological Sciences and Faculty of Medicine Universidad Andres Bello.[4]Laboratorio de Neurociencias, Centro de Investigaciones Biomédicas Universidad de los Andes.[5]Laboratorio de Neurobiología, Centro de Investigaciones Biomédicas, Facultad de Ciencias Biológicas and Facultad de Medicina Universidad Andrés Bello.

Fluoxetine has been prescribed to treat depression in pregnant women for over 30 years. Some studies have suggested that administration of fluoxetine during early development in rodents may induce persistent changes in emotional behavior of the offspring. However, the effect of prenatal fluoxetine on memory is less studied, although studies in adult rats treated with clinically relevant doses of fluoxetine (0.7 mg/kg) suggest long-lasting memory impairments. The objective of this study is to evaluate the effects of in utero exposure to fluoxetine on hippocampal- and non-hippocampal-dependent memory of the adult male offspring, using Morris Water Maze and the Novel Object Recognition memory, respectively. Anxiety- and depressive-like symptoms were also evaluated.Fluoxetine treated offspring showednovel object recognition memory impairments 24 hours post-training, as well as increased anxiety and depressive-like symptoms. Interestingly, treated animals did not show significant differences in learning capacity or retention 24 hrs post training in comparison to a control group in the Morris Water Maze, but showed retention impairments when tested 15 days after training.Our data suggests that prenatal exposure to fluoxetine may induce long-lasting, detrimental effects on memory and emotional behavior in the adult male offspring, and warrants the need for studies to assess human memory in adults born to mothers who took Fluoxetine during pregnancy.

9) **Effects of the alkaloid gelsemine on recombinant glycine receptors**

Marileo, Ana[1]., Lara, Cesar[1].,Burgos, Carlos[1].,Yévenes, Gonzalo[1].,[1]Department of Physiology Universidad De Concepción.
(Sponsored by Supported By FONDECYT 1140515. Sponsored By Dr. Jorge Fuentealba A.)

Glycine receptors (GlyRs) are transmitter-gated anion channels that mediate synaptic inhibition in the central nervous system. Despite their pivotal role in many physiological and pathophysiological processes, the GlyR pharmacology is still very limited. Recent studies have shown that gelsemine, a natural alkaloid from *Gelsemium sempervirens*, binds to spinal GlyRs and exerts antihyperalgesic actions in rodents. However, the functional effects of gelsemine on GlyRs are still largely unknown. In this study, we characterize the functional effects of gelsemine on GlyRs using patch-clamp recordings of HEK293 cells transiently transfected with plasmids encoding the three main GlyR alpha subunits (α1, α2 and α3, i.e. homomeric αGlyRs) in the absence or the presence of the beta GlyR subunit (i.e. heteromeric αβGlyRs). We first evaluated the sensitivity of homomeric α1GlyRs to different concentrations of gelsemine. We found that low micromolar concentrations (0.1-50 μM) of gelsemine potentiated the glycine-activated currents of α1GlyRs. The potentiation displayed a bell-shaped profile, with a peak potentiation of 80± 30% using 25 μM of gelsemine. We next investigated whether the α2 and α3 GlyRs were similarly modulated by gelsemine. Unexpectedly, the glycine-evoked chloride currents through these receptors were significantly inhibited in a concentration-dependent manner by gelsemine (1-100 μM). We next analyzed the influence of the β subunit on these pharmacological profiles by studying the corresponding heteromeric αβGlyRs. Our results showed that the presence of the β subunit did not affect the sensitivity α2 and α3GlyRs to gelsemine. However, the gelsemine-induced potentiation of α1GlyRs was significantly diminished by the expression of β subunits (-8±7% using 25μM of gelsemine in α1βGlyRs). These results indicate that the actions of gelsemine are subunit-specific and that are influenced by the presence of β subunits. These data thus suggests the presence of specific molecular determinants for the alkaloid effects on several GlyR subtypes. Ongoing studies using chimeric and point-mutated GlyRs will define the residues involved in these pharmacological differences. Future efforts aiming to identify the gelsemine binding and modulatory sites may facilitate the development of new subunit-specific GlyR modulators with therapeutic potential.



10) **Modulation of spinal glycine receptors by the alkaloid gelsemine.**

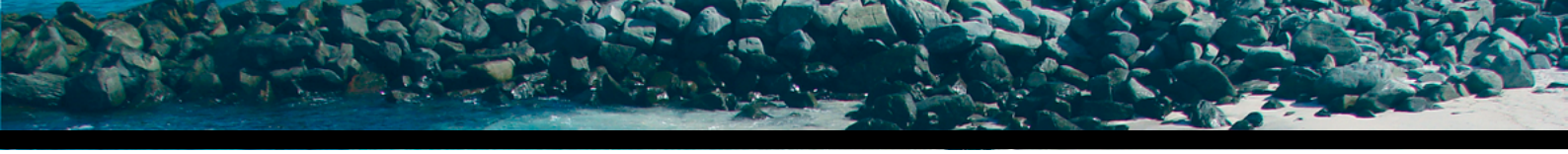
Murath, Pablo[1]., Lara, Cesar[1].,Yévenes, Gonzalo[1].,[1]Department of Physiology Universidad De Concepción. (Sponsored by Supported By FONDECYT 1140515. Sponsored By Dr. Jorge Fuentealba A.)

Glycine receptors (GlyRs) are transmitter-gated anion channels that mediate synaptic inhibition in the central nervous system. GlyR inhibitory function is particularly critical in the processing of nociceptive and sensory signals at the level of the spinal dorsal horn. Recent studies have shown that gelsemine, a natural alkaloid from *Gelsemium sempervirens*, exert potent analgesic actions on behavioral models of pain. These evidences suggest that gelsemine may interact with spinal GlyRs and thus modulate the dorsal horn inhibitory synaptic transmission. Here we studied the sensitivity of spinal GlyRs to gelsemine using cultured mouse spinal cord neurons and whole-cell patch-clamp recordings. We first analyzed the effect of different concentrations of gelsemine on the glycine-evoked currents. We found that gelsemine (0.1-200 µM) inhibited the glycine-activated currents in a concentration-dependent fashion. The inhibition displayed a peak inhibition of -79±3% elicited by 200 µM of gelsemine with an IC_{50} of 48±7 µM. We next investigated the effects of the alkaloid on the GABA-evoked or AMPA-evoked currents on these neurons. Our results showed that the agonist-evoked GABAergic or AMPAergic currents were not significantly modified by 50 µM of gelsemine. We finally studied the effects of gelsemine on the glycinergic synaptic activity by studying miniature glycinergic post-synaptic currents (mIPSCs). Our results showed that gelsemine (50 µM) dramatically decreased the frequency of glycinergic mIPSCs (-89±4 %). The alkaloid however did not significantly altered the glycinergic mIPSC amplitude (13±6%). These results indicate that gelsemine negatively modulates spinal GlyRs. Furthermore, our data showed that the alkaloid strongly modify the glycinergic synaptic activity of cultured spinal neurons. Collectively, these data suggest that the alkaloid may modulate inhibitory synaptic networks at the level of the spinal dorsal horn. These synaptic actions may contribute to explain the analgesic activity of gelsemine in behavioral pain models.

11) **TOLL-LIKE-RECEPTOR (TLR4) INDUCES AN INCREASE IN PROINFLAMMATORY CYTOKINES AND ADHESION MOLECULES IN CARDIAC FIBROBLAST AND MYOFIBROBLAST**

ANFOSSI, Renatto[1]., Humeres, Diego[1].,Boza, Pía[1].,Muñoz, Claudia[1].,Vivar, Raúl[1].,Díaz-Araya, Guillermo[1].,[1]FARMACOLOGÍA Y TOXICOLOGÍA, CIENCIAS QUÍMICAS Y FARMACÉUTICAS, Universidad De Chile. (Sponsored by FONDECYT Regular 1130300 (GDA). BECA GASTOS OPERACIONALES 21120406)

Introduction: Toll-like-receptor (TLR4) plays a critical role on the onset and resolution of inflammatory cardiovascular diseases such as cardiac fibrosis. This receptor has been extensively studied in immune cells present on the site of cardiac injury. However, its role on cardiac fibroblasts (CF) and myofibroblasts (CMF); which are central mediators of inflammatory and fibrotic myocardial remodeling, remains unknown. We hypothesize that this receptor plays an important role in CF/CMF proinflammatory cytokine secretion and adhesion molecule expression. Methods: Adult rat CF and CMF were treated with LPS (1 µg/ml 8-48 h) in presence/absence of TLR4 inhibitor TAK-242. 9 cytokines secreted to the extracellular medium by CF and CMF (TNF-α, IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IFN-γ, MCP-1) were quantified by LUMINEX after treatment. Protein levels of intercellular adhesion molecule (ICAM-1) and vascular cellular adhesion molecule (VCAM-1) were measured by Western blot. To assess if VCAM-1/ICAM-1 were functional, we cultured monolayers of CF and CMF in presence/absence of LPS and TAK-242 during 24h, and performed adhesion assays of monocytes over those cell monolayers. Results: TLR4 activation induces an increase in the secretion of TNF-α, IL-10 and MCP-1 in both CF and CMF at 24/48 h of LPS stimulation. TLR4 activation also generated an increase of ICAM-1/VCAM-1 proteins expression in CF and even greater levels were observed in CMF. These effects were observed from 8h of LPS stimulation and onwards. Both ICAM-1/VCAM-1 were necessary for adhesion of monocytes to layers of CF and CMF, because the inhibition of these adhesion molecules decreased the number of monocytes adhered. These results suggest that activation of TLR4 in CF/CMF can act as a key component in the crosstalk between immune and cardiac cells by contributing to the proinflammatory cytokine milieu and by recruiting immune cells to the site of injury through the up-regulation of ICAM-1/VCAM-1.



12) **Characterization of toxicity and antioxidant effects of selenium nanoparticles biosynthesized by *Pantoea agglomerans* in HUVEC**

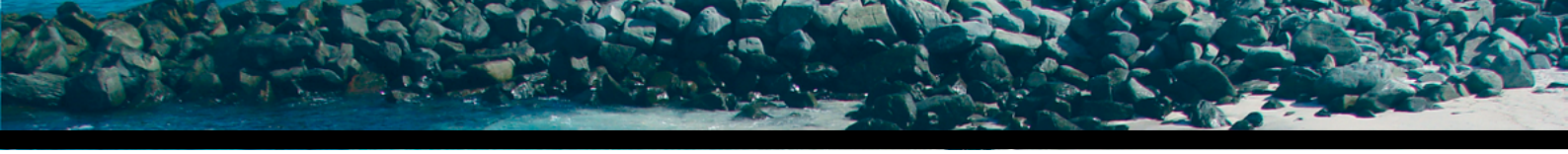
Haensgen , A.[1].,Saavedra, A.[1].,González, M.[1].,Rojas, S.[1].,Carrasco, I.[2].,Rodríguez, S.[3].,Rojas, C.[3].,[1]Physiology Universidad De Concepción.[2]Microbiology Universidad De Concepción.[3]CIPA Universidad De Concepción. (Sponsored by FONDEF CA12-I-10374 , Basal Conicyt-Regional R08C1002)

It has been found that selenium (Se) is an essential micronutrient that has an antioxidant and cardio protective role. Selenium deficiency causes cardiovascular diseases and this condition is associated with lower concentration of Se in soil, affecting the nutritional properties of grown vegetables for human and animal consumption. One of the main difficulties about the use of Se like a nutritional supplement is that the soluble forms of Se, selenite (Se IV) or selenate (Se VI), are toxic and unable for use in humans. The elemental Se (Se 0) has lower toxicity, but is insoluble in water. To solve this problem we propose the use of *Pantoea agglomerans,* a bacterium capable of coexisting in enriched medium with selenite/selenite, because reduces the metalloid. *P. agglomerans* synthetizes and releases Se 0 nanoparticles (SeNPs), these SeNPs can be obtained, filtered, stabilizes with L-cysteine and encapsulates with chitosan and tripolyphosphate for potential application as a nutritional supplement. Our aim was determine the toxicity and antioxidant capacity of biosynthesized SeNPs on human endothelial cells. Human umbilical vein endothelial cells (HUVEC) were isolated (collagenase digestion) and maintained in medium 199 (M199) with sera (20%). HUVEC were incubated (37°C, 24 h) with different forms of Se and SeNPs and cell viability and reactive oxygen species (ROS) synthesis were determined with Vybrant® MTT Cell Proliferation Kit and 2',7'-dichlorofluorescein (DCF) dye, respectively. The incubation of HUVEC with selenite (1 µg/ml) reduced the cell viability to 20%, meanwhile the incubation with biosynthetized SeNPs (1 µg/ml) did not reduced the cell viability, compared to control. In regards with ROS synthesis, incubation with selenite (1 µg/ml) increased the oxidative stress 2.9-fold related to control, filtered SeNPs did not change the ROS levels, meanwhile the encapsulated SeNPs induced a significant reduction of ROS in HUVEC exposed to high concentration of D-glucose. In conclusion, selenite enhances ROS synthesis and diminished the viability of human endothelial cells. These alterations are avoided when we used biosynthesized SeNPs, especially the stabilized and encapsulated form.

13) **Prolonged activation of connexin-formed hemichannels by angiotensin II-induced NADPH oxidase-mediated superoxide production in endothelial cells**

Lazo, V[1]., Poblete, Inés[1].,Figueroa, Xavier[1].,[1]Departamento de fisiología, Facultad de ciencias biológicas, Pontificia Universidad Católica De Chile.

Increases in superoxide ($O_2^{\cdot-}$) production by the NADPH oxidase are associated with the development of endothelial dysfunction and hypertension. It is thought that the detriment of vascular function observed in response to angiotensin II (AII) is mediated by the increase in NADPH oxidase-derived $O_2^{\cdot-}$ and the further rapid reaction of this reactive oxygen specie with nitric oxide (NO), which subsequently attenuates the endothelium-dependent vasodilator responses. However, in addition to NO, connexin-formed channels also play an important role in the control and coordination of vascular signaling. Connexin proteins can form gap junction channels or hemichannels (half of a gap junction channel) and are express in endothelial and smooth muscle cells. It has been shown that prolonged activation of hemichannels leads to the development of cell dysfunction, but the effect of $O_2^{\cdot-}$ on hemichannel activity in the vascular wall of resistance arteries has not been determined. In this work, we analyzed the involvement of NADPH oxidase-mediated $O_2^{\cdot-}$ production in the regulation of hemichannel function in mesenteric resistance arteries and primary cultures of mesenteric endothelial cells. Hemichannel opening was assessed by measuring ethidium uptake in response to AII (10 nM) or directly to the increment of $O_2^{\cdot-}$ formation evoked by the application of the NADPH oxidase high affinity substrate, NADH (100 µM). As expected, AII and NADH application resulted in a prolonged increase of $O_2^{\cdot-}$ formation and ethidium uptake in intact arteries and cultured endothelial cells. Both the increase in $O_2^{\cdot-}$ production and ethidium uptake were blocked by apocynin, a NADPH oxidase blocker, or TEMPOL, a $O_2^{\cdot-}$ scavenger. In addition, the connexin-formed channel blocker, carbenoxolone, also abolished the increment in ethidium uptake. These results suggest that NADPH oxidase-derived $O_2^{\cdot-}$ formation leads to prolonged activation of connexin hemichannels in endothelial cells of resistance arteries, which may explain, at least in part, the development of endothelial dysfunction typically observed in response to AII. Proyecto FONDECYT 1150530



14) **NADPH oxidase regulation by polycystin-1 in cardiomyocytes**

Córdova-Casanova, A[1]., Olmedo, I[1].,Donoso, P[1].,Sánchez, G[1].,Pedrozo, Z[1,2].,[1]Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad De Chile.[2]Advanced Center for Chronic Diseases (ACCDiS), Facultad de Medicina, Universidad De Chile.
(Sponsored by Fondecyt 1150887, 1130407, 3140449. Programa U-Inicia Concurso De Reforzamiento De Inserción Productiva De Nuevos Académicos VID 2014 Universidad De Chile, FONDAP 15130011)

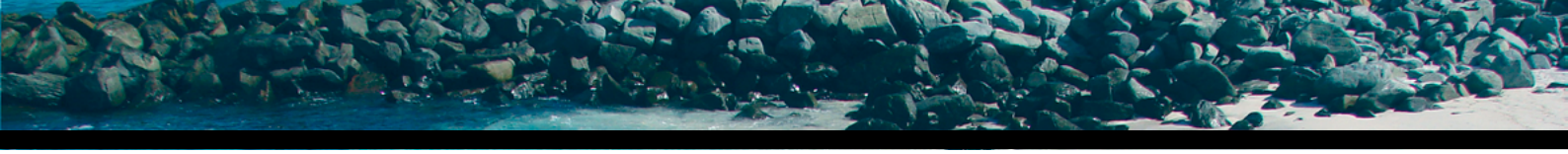
NADPH oxidase 2 (Nox2), is one of the most important sources of reactive oxygen species (ROS) in the cardiomyocyte and plays a crucial role in cardiac physiology and pathology. Mechanical stretch activates Nox2 and increases ROS generation but the mechanosensor implicated in this activation is unknown. Polycystin-1 (PC1), a transmembrane protein that acts as a mechanosensor in different cells, is also expressed in cardiomyocytes but its physiological function is not fully understood. To investigate the role of PC1 in the regulation of Nox2 activity we measured nitrated proteins, as an indication of ROS generation, in neonatal rat ventricular cardiomyocytes transfected with siRNA specific to PC1 or in heart homogenates from PC1 knockout mice.

We observed that the decreased PC1 expression in the neonatal cardiomyocytes transfected with the PC1 siRNA caused a significant increase in nitrated proteins. Apocynin, a Nox2 inhibitor, prevented this increase. Heart homogenates from PC1 KO mice also showed an increase in nitrated proteins as compared to controls, confirming the data obtained in cardiomyocytes. Our results suggest that PC-1 is a new regulator of Nox2 in cardiomyocytes which inhibits Nox2, and prevents the generation of ROS in baseline conditions.

15) **Effect of oxHDL on the expression and distribution of endothelial proteins involved in coagulation and fibrinolysis**

Pérez, Lorena[1]., Simon, Felipe[1,2].,[1]Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas y Medicina, Universidad Andrés Bello.[2]Millennium Institute on Immunology and Immunotherapy Santiago, Chile. (Sponsored by Fondecyt 1121078 And MII P09-016-F)

High density lipoprotein (HDL) has been always known as a protective agent against cardiovascular diseases such as atherosclerosis and thrombosis. However, there is controversy about the role played by the oxidized form of HDL (oxHDL) on these diseases. Oxidative modification of native HDL can take place as a consequence of high oxidative stress mainly due to inflammatory stimuli. On the other hand, the endothelium plays a key role on thrombosis regulation, through expression and/or secretion of proteins involved in coagulation (thrombus formation) and fibrinolysis (thrombus degradation). During pathological condition, the endothelium is in permanent contact with several mediators of inflammation including oxHDL. Nevertheless, the effect produced by oxHDL on thrombosis regulation is not known. Thus, the aim of this work is to study the effect of oxHDL on the expression and distribution of endothelial proteins involved in thrombosis regulation. In order to assess that issue, rat primary cultures of mesenteric endothelial cells (RMEC) were exposed to 50 µg/mL of native HDL or oxHDL for 18 h, and the protein expression and distribution was assessed by western blot and immunofluorescence, respectively. Tissue factor (TF) and tissue factor pathway inhibitor (TFPI), were evaluated as indicators of coagulation. Plasminogen activator (t-PA), plasminogen activator inhibitor (PAI-1) and thrombin-activatable fibrinolysis inhibitor (TAFI) were evaluated as indicators of fibrinolytic activity. Our results show that RMEC exposed to oxHDL changes the expression and distribution of TF, TFPI, t-PA, PAI-1, and TAFI, suggesting a potential modulation on thrombus formation/degradation rate. We conclude that oxHDL is involved in the expression and distribution of TF, TFPI, t-PA, PAI-1, and TAFI on RMEC.



16) **Inhibition of signal transducer and activator of transcription 3 expression induces ALK-5-dependent SMAD4 mobilization in endothelial cells.**

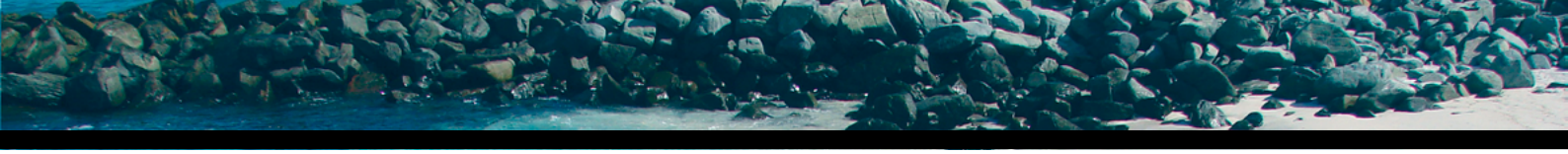
Rojas, Macarena[1]., Becerra, Alvaro[1].,Simon, Felipe[1,2].,[1]Ciencias biológicas, Ciencias biológicas, Universidad Andrés Bello.[2]IMII Millennium Institute on Immunology and Immunotherapy. (Sponsored by Fondecyt 1121078 And MII P09-016-F)

Introduction: It has been demonstrated that during the conversion of endothelial cells (ECs) into activated fibroblast, the pro-fibrotic cytokine transforming growth factor-β1 (TGF-β1) is increased. Upon binding of TGF-β1 to its type I receptor activing receptor-like kinase 5 (ALK-5), SMAD proteins conform an heterologous complex with the common-mediator SMAD4. Subsequently this protein complex translocate into the nucleus where it regulates the expression of pro-fibrotic genes promoting fibrosis. On the other hand, the signal transducer and activator of transcription 3 (STAT3) is a transcription factor which is activated in response to cytokines and growth factors. It has been described that inhibition of STAT3 expression induces spontaneous fibrosis, however, the underlying mechanism has not been described. Therefore our aim was to study whether inhibition of STAT3 expression induces the mobilization of SMAD4 into the nucleus, mediated by a TGF-β1/ALK-5 signaling pathway mechanism. **Methods and Results:** Using primary cultures of rat mesenteric endothelial cells (RMEC), we demonstrated that expression inhibition of STAT3 using siRNA-STAT3 induced the synthesis and secretion of TGF-β1. Moreover, expression inhibition of STAT3 increased SMAD4 mobilization to nucleus. Furthermore, when we used the siRNA-STAT3 combined with an inhibitor of ALK-5 (SB431542) the translocation of SMAD4 into the nucleus was blocked. **Conclusion:**We demonstrate that the inhibition of STAT3 expression induces increased translocation of SMAD4 into the nucleus dependent on ALK-5 activity. Furthermore, STAT3 suppression induces TGF-β1 secretion suggesting its participation on ALK-5-dependent SMAD4 translocation process.

17) **The inhibition of endoplasmic reticulum stress do not reverts the fetoplacental endothelial dysfunction in maternal obesity.**

Saavedra, Andrea[1]., Rojas, Susana[2].,Valdivia, Luz[1].,Haensgen, Astrid[2].,Cid , Marcela[3].,Gonzalez, Marcelo[1].,Farias, Marcelo[4].,[1]Fisiología , Ciencias Biologicas., Universidad De Concepción.[2]fisiologia, ciencias biologicas, Universidad De Concepción.[3]Obstetricia, Medicina, Universidad De Concepción.[4]Obstetricia y Ginecología, Medicina, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 1121145, 11100192.)

The maternal obesity (MO) is a growing syndrome in our population, with a prevalence of 30.7% in Chilean pregnant in according to Chilean Ministry of Health (MINSAL). In animal models of obesity, have been determined that there is a deleterious effect of endoplasmic reticulum stress (ERS) on insulin signaling pathway. In other hand, is well known that insulin increases the nitric oxide (NO) synthesis in human endothelium and insulin resistance (IR) is associated with endothelial dysfunction. We have hypothesized that there is an association between MO, IR and ERS in human endothelial dysfunction. **Objectives**: To determine the effect of ERS inhibitor (TUDCA, tauro urso-deoxycholic acid) and/or insulin on placental vascular reactivity, phosphorylation of endothelial nitric oxide synthase (eNOS) and levels of nitric oxide (NO) and reactive oxygen species (ROS) in HUVEC (human umbilical vein endothelial cells) from normal and obesity pregnant. **Methods:** Chorionic veins and HUVEC were isolated (collagenase digestion) from placenta and umbilical cord. Samples were classified as normal(N) or obese (ob) in accord with pregnant body mass index at end of gestation. Isometric tension was determined in vessels pre-constricted with increasing concentrations of U46619 ($5x10^{-8}$-$5x10^{-4}$) and exposed to insulin (1nM) and/or TUDCA (500µM). HUVEC were incubated in presence or absence of insulin (8h) and/or TUDCA (100µM, 24h) to measure total and phosphorylated eNOS ratio (*p*-eNOS/eNOS) by western blotting, fluorescence measure for NO and ROS used DAF and DCF probes, respectively. **Results**: In normal chorionic vein, the constriction induced by U46619 was decreased ($p<0.05$) by insulin (41% of maximal constriction). When vessels were exposed to TUDCA, vasoconstriction decreased 7% and 22% in absence or presence of insulin, respectively**.** In HUVEC-N insulin increase the *p*-eNOS/eNOS ratio, without changes in cells co-incubated with TUDCA. In HUVEC-ob was determined higher *p*-eNOS/eNOS ratio compare with HUVEC-N. Insulin reduces *p*-eNOS/eNOS ratio in absence (32%) or presence (41%) of TUDCA, until levels similar to normal control. In HUVEC-ob there is an increase of NO production (66%) and ROS (138%), which is not reversed by insulin and/or TUDCA. **Conclusions**: The ERS inhibition with TUDCA is not capable of potentiate the vasorelaxation induced by insulin in normal patients, in fact there is a reduction of insulin relaxation in presence of the inhibitor. These results are correlated with p-eNOS. In obesity, insulin restores the normal abundance of p-eNOS in HUVEC, but there are no changes in cells incubated with TUDCA. ROS and NO production are increased in obesity patients, but TUDCA is unable to increase or decrease effects of insulin.



18) **Allosteric regulation of arginase II by inhibitors of NO synthesis. A new mechanism of regulation of NO synthesis in hypertensive processes?**

Taborda, María[1]., Betancur, Johana[1].,Moraga, Fernando[1].,López, Vasthi[1].,[1]Ciencias Biomédicas, Medicina, Universidad Católica Del Norte. (Sponsored by This Investigation Was Made With Funding From The Regional Government And The FIC R 30137774-0 Project.)

INTRODUCTION: The mining exploitation in the north of Chile has been settled at sites located over 4000 m, generating the exposure of human to chronic intermittent hypobaric hypoxia (CIHH). Has been described, that hypoxia produces vasoconstriction of the cardiovascular system, with the purpose of improve the difusion of the O_2. Although, if the hypoxia is chronic, would develop hypertension. Described in several types the hypertension, an decrease of plasmatic of NO, would be associated with increased concentrations inhibitors of eNOS, such as asymmetric dimethylarginine (ADMA) and homocysteine and a minor bioavailability of L-arginine or is the results of the competition between nitric oxide synthase (INOS) and arginase. The main purpuse of this study was to determine the effect the ADMA and homocysteine on the arginase via, and its possible rol in the development the hypertension induced by hypoxia. **METHODS:** The hypobaric hypoxia was simulated in a hypobaric chamber at 428 torr (4600m). Subsequently, once sacrificed and identified rats *tolerants* and *intolerants* to CIHH according to methodology described on earlier studies. The arginase activity and inhibitors concentrations were measured. **RESULTS AND CONCLUSION**: Hypoxia-induced hypertension would develop by increased concentrations of ADMA and homocysteine, producing an activation of arginase II, generating a minor availability of L-arginine for NO synthesis.

19) **Comparative study of the protein expression and activity of inflammasome NLRP3 in cardiac fibroblast and myofibroblast.**

Tapia, Felipe[1]., Boza, Pia[1].,Díaz, Guillermo[1].,[1]Departamento de Farmacología y Toxicología, Ciencias Químicas y Farmacéuticas, Universidad De Chile. (Sponsored by Project FONDECYT 1130300. Sponsor Guillermo Díaz.)

The inflammasome is a multiprotein complex that includes pattern-recognition receptors (PRRs). These receptors can detect a wide range of molecular pattern exclusiveof microorganisms and can also recognize chemical signals associated to infection or tissue damage. The inflammasome NLRP3 has been widely studied, along with the receptor, the complex also includes the adapter protein ASC and pro-caspase-1. The purpose of this complex it is to activate and regulate the innate immune system through the secretion of the pro-inflammatory cytokine IL-1b.

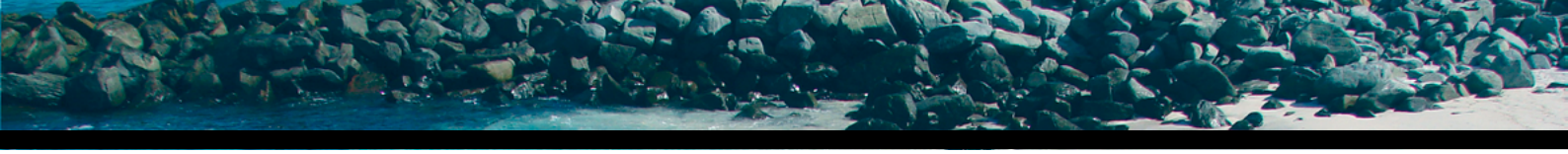
Cardiac Fibroblasts (CF) equals to approximately two-thirds of the myocardial tissue volume and are involved in the maintenance and homeostasis of the extracellular matrix (ECM). In addition, they also participate in the heart repair process by differentiating into cardiac myofibroblasts (CMF) which are cells involved in the inflammatory response to injury. Myofibroblasts are large cells with specific stress fibers that distinguish them from CF and they normally are present following cardiac injury.

Even though the inflammasome NLRP3 has been studied in different cellular types, until now there are no evidence about comparative studies of protein expression and activity of this inflammasome in CF and CMF.

Primary cultures of CF obtained from neonatal rats in passage 1 were realized for the experiments. CMF were obtained by incubating CF with TGF-b_1 (5 ng/ml) for 96 hours. Both cell types were stimulated with LPS (1 mg/ml) for 8 hours. The protein levels of the NLRP3, ASC, pro-caspase-1 and pro-IL-1b were measured by Western Blot . The activity of caspase 1 was obtained by fluorometric assays and the IL-1b secretion was measured by an ELISA Kit.

The results obtained showed higher levels of pro-IL-1b in CF than in CMF. The protein levels of pro-caspase-1 and the secretion of IL-1b were higher in CMF than in CF. CMF also showed a greater activity of caspase 1 than CF. Protein levels of ASC and NLRP3 did not change between either cell types.

Finally, we can conclude on the basis of the results obtained that CF have a pro-inflammatory role in acute states in comparison with CMF. This can be evaluated through the higher levels obtained of pro-IL-1b. On the other hand, the CMF present higher levels of protein expression and activity of inflammasome NLRP3 in CMF.



20) **Participation of signal transducer and activator of transcription 3 in fibrosis of vascular endothelial cells**

Vallejos, Alejandro[1]., Rojas, Macarena[1].,Becerra, Álvaro[1].,Simon, Felipe[1,2].,[1]Departamento Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad Andrés Bello.[2]IMII Millennium Institute on Immunology and Immunotherapy. (Sponsored by Fondecyt 1121078 And MII P09-016-F)

Introduction: Most cardiovascular diseases have common inflammatory conditions which induces endothelial dysfunction through immune system activation. Inflammatory conditions prompts endothelial cells (ECs) to adopt fibroblast-like features characterized by loss their endothelial specific markers VE-cadherin and CD-31. In addition, ECs acquire fibrotic markers as fibroblast – specific protein 1 (FSP-1) and α-smooth muscle actin (α-SMA). Furthermore, by means of endothelial fibrosis, ECs gain the extracellular matrix (ECM) proteins fibronectin (FN) and collagen type III (Col III). Concordantly, morphological changes have been observed. Normal ECs show a short-spindle morphology with cobblestone appearance, whereas fibrotic endothelium acquires a fibroblast-like spindle shaped phenotype. Moreover, endothelial cell–cell junctions are loss. Signal transducer and activator of transcription 3 (STAT-3) is a transcriptional factor implicated in the growth of tumors, in processes like apoptosis and cellular proliferation. It has been described that inhibition of STAT3 expression induces spontaneous fibrosis. However, its participation on endothelial fibrosis is not known. Thus, our aim was to study whether inhibition of STAT3 expression induces endothelial fibrosis. **Methods and Results:** Using primary cultures of rat mesenteric endothelial cells (RMEC), we demonstrated that expression inhibition of STAT3 using a pharmacological inhibitor and siRNA against STAT3 induced endothelial fibrosis. Expression inhibition of STAT3 decreased expression of VE-cadherin and CD-31 and increased expression of the fibrotic markers, FSP-1 and α-SMA. Furthermore, ECM proteins, FN and Col III, were severely increased. **Conclusion:** We demonstrate that the inhibition of STAT3 expression induces a change in the endothelial expression pattern producing endothelial fibrosis.

21) **NADPH oxidase blockade reduces Snitrosylation and opening of Cx43 hemichannels improving heart contractility and rhythmicity in *mdx* mice**

Vielma Z, Alejandra[1]., Boric P, Mauricio[1].,Gonzalez R, Daniel[2].,[1]Ciencias Fisiologicas, Ciencias Biologicas, Pontificia Universidad Católica De Chile.[2]Ciencias Basicas Biomedicas, Ciencias de la Salud, Universidad De Talca. (Sponsored by FONDECYT 1120595)

Duchenne muscular dystrophy, a fatal progressive genetic disease, causes dystrophic cardiomyopathy altering intracellular calcium and oxidative stress. One of the most important sources of reactive oxygen species (ROS) in the cardiovascular system is NADPH oxidase (NOX). It has been shown that ROS interferes with connexin 43 (Cx43) location to the intercalated discs; and hemichannels formed by connexins (Cx) or pannexins (Px) constitute a potential pathway for dissipation of ionic gradients and tissue damage.

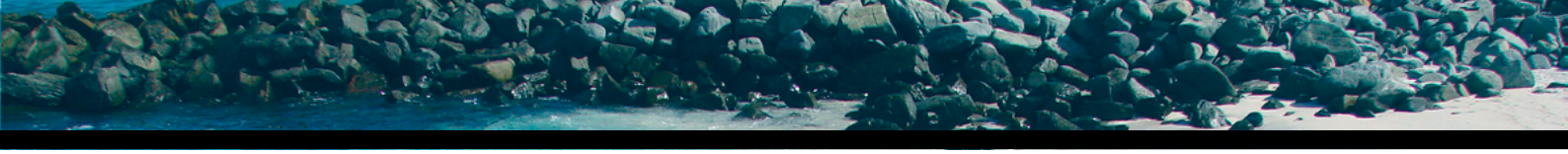
Using *mdx* mice, a model of Duchenne disease, we tested the hypothesis that increased oxidative stress due to increased NOX activity causes S-nitrosylation and lateralization of Cxs and/or deregulating of Cxs or Pxs hemichannel activity, leading to decreased inotropism and increasing arrhythmogenicity.

Isolated hearts from 2 and 10 months of age *mdx* mice(Langendorf) showed a reduced contractility, decreased response to β-adrenergic stimulation, higher number of arrhythmic episodes, and increased fibrosis (Masson trichrome stain) and increased number of apoptotic cells (TUNEL), as compared to controls. At both ages, *mdx* showed increased expression of cardiac $p22^{phox}$ and $gp91^{phox}$ subunits, and higher NOX activity, associated with increased lipid peroxidation in serum, skeletal and cardiac muscle. All these conditions were reversed to control levels when *mdx* animals were treated chronically with NOX inhibitor apocynin (1 month orally).

While total cardiac Cx43 content was unchanged, immunofluorescence and Western blot analysis demonstrated higher presence of Cx43 at lateral membranes in 2- and 10-month *mdx* mice, indicating that Cx43 re-localizes from intercalated discs to sarcolemma. In addition, biotin-switch assays showed increased S-nitrosylation of Cx43 and Px1 proteins.

Hemichannels opening, evaluated using ethidium permeability was substantially higher in *mdx* hearts and this condition was normalized when mice were treated by apocynin or acutely, using hemichannel blockers carbenoxolone (for Cx) and probenecid (for Px).

These results suggest that, in Duchenne disease, increased NOX activity deregulates Cx43 distribution and S-nitrosylation, causing hemichannels formation and/or activation, which may contribute to increased apoptosis and cardiac dysfunction.



22) **Daily variation of salivary melatonin acute exposure to altitude of 3270 m.**

Tapia, Marcelo[1]., Wulff, Cristian[2].,Silva, Juan[2].,De Gregorio , Nicole[3].,Behn, Claus[3].,[1]Kinesiologia, Ciencias de la Salud, Universidad De Antofagasta.[2]Biomédico, Ciencias de la Salud, Universidad De Antofagasta.[3]ICBM, Facultad de Medicina, Universidad De Chile.

Altitude is an external environment affecting multifactorial as living organisms that are exposed it acutely or chronically. The most immediate responses to altitude are generated respiratory and cardiovascular level and are intended to ensure the availability of oxygen to the tissues. There is an increase in altitude insomnia or trouble sleeping, either conciliation deficit in sleep or increased number of awakening, is associated with subsequent sleepiness during working periods, affecting the performance of cognitive motor functions and status of persons focusing on productivity and quality of work performed, and parallel critically manifesting in increased risk of accidents. In the sleep/wake cycle plays a fundamental role melatonin, known as the sleep hormone which also has antioxidant effect and is involved in energy metabolism. Melatonin synthesis is inhibited by exposure to light, especially blue light spectrum, concomitantly raised the perception of blue light maybe diminished by the effects of altitude. From this back ground to assess the effects on melatonin secretion during acute exposure to altitude appears as an interesting problem that may yield new insight for understanding the changes occurring in the control of this hormone on physiological functions and the sleep / wake cycle in altitude. **Objective:** The objective of this project was to evaluate the effect of acute exposure to altitude of 3270 m in the concentration of salivary melatonin in eleven young subjects residents sea level and relationship to physiological parameters. **Methodology:** The sample population is characterized by its anthropometric surveys and right through sleep habits, smoking and stress level. Profile, respiratory and cardiovascular parameters were recorded at rest at sea level on 24 h after exposure to altitude of 3270 m, using portable ergospirometer. Melatonin measurements for collection saliva samples was performed on each subject with bucal with bucal swords, daytime (12:00 h) and nighttime (0:00 h) to sea level and after 24 h of exposure to 3270 m. Melatonin level were determined by inmunoassay for the thest of ELISA. **Results:** Acute exposure to altitude (3270 m) caused increased melatonin concentrations in saliva samples compared to the values of metabolite collected at sea level. Also states that there is an inverse linear correlation with changes in respiratory quotient (RQ) with the differences between the level of daytime and nighttime melatonin of sea level. However the aforementioned correlation is lost when individuals are exposed to acute hypoxia conditions.

23) **Low birth weight children associate low 11BHSD2 activity and high lipocaline-2/NGAL**

Carvajal, C[1]., Tapia-Castillo, Alejandra[2].,Lizama-Gonzalez , Jaime[1].,Valdivia, Carolina[1].,Villarzu, Paula[1].,Martinez-Aguayo , Alejandro[3].,Fardella, Carlos[1].,[1]Endocrinology, Medicine, Pontificia Universidad Católica De Chile.[2]Genetica, Medicina, Universidad Del Desarrollo.[3]Pediatria, Medicina, Pontificia Universidad Católica De Chile.

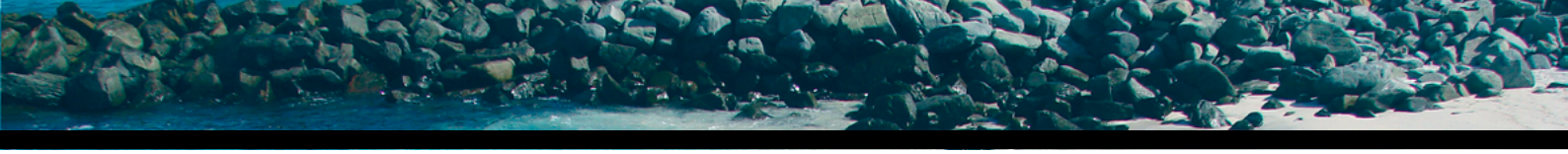
Low birth weight (LBW) has been associated with the risk of hypertension in adults. Recently has been proposed that increased in glucocorticoid exposure is likely to be a critical determinant of growth in early life. The amount of fetal exposure to maternal glucocorticoids depends on the expression of 11beta-hydroxysteroid dehydrogenase type 2 enzyme (11beta-HSD2), that inactivates cortisol (F) to cortisone (E). Thus, impairment of the 11beta-HSD2 would allows the activation of the mineralocorticoid receptor (MR) by cortisol, which affect the release of cytokine dependent MR as NGAL, that has been linked to progression of renal disease.

Aim: To characterize the associations between birth weight (BW) and HSD11B2 expression, F/E ratio in serum, and NGAL as renal marker in pediatrics subjects.

Methods: We studied 12 pediatric patients and 16 control subjects (0-18 years-old). We carried out a whole clinical examination, and measurement of serum aldosterone, plasma renin activity (PRA), free cortisol (F)(serum), free cortisone (E) (serum), free NGAL. RNA was isolated from peripheral leukocytes. The gene expression was quantified by RT-qPCR and expressed in relative units (RU). Statistical analyses were performed by Mann Whitney test to evaluate differences between groups. Data were expressed as median [Q1-Q3] and compared with Mann Whitney test.

Results: We found that the HSD11B2 expression is decreased in subjects with LBW vs NBW (0.0017[0.00061-0.0036] vs 0.01998 [0.002912-0.1211] RU; p 0.03). Interestingly, F/E in serum isincreased in subjects with LBW vs NBW (5.15 [2.72 -8.67] vs 2.56 [2.19 – 3.73] RU; p 0,01) and the NGAL is increased in subjects whit LBW vs NBW (108.4[91.79-133.1] vs 78.32[59.72-83.70] ng/ml; p 0.04). We not found differences with the other variables studied.

Conclusions: Our results suggest that children with lower birth weight presentedreduced 11β-hydroxysteroid dehydrogenase type 2 activity is likely to be a critical determinant of growth in early life, renal damage and the hypertension development.



24) **Aldosterone stimulates immune markers expression related to steroid receptors activation in adipose LS14 but not in SW872 cell line**

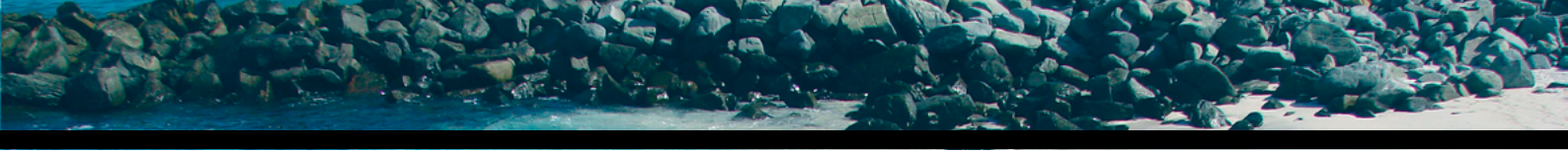
Gonzalez-Gomez, Luis Martin[1]., Fuentes, Cristobal[1].,Allende, Fidel[2].,Fuentes-Ibacache, Nataly[1].,Ortiz-Canales, David[1].,Muñoz-Durango, Natalia[3].,Campino, Carmen[1,3].,Solari, Sandra[2].,Cifuentes, Mariana[4].,Carvajal, Cristian[1,3].,Kalergis, Alexis[3].,Lagos, Carlos[1,3].,Vecchiola, Andrea[1,3].,Fardella, Carlos[1,3].,[1]Department of Endocrinology, School of Medicine, Pontificia Universidad Catolica de Chile.[2]Department of Clinical Laboratories, School of Medicine, Pontificia Universidad Catolica de Chile.[3]IMII Millennium Institute on Immunology and Immunotherapy.[4]Institute of Nutrition and Food Technology (INTA), Universidad de Chile. (Sponsored by Supported By Proyecto SOCHED 13-6, CORFO 13CTI-21526-P1, FONDECYT 1150437 & 1130427, IMII P09/016-F Grants.)

Background. Clinical data support the notion that aldosterone is associated with an inflammatory state, and recent reports show that this hormone is naturally synthesized in the adipose tissue. On the other hand several reports show that aldosterone modulates T cell polarization, including an inflammatory Th17 phenotype associated with end-organ damage.Thereforealdosterone's interaction with the adipose tissue may alter the function of the immune system and enhance a systemic low-grade inflammation, leading to end-organ damage. **Aim.** To assess *in vitro* whether aldosterone modulates immunogenic activity, using 2 different adipose cell lines. **Methods.** For LS14 and SW872 preadipocytes differentiation, cells were serum-starved overnight, and then treated with an adipogenic cocktail for 8 days. SW872 and LS14 preadipocytes and adipocytes were treated with increasing doses of aldosterone (0.1–100 nM), LPS (100 ng/mL) or vehicle for 24 hrs. RNA was isolated, and steroid receptors (MR and GR) and inflammation markers expression (IL-6, IL-1β, HSP-90 and TLR-4) were determined by qRT-PCR. **Results.** SW872 cultures proliferate much faster than LS14 cultures, but differentiation generated mature adipocytes at similar times, showing a morphological shift, from fibroblast-like shapes to a wider and rounder shape. Upon differentiation, LS14 show a significant decrease in MR (p=0.0204). SW872 increase both MR and GR expression (p, which are also significantly higher than LS14 (p). 100 nM aldosterone treatment tends to decrease MR and increase GR expression in LS14 cells, whereas SW872 tend to decrease both MR and GR expression after stimulation, where only GR significantly differs between LS14 and SW872 preadipocytes (p=0.0153). Inflammation marker studies show that 10 nM aldosterone treatments significantly increased HSP-90 expression in LS14 preadipocytes ($p<0.05$). In LS14 adipocytes, TLR-4 expression positively correlates with aldosterone treatment concentrations (p=0.0264) as well as HSP90 (p=0.023), whereas IL-1β expression shows a negative correlation with these treatments (p=0.0402). LPS treatment increased the expression of IL-1β in LS14 preadipocytes and both TLR-4 and IL-6 expression in LS14 adipocytes (p, where it also tends to increase IL-1β expression. SW872 cell line did not change its immune markers expression in response to aldosterone or LPS. **Conclusion.** Aldosterone and LPS differentially stimulates immune markers expression associated with steroid receptors in LS14 cell cultures, whereas SW872 cell cultures do not respond either aldosterone nor to LPS stimulation.

25) **Expression of free fatty acid receptors 1 and 4 in bovine epithelial endometrial cells**

Hidalgo, Maria[1]., Larrazabal , Camilo[1].,Teuber, Stefanie[1].,Loncoman, Carlos[1].,Manosalva, Carolina[2].,Burgos, Rafael[1].,[1]Instituto de Farmacologia, Facultad de Ciencias Veterinarias, Universidad Austral De Chile.[2]Instituto de Farmacia, Facultad de Ciencias , Universidad Austral De Chile. (Sponsored by Fondecyt 1151047, FONDEF ID14I10050 And DID-UACH S-2014-23)

Free fatty acids are increased in plasma around partum in cows, period which there is a high incidence of infectious disease, such as metritis. Long chain fatty acids bind to G-protein coupled receptors, such as free fatty acid receptor 1 (FFAR1/GPR40) and FFAR4/GPR120, and a role of these receptors on innate immune response has been suggested. Recently, we cloned and demonstrated the presence of the functional FFAR1 in bovine neutrophils, cells with a key role in immune response in endometrium. The aim of this study was to determine the presence of FFAR1 and FFAR4 in epithelial endometrial cells. Bovine epithelial endometrial (BEND) cells line were cultured and total RNA and proteins were isolated. By RT-PCR and using specific primers to bovine FFAR1 and FFAR4 we obtained a product of amplification of the expected size, 254 and 134 bp respectively, which correspond to both receptors. Using antibodies against FFAR1 and FFAR4, two proteins of approximately 31 and 42 kDa, the predicted size for FFAR1 and FFAR4, were detected by immunoblot. Also, it was possible to observe the presence of FFAR1 and FFAR4 in BEND cells by immunofluorescence. We showed that the agonists of FFAR1 and FFAR4, linoleic and docosahexaenoic acid, respectively, induced intracellular calcium mobilization in Fura-2AM-loaded BEND cells by spectrofluorometric assay. In conclusion, our results show that BEND cells express FFAR1 and FFAR4, and agonists of both receptors induce intracellular calcium mobilization, thus suggesting that BEND cells function could be modulated in the presence of fatty acids. (Supported by Fondecyt 1151047, FONDEF ID14I10050 and DID-UACh S-2014-23).



26) ***Ugni molinae* extracts and its triterpenoids: modulatory effects on β-amyloid aggregation**

Jara, D[1]., Ana, Riveros[1].,Marcelo, Kogan[2].,Carla, Delporte[2].,[1]Química Farmacológica y Toxicológica, Ciencias Químicas y Farmacéuticas, Universidad De Chile.[2]Química Farmacológica y Toxicológica Universidad De Chile. (Sponsored by FONDECYT 1130155 Y 1130425, Beca CONICYT N°21130380)

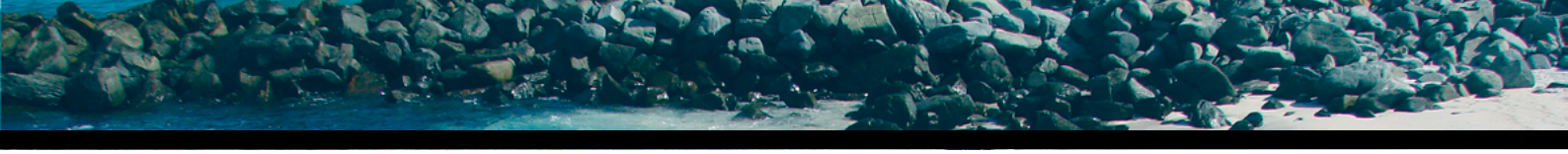
The β-amyloid peptide is a 40-42 amino acid macromolecule and is considered one of the major etiological factors in Alzheimer's disease. This protein is able to aggregate into more complex structures such as oligomers, protofibrils, fibrils or other possessing varying degrees of neurotoxicity and directly influence the development of the mentioned pathology. Therefore, it is highly attractive finding molecules capable of modulating the aggregation process of this peptide in order to avoid its toxic effects. *Ugni molinae* is a native shrub from the south of Chile. The extracts of the leaves of this wild species have shown anti-inflammatory, analgesic and antioxidant effects that would support its use in folk medicine[1,2]. Chemical studies of murtilla leaves have shown the presence of two major groups of constituents: phenolic compounds such as flavonoids, gallic acid, tannins and the derivates compounds of all of them; pentacyclic triterpenoid acids derived from ursane, oleanane and lupane such as asiatic, madecassic, ursolic, oleanolic, corosolic, betulinic, alphitolic and maslinic acids. Some of the phenolic compounds and triterpenoids present in the leaves of murtilla have shown modulatory effects on β-amyloid aggregation and as a result, the modulatory effects of the ethanolic extracts (EET), ethyl acetate (EAE), the triterpenoids ursolic and madecassic acids were studied in the mentioned peptide aggregation process. For the above, the T thioflavin assay was used. The emission of fluorescence of thioflavin (450/480 nm) is directly proportional to the concentration of amyloid aggregates[3].

1 Aguirre, M. C., Delporte, C., Backhouse, N., Erazo, S., Letelier, M. E., Cassels, B. K., Negrete, R. Topical anti-inflammatory activity of 2α-hydroxy pentacyclic triterpene acids from the leaves of *Ugni molinae*. **Bioorganic & medicinal chemistry**, *14*(16):5673-5677, 2006. 2 Rubilar, M., Pinelo, M., Scheuermann, E., Sinero, J., Nuñez, M.J. Murta leaves (*Ugni molinae* Turcz) as a source of antioxidant polyphenols. **Journal of Agricultural and Food Chemistry**, 54(1):59-64, 2006. 3 Levine, H. **Protein Science**, 2:404−410, 1993.

27) **Structure-based virtual screening identification of a novel selective connexin hemichannel blocker**

Lagos, Carlos F[1]., Fernandez, Paola[2].,Vargas, Anibal[2].,Perez-Acle, Tomás[3,4].,Sáez, Juan C[2,4].,[1]Department of Endocrinology, School of Medicine, Pontificia Universidad Católica de Chile.[2]Department of Physiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile.[3]Computational Biology Lab (DLab) Fundacion Ciencia y Vida.[4]CINV Instituto Milenio Centro Interdisciplinario de Neurociencia de Valparaíso. (Sponsored by Supported By FONDECYT Projects 1150291& 1130652, Programa De Financiamiento Basal PFB16 And ICM09-022-P Grants.)

Connexins are membrane channel proteins, which form hexagonal arrays in the plasma membrane called hemichannels or connexons. During the last decade, it has been demonstrated that under physiological conditions hemichannels play relevant roles in cell-cell signaling acting as membrane pathway for releasing extracellular signaling molecules such as ATP and NAD^+. Thus, they are currently considered as autocrine and paracrine pathway for intercellular communication. However, in diverse pathological conditions the activity of connexin hemichannels is up regulated and contributes to the outcome of cellular degeneration. Currently, the available hemichannel blockers also inhibit gap junction channels, which play relevant roles in coordinating numerous electrical and metabolic responses of cellular communities. Therefore, it is important to discover specific hemichannel blocker without effect on gap junction channels, which might be useful to design rational therapeutic treatments of diverse diseases. In the present work, we report the successful use of structure-based virtual screening for the identification of novel chemical entities targeting connexin hemichannels. In particular we have identified a connexin hemichannel blocker (D4) which block hemichannels formed by connexins 26, 32, 43 and 45, but not gap junction channels formed by these connexins or pannexin 1 related channels. The identified compounds may serve as starting point for the development of novel generation of more potent and specific connexin hemichannel modulators.



28) **Linoleic acid increases cell migration, MMP-9 activity and MAPK phosphorylation in human keratinocytes**

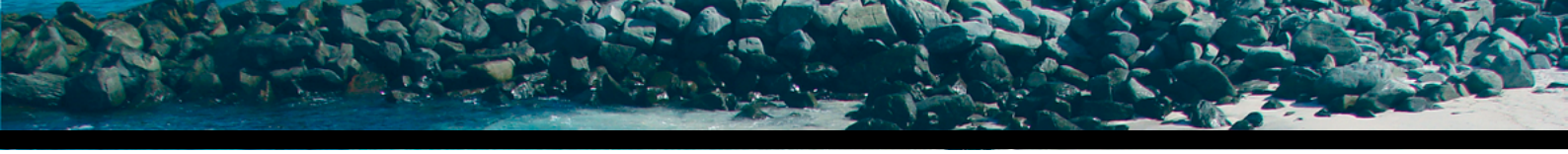
Manosalva, Carolina[1]., Mena, Jaqueline[2].,Burgos, Rafael[3].,Hidalgo, María Angélica[3].,[1]Institute of Pharmacy, Faculty of Science, Universidad Austral De Chile, Valdivia, Chile.[2]Department of Biology, Faculty of Exact and Natural Sciences , Universidad de Nariño, Pasto, Colombia.[3]Institute of Pharmacology, Faculty of Veterinary Science, Universidad Austral De Chile, Valdivia, Chile.
(Sponsored by Financed By DID 2014-S13-UACh)

Wound healing plays a vital role in the maintenance of the integrity of the skin and mucosal membranes. Indeed, there are three major skin responses after injury, including inflammation, reepithelization (migration of keratinocytes) and remodeling (formation of granulation tissues). The inflammatory response is a key event for a correct reepithelization and wound closure. Neutrophils and macrophages are predominant in the inflammation phase producing cytokines and a great variety of growth factors that stimulate migration and proliferation of keratinocytes. The directed migration of keratinocytes is in turn essential for reepithelization and defects in this function are associated with chronic non-healing wounds, such as diabetic ulcers. It has been demonstrated that long chain free fatty acids, such as oleic acid (OA) and linoleic acid (LA), induces an increase in wound healing by influencing the inflammatory phase, increasing the number of neutrophils in the wound, and by affecting the reepithelization phase. However, the effect of LA on keratinocyte migration has not yet been explored. This study demonstrated that LA significantly increases migration of an immortal keratinocyte cell line from adult human skin, known as HaCat in a scratch wound healing assay and a transwell migration assay. Matrix metalloproteinase (MMP)-9 is key for the migration of keratinocytes during the wound healing process. We have demonstrated that LA induces an increase of MMP-9 activity and protein expression, analyzed through zymography and western blot respectively. Additionally, we demonstrated that LA rapidly (2-5 min) stimulated phosphorylation of ERK1/2 and p38 MAPK, evaluated by immunoblot. Furthermore, the presence of the free fatty acid receptor-1 (FFAR1), an LA receptor, was assessed by RT-PCR, immunoblotting and fluorescence microscopy. In conclusion, we report here, for the first time, that LA plays a key role in cell migration and MMP-9 activation in keratinocytes, therefore supporting its potential application in regenerative medicine; furthermore, these responses could be induced by FFAR1 activation.

29) **Nitric oxide synthase, a target for polyphenols derived from the diet: a molecular approach to the French paradox.**

Mateluna, Carlos[1]., Calfío , Camila[1].,Huidobro-Toro, J Pablo[1].,Mascayano, Carolina[2].,[1]Departamento de Biologia, Facultad de Química y Biología, Universidad de Santiago de Chile.[2]Departamento de Ciencias del Ambiente, Facultad de Química y Biología, Universidad de Santiago de Chile. (Sponsored by Funded FONCECYT Grant 114-1132, FPB 087, CEDENNA)

The French paradox is based on the observation that French's, in spite of a high lipid diet, are less prone to cardiovascular diseases. Since French drink more wine than other Europeans; the hypothesis was rose that wine might protect from the development of vascular diseases. Wines, fresh fruits and vegetables are rich in polyphenols including flavonoids and related compounds. We infer that endothelial nitric oxide synthase (eNOS) might be an interesting molecular target to examine for the action of these chemicals. In support of our proposal, NO, the gas signal derived from eNOS activity, is an important signaling molecule in blood vessels associated to a potent cGMP-dependent vasodilatation. Polyphenols may affect the bioavailability of NO. Although studies postulate different pathways for polyphenol action, none has been clarified molecularly. Using bioinformatics, we assessed whether polyphenols and flavonoids modulate human eNOS, acting a unique site common to these chemicals. This hypothesis was examined using molecular docking based in AutoDock 4.2. For the protein model, we used the oxygenase domain of the dimer of human eNOS crystallographic structure. We examined 10 flavonoids plus trans/cis resveratrol and piceatanol as model of natural stilbenes. Chemical structures were minimized (MMF94x hybrid force field) on MOE program. Results are consistent with a single site in the extracellular enzyme surface suggesting a defined and relevant "pocket" in the eNOS oxygenase domain. Polyphenols binding energies ranged from -8.2 to -5.7 kcal/mol; which correlated positively with endothelium-dependent vasodilator effects reported in literature ($r=0.81$, $p<0.01$).Moreover, eNOS Arg^{107} and Glu^{347} were found essential for the interaction with 3'-OH, 5-OH and 7-OH of these polyphenols. Furthermore, when Arg^{107} was artificially mutated to Ala^{107} in the eNOS template; the affinities for the proposed pocket decreased resulting in random interactions at multiple sites with increased binding energies. Likewise, when Glu^{347} was mutated for Ala^{107}, the binding energy increased in the range of 0.3-2.2 kcal/mol, but the polyphenols still interact at the proposed site with higher energies, illustrating the relevance of Arg^{107} at the described pocket. Binding energies of synthetic polyphenols either lacking the hydroxyl groups or with additional OHs, or OH substitutions by methyl or methoxyl are compatible with the model. We propose a positive allosteric modulator site in the external surface of the eNOS for diet polyphenols. The polyphenol pocket is close to the biopterin binding site, allowing us to infer its role favoring NO genesis by eNOS activity.



30) **Effect of Simvastatin upon murine chronic Chagas cardiopathy therapy with benznidazole. Role of simvastatin on endothelial adhesion molecules**

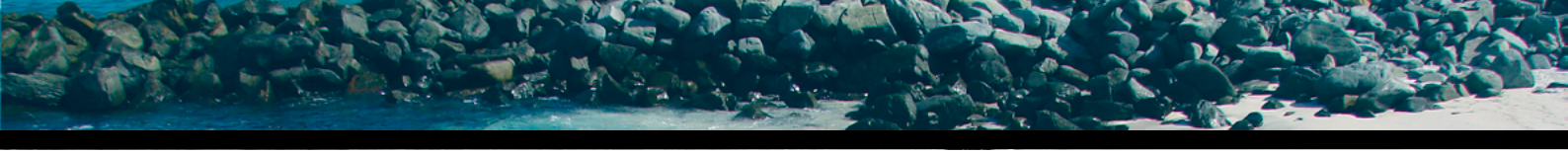
Gonzalez-Herrera, Fabiola., Castillo, Christian[1].,Liempi, Ana[1].,Kemmerling, Ulrike[1].,Maya, Juan [2].,[1]Programa de Anatomía y Biología del Desarrollo - ICBM, Facultad de Medicina, Universidad De Chile.[2]Programa de Farmacología Molecular y Clínica - ICBM, Facultad de Medicina, Universidad De Chile. (Sponsored by Proyectos FONDECYT: 1130189 (JDM), 1120230 (UK))

Trypanosoma cruzi causes chronic Chagas heart disease (CCC). The physiopathology of CCC includes endothelial dysfunction and microvascular damage that lead to focal ischemic areas in cardiac tissue. During infection with *T. cruzi*, vascular endothelium is activated, and expression of proinflammatory cytokines increases. This proinflammatory state is mediated by activation of nuclear factor kappa B (NFκB) that is involved in the increased expression of cellular adhesion molecules (CAMs), among other effects, to encourage cell recruitment inflammatory. On the other hand, statins reduce inflammation in the vascular endothelium, NFκB activation, E-CAMs expression, and inflammatory cells recruitment. It has been described that these effects are mediated by the production of 15-epi-lipoxin A4, a pro-resolutory inflammation eicosanoid, which inhibits NFkB activation, and CAMs expression. Benznidazole is the unique drug that has proven a relative efficacy in treating Chagas disease, mainly during the acute phase. However, it is possible that through modulating key physiopathological factors, the efficacy of benznidazole could be increased during the chronic phase. Therefore, we studied the effect of simvastatin on benznidazole therapy in an *in vivo* model of Chagas's disease. We evaluated the impact of this therapeutic approach on CAMs expression, and on the benznidazole efficacy to treat this disease. Balb/c mice infected with 1000 trypomastigotye of Dm28c strain.were treated with simvastain 4 and 40 mg, benznidazole 30 and 100 mg/Kg/day, and the combination of or simvastatin 4 and benznidazole 30 mg/Kg/day for 20 days, starting at the 30th day post infection. Animals were followed during a 90 days period, and were euthanized to obtain blood and cardiac tissue for parasite load, inflammatory infiltrate and CAMs expression analysis. Mortality rates were similar among all gropus studied. As expected, benznidazole 100 mg/Kg/day decreased parasite load, CAMs expression and inflammation on the cardiac tissue. Similarly, Simvastatin 40 mg/kg/day, and at a lesser extent, 4 mg/Kg/day, was able to decrease expression of VCAM-1, ICAM-1 and E-Selectin on th eimmunohistochemical studies. Interestingly, this drug decreased significantly parasite load on cardica tissue. When the combination of simvastatin plus benznidazole was analyzed, parasite load decreased to almost zero, and cardiac tissue and ECAMs expression was similar to control. In conclussion, Simvastatin alone provides an anti-inflammatory ¡¡enviroment on cardiac tissue infected with *T. cruzi*, and the combination with benznidazole could improve Chagas treatment.

31) **The inhibition of proteasome prevents Mitofusin 2 and Miro 1 degradation in cardiomyocytes during ischemia -reperfusion.**

Olmedo, I[1]., Pino, G[1].,Anríquez, C[1].,Pedrozo, Z[1,2].,Donoso, P[1].,Sánchez, G[1].,[1]Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad De Chile.[2]Advanced Center for Chronic Diseases, Facultad de Medicina, Universidad De Chile. (Sponsored by FONDECYT Postdoctorado 3140449, FONDECYT 1150887, 1130407, FONDAP 15130011 .)

During cardiac ischemia reperfusion (I/R) diverse proteins are degraded, among them mitochondrial proteins such as mitofusin 2 (Mfn2). This event is responsible for a change in mitochondrial dynamics inducing fragmented mitochondria (fission) and impairment of its function, causing cardiomyocyte death. One strategy to reduce heart damage during I/R is proteasome inhibition, however the mechanism by which this inhibition induce such protection during I/R is still unknown. Miro 1 is another protein located in mitochondrial membrane implicated in transport and dynamics of the mitochondria, nevertheless the consequences of I/R on Miro1 content in cardiomyocytes have not been studied. The objective of this work was to evaluate whether inhibition of the proteasome is able to protect the mitochondria from I/R injury and preserve the content of Mfn2 and Miro1. Cultured neonatal rat cardiomyocytes were subjected to simulated I/R (sI/R) in the absence or the presence of the proteasome inhibitor MG132. Cell death was evaluated by lactate dehydrogenase release (LDH) and the relative content of mitochondria was determined by qPCR. Mitochondrial fusion and fission were evaluated by confocal microscopy using mitotracker green and the protein levels of Mfn2 and Miro1 were evaluated by inmunowesternblot (WB). In the absence of proteasome inhibitor, sI/R decreased the relative content of mitochondria, decreased mitochondrial fusion and metabolism and produced cardiomyocyte death. Also, sI/R decreased the protein content of Mfn2 and Miro1. The inhibition of the proteosome by MG132 avoided all the mitochondrial changes induced by sI/R mentioned above, preserved the content of Mfn2 and Miro1 and prevented cardiomyocyte death. Taken together, these data suggest that inhibition of the proteasome preserves mitochondrial function which explains at least in part the protective effect of proteasome inhibition in I/R injury.



32) **PARTICIPATION OF TLR4 IN THE ANTIFIBROTIC RESPONSE OF KININS BY AN INCREASE OF PGI2 AND NITRIC OXIDE LEVELS IN CARDIAC FIBROBLASTS**

Osorio, José Miguel[1]., Muñoz, Claudia[1].,Díaz-Araya, Guillermo[1].,[1]Química Farmacológica y Toxicológica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile. (Sponsored by Fondecyt 1130300 - Beca CONICYT Doctorado Nacional 21120401)

Toll like receptors (TLR) are involved in immune innate response and are capable to sense danger associated molecular patterns. TLR4 has been described as a key mediator of cardiac function due to its ability to promote cell survival and inflammatory response by regulation of cytokines secretion, adhesion proteins, etc, after injury. On the other hand, kinins are peptides, which are capable to bind their own receptors (B1R and B2R) and reduce blood pressure and collagen secretion, preventing the progress of cardiac fibrosis through activation of kinin signaling cascade, including eNOS and COXs (by production of nitric oxide (NO) and PGI_2). Previously, we demonstrated that TLR4 activation by LPS increase COX-2 and iNOS protein levels in cardiac fibroblasts (CF) and myofibroblasts (CMF), and LPS pretreatment increases the reduction of collagen I protein levels induced by DAKD (B1R agonist) in CF. We hypothesize that the increase in PGI_2 and NO levels are responsible for the enhanced reduction of collagen I levels induced by DAKD in CF pretreated with LPS. The purpose of this work is to demonstrate if TLR4 increases PGI_2 and NO secretion, enhancing kinin antifibrotic response. CF and CMF were obtained from 1-3 days-old Sprague Dawley rats. Both phenotypes were stimulated with LPS 1µg/ml, TAK-242 2 µM, and both stimuli during 0, 24, 48 and 72 hours. After treatment COXs and iNOS levels were measured by western blot (IWB). PGI_2 and NO levels were measured by ELISA, for these, CF were pretreated with LPS 1µg/ml during 24 and 48 hours, and then stimulated with DAKD 100 nM during 24 and 48 hours. We demonstrate that TLR4 activation by LPS induces an increase COX-2 and iNOS protein levels in fibroblasts (CF) and myofibroblasts (CMF) by IWB, and in a major secretion of PGI_2 and NO to the extracellular medium in CF, resulting in a decrease of collagen I levels. Finally, we can conclude that TLR4 has an antifibrotic role by increasing COX-2 and iNOS protein levels, and PGI_2 and NO secretion, which contributes to reduce collagen levels in CF.

33) **Effect of recurrent metabolic insults on organotypic cultures from asphyxia-exposed rat pups**

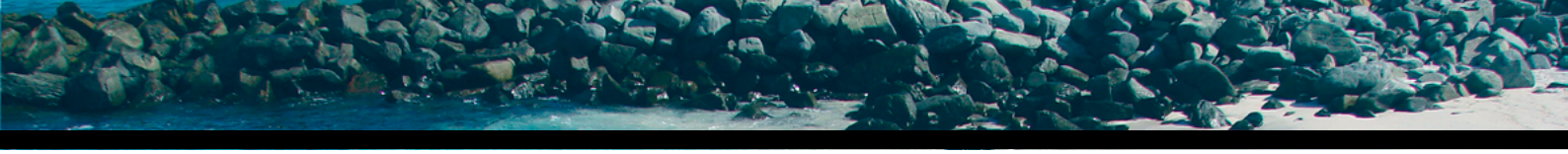
Palacios, E[1]., Pérez-Lobos, R[2].,Lespay-Rebolledo, C[2].,Bustamante, D[3].,Morales, P[4].,Herrera-Marschitz, M[5].,[1]Farmacología Molecular y Clinica, Medicina, Universidad De Chile.[2]Programme of Molecular & Clinical Pharmacology, ICBM, Faculty of Medicine, Universidad De Chile.[3]Programme of Molecular & Clinical Pharmacology, ICBM, Medicine, Universidad De Chile.[4]Millenium Institute BNI-Chile, Programme of Molecular & Clinical Pharmacology, ICBM, , Medicine, Universidad De Chile.[5]Millenium Institute BNI-Chile; Programme of Molecular & Clinical Pharmacology, ICBM, medicine, Universidad De Chile.
(Sponsored by Supported By Millennium Institute Initiative-Chile (BNI P09-015-F); FONDECYT (1120079, 1110263). The Excellent Technical Support From Mr. Juan Santibáñez, Mr. Alejandro Leiva And Ms. Carmen Almeyda Is Acknowledged.)

Perinatal asphyxia is a relevant clinical issue associated to long-term disabilities by mechanisms not yet identified. We have speculated that the long-term effects are not directly due to deficits produced by the primary insult, but to a permanent vulnerability to recurrent metabolic or environmental depending insults that can or cannot occur a long the life of the particular individual.

Thus, we have studied the issue by profiting of the organotypic culture model, allowing to reconstruct specific brain pathways *in vitro*, to be monitored along development, evaluating cell death, cell and neurochemical phenotype and the re-establishing of proper neurocircuitries. We sampled brain tissue from 2-3 days old rat pups, subjected to perinatal asphyxia, reconstructing the basal ganglia by putting together on a coverslip tissue from substantia nigra, neostriatum and neocortex, grown together on a culture tube containing 0.75 ml of a culture medium in cell incubator at 10% CO_2 and 35^o C in a roller wheel turning 1 time/min for exposing the samples to aqueous and gaseous phases.

At day *in vitro* 18 (DIV17), the cultures from asphyxia-exposed and control animals were exposed to a metabolic insult consisting of a high concentration of H_2O_2 for 18h, containing or not 0.5% glucose. After a 48h recovery period, treated and control cultures were assayed for cell viability using the LIVE/DEAD viability kit (Molecular Probes, Eugene, OR, USA), or formalin fixed for further immunocytochemistry and confocal microscopy, focusing on cell (glial/neuronal; GFAP/ MAP2) and neurochemical (tyrosine hydroxylase, TH; NOS) phenotype.

The recurrent metabolic insult increased cell death in cultures from both asphyxia-exposed and control pups, with a selective regional vulnerability. The specificity of the effect on cell and neurochemical phenotype was stereologically quantified in pictures taken by a Fv10i confocal microscope (Olympus, Japan).



34) **Histone deacetylase inhibitors: Synthesis, docking and citotoxicity of thiazolylcoumarins derivatives.**

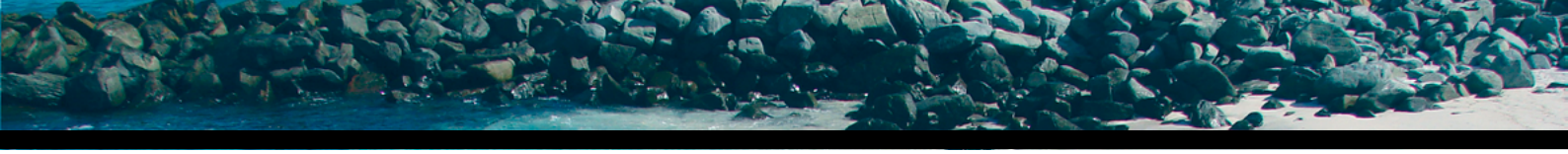
Pardo-Jiménez, Viviana[1]., Díaz-Araya, Guillermo[2].,Navarrete-Encina, Patricio[3].,[1]Química Orgánica y Fisicoquímica, Química Farmacológica y Toxicológica, Ciencias Químicas y Farmacéuticas, Universidad De Chile.[2]Química Farmacológica y Toxicológica, Ciencias Químicas y Farmacéuticas, Universidad De Chile.[3]Química Orgánica y Fisicoquímica, Ciencias Químicas y Farmacéuticas, Universidad De Chile. (Sponsored by Beca Doctorado Nacional Conicyt Nº 21140371)

Histone deacetylases (HDACs) are a family of zinc-dependent enzymes. They are involved in gene expression through regulation of transcription and other cellular processes including cell cycle arrest, cell proliferation, apoptosis and terminal differentiation of various cell types. HDAC inhibitors are considered a new class of drugs with multiple possibilities of therapeutic action, among others we have antineoplastic, anti-inflammatory and anti-fibrotic activities. The structure of these inhibitors fits a pharmacophore structure including three zones: a hydrophobic head group, a 6 methylene or heteroatoms hydrophobic spacer chain and a Zn^{+2} binding terminal group. Based on this general structure, N-adipoyl and N-lipoyl-thiazolylcoumarins derivatives were designed, using the thiazolylcoumarin scaffold as head group, lipoic and adipic acid spacers and terminal Zn^{+2} chelating carboxilate and disulfide groups. The synthesis comprises the preparation of the coumarin nucleus (through a modified Perkin reaction), thiazolyl ring formation and a further condensation reaction with lipoic and adipic acid to provide the final compounds. A docking analysis of the preferred conformations adopted by these compounds in the catalytic site of HDACs was done using the enzyme HDAC8 crystal structure as reference. Thus we check that our inhibitors design meet the adequate conditions for enzyme interaction and are similar to the pan-inhibitor trichostatin having inhibition constants energies between -10 and -15 kcal / mole. To determine that the synthesized compounds are not cytotoxic in non-tumor cells, a viability test was performed on neonatal rat cardiac fibroblasts and we found that thiazolylcoumarins derivatives in the concentration range of 100 nM to 10 uM, are not cytotoxic.

35) **New semi-synthetic derivatives of natural catechins: antioxidant-anti-inflammatory activity and effect upon Helicobacter pylori carbonic anhydrase**

Pastene, Edgar[1]., Parada, Víctor[1].,Torres, Eillen[2].,Avello, Marcia[1].,Alarcón, Julio[3].,Felipe, Zuñiga[4].,Ormazabal, Valeska[5].,Ariel, Saavedra[1].,Aranda, Mario[6].,García, Apolinaria[2].,[1]Farmacia, Farmacia, Universidad De Concepción.[2]Microbiologia, Ciencias Biológicas, Universidad De Concepción.[3]Química, Ciencias Básicas, Universidad Del Bío-bío.[4]Bioquímica Clínica e Inmunología, Farmacia, Universidad De Concepción.[5]Farmacología, Ciencias Biológicas, Universidad De Concepción.[6]Ciencia y Tecnologia de Alimentos, Farmacia, Universidad De Concepción.

Several semi-synthetic derivatives of natural catechins were prepared from polymeric proanthocyanidins (PACs) extracted from cranberry pomace, Boldus leaves, avocado peels and grape skins. Through acid-catalyzed cleavage and depolymerization of such PACs, formed carbocations were attacked with different phenolic nucleophiles (phloroglucinol, resorcinol and pyrogallol), giving three adducts of catechin and epicatechin, respectively. The new semi-synthetic compounds were purified and isolated by preparative techniques such as CPC and HPLC and their formation was followed and confirmed by HPLC-DAD-ESI-MS/MS. Introduction of groups harboring phenolic hydroxyls on the catechins structure increase its antioxidant activity in the DPPH, CUPRAC, ORAC-FL and red blood cells protection assays. Such derivatives also were able to reduce IL-8 secretion in H. pylori-infected AGS cells. Finally, we evaluate whether the introduction of additional phenolic groups in natural catechins acts as modification that favors the bind of the new compounds to the active-site zinc atom of the bacterial enzyme carbonic anhydrase (CA), therefore increasing its inhibitory properties. Using an in silico approach (Docking) the most probable binding mode of these compounds is proposed. Our preliminary results suggest that these semi-synthetic phenolic compounds could be used as chemical framework for the design of new drugs that help to the prevention and treatment of the colonization by H. pylori and the inflammatory damage exerted upon gastric mucosa (Financial support: Fondecyt 1150948; Fondequip N° EQM 130209).



36) **Salsolinol, an alcohol-derived metabolite, acts as an agonist of the mu-opioid receptor.**

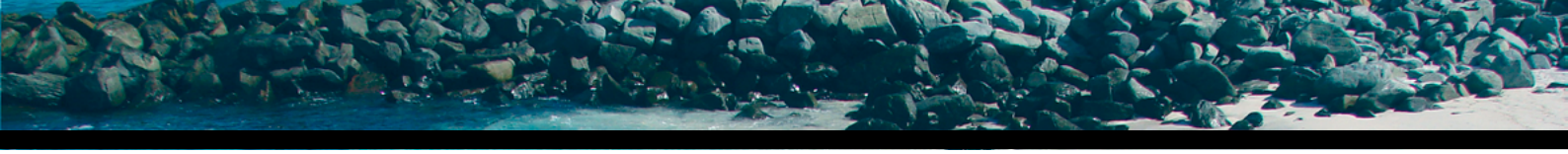
Rivera-Meza, Mario[1,2]., Berrios-Cárcamo, Pablo[1].,Herrera-Marschitz, Mario[1,2].,Quintanilla, María Elena[1].,[1]Programa de Farmacología Molecular y Clínica, Facultad de Medicina, Universidad De Chile.[2]Millenium Scientific Initiative Biomedical Neuroscience Institute. (Sponsored by Supported By: FONDECYT #11130241: BNI P09-015-F.)

Animal studies have showed that acetaldehyde, the first metabolite of ethanol, is a motivational and reinforcing molecule. In the brain, ethanol-derived acetaldehyde can condense with dopamine to generate salsolinol. This compound is self-administered intracranially by animals, suggesting that salsolinol is the molecule mediating the rewarding effects of acetaldehyde and ethanol. Recent evidence indirectly suggests that salsolinol may exert its action through an opiate mechanism, which may explain the efficacy of naltrexone (an opioid-receptor antagonist) to inhibit ethanol intake. However, there are no studies showing that salsolinol acts directly on opioid receptors. To address this question we studied the capacity of different concentrations of salsolinol (10^{-3} to 10^{-9} M) to activate the human mu-opioid receptor in a cell based receptor assay. Since the mu-opiod receptor is coupled to an inhibitory G protein (Gi), its activation by an agonist results in a reduction of the intracellular levels of cAMP induced previously by the adenylate cyclase activator forskolin. Results showed that salsolinol is effective to activate the mu-opioid receptor but posses a lower potency compared to the full agonist [Met5]-Enkephalin, measured as the capacity to activate Gi and inhibit the generation of cAMP. The apparent half maximal effective concentrations (EC50) for salsolinol and [Met5]-Enkephalin were $7x10^{-5}$ Mand $2x10^{-8}$ M, respectively. These pharmacodynamic studies showed that salsolinol acts as an agonist of the mu-opioid receptor, showing the same efficacy, but a lower potency compared to the full opioid agonist [Met5]-Enkephalin.

37) **Anthocyanins from *Aristotelia chilensis* inhibit Olanzapine-induced adipogenesis in 3T3-adipocytes**

Arena, Pamela[1].,Jara, Belen[1].,Cubillos-Robles, Karen[1].,Tordoya, Isis[1].,Gonzalez, Paula[1].,Rojo, Leonel[1].,[1]Laboratorio de Farmacología y Neurociencias, FACULTAD DE CIENCIAS DE LA SALUD, Universidad Arturo Prat. (Sponsored by Acknowledgements: This Project Was Funded By FODECYT Project N° 11140915)

Second-generation antipsychotics (SGAs), clozapine, olanzapine and risperidone, have improved quality of life of billions patients worldwide and are essential for treatment of schizophrenia (SZ). Off-label applications of SGAs are increasing dramatically, including millions of non-SZ adults, children and adolescents. SGAs can cause devastating cardiometabolic side effects leading to increased premature morbi-mortality in as short as three months after initiation of the pharmacotherapy. The SGAs-induced hyperlipidemia is associated with the up regulation of sterol regulatory element-binding protein 1c (SREBP1c), a central regulator of lipid biosynthesis in liver and fat tissue. There is a growing body of evidence demonstrating that anthocyanins from fruits and vegetables ameliorate insulin resistance, inflammation and obesity. Our research is focused on the evaluation of the effect of anthocyanins from maqui berry (*Aristotelia chilensis*) on the olanzapine-induced lipid accumulation in adipocytes. Isolation of anthocyanins from Maqui (MBA) was done as described by Rojo et al (2012). Briefly, freeze dried maqui berries from commercial sources were blended with acidified 80% ethanol, lipophilic compound were removed with ethyl acetate. Anthocyanins were then purified through Amberlite and Sephadex LH-20 columns. The anthocyanins contained in this extract are mainly cyanidin and delphinidin glucosides and the characteristic marker compound of the extract is delphinidin 3-sambubioside-5-glucoside (D3S5G). We observed that olanzapine, at 20 µM concentration, induced significant cell differentiation and lipid accumulation in cultured 3T3 adipocytes. Cellular differentiation and lipid accumulation was analyzed by digital microscopy and UV spectrometry after staining with Oil Red. Co-incubation of 3T3 cells with MBA, at 5, 10, 20, 40, 60 and 100 µg/mL, produced a significant reduction of the intracellular lipid accumulation in differentiated adipocytes. The cytotoxicity of the MBA was also tested by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay. Cellular toxicity was observed with MBA at concentrations above at 60 µg/mL.



38) **Ethyl acetate extracts of different *Ugni molinae* Turcz. genotypes able to inhibit α-glucosidase**

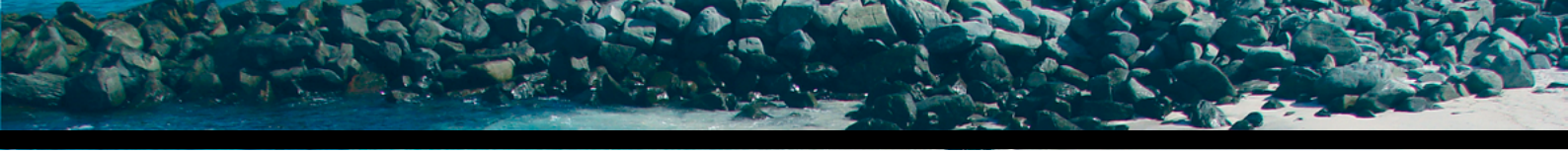
Veas, Rubén[1]., Arancibia-Radich, Jorge[1].,Peña-Cerda, Marcelo[1].,Cortez, Giovanni[1].,Seguel, Ivette[2].,Delporte, Carla[1].,[1]Química Farmacológica y Toxicológica, Ciencias Químicas y Farmacéuticas, Universidad De Chile.[2]Instituto de Investigación Agropecuaria INIA, Carillanca, IX Región, Chile. (Sponsored by Acknowledgments: We Are Grateful To FONDECYT N° 1130155, CONICYT Grants N°21130672 And N°21120377, Chile. Thank You To INIA (Carillanca, Temuco) For The Genotypes.)

Previous studies in our laboratory shown pentacyclic triterpenic acids in different extracts of murtilla leaves (*Ugni molinae* Turcz., Myrtaceae) responsible for inhibitory activity against various enzymes. Between these secondary metabolites can find: asiatic, ursolic, madecassic and corosolic acids, derivatives from ursane; betulinic and alfitolic acids derivatives from lupano and oleanolic and maslinic acids derivatives from oleanane[1]. Studies carried out in parallel in our laboratory indicate a high concentration of these metabolites in ethyl acetate extracts (EAEs) of leaves. Chilean folk medicine attributes many properties to the murtilla and among its uses includes infusions from its leaves and branches to treat diabetes[2]. This use can be explained by way of inhibiting α-glucosidase, an exo-carbohydrase that catalyzes the hydrolysis of complex sugars to monosaccharides in the brush border of enterocytes, and if it is inhibited decreases postprandial glucose peak[3]. Therefore the aim of this study was to benchmarking of EAEs of leaves from seven genotypes of murtilla grown in equal conditions of soil and weather, and same agronomic management, from the gene bank of the Institute of Agricultural Research (IAR) through the methodology descrite by Kim et al. (2005) with slight modifications. Statistical analysis was performed using ANOVA and Tukey multiple comparisons method. The results indicate that the genotype 19-1 was the most potent inhibitor against α-glucosidase from *Saccharomyces cerevisiae* with IC_{50} equal to 12.7 ± 0.2 µg/mL. Genotype 19-1ha was the less potent with IC_{50} of 41.0 ± 2.0 µg/mL. However all genotypes showed better potency than the reference drug, acarbose (IC_{50} of 267.2 ± 35 µg/mL). Previous studies with ethanol extracts showed a similar behavior of genotypes, where the 19-1 is part of genotypes with acceptable power and 19-1ha as less potent. References: 1 GOITY L. E., Queupil M. J., Jara D., Alegría S. E., Peña M., Barriga A., Aguirre M. C. y Delporte C. An HPLC-UV and HPLC-ESI-MS based method for identification of anti-inflammatory triterpenoids from the extracts of Ugni molinae. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 12 (1): 108 – 116 3 RUBILAR M., Pinelo M., Ihl M., Scheuermann E., Sineiro J. y Núñez M. J. Murta Leaves (Ugni molinae Turcz) as a Source of Antioxidant Polyphenols. Journal of Agricultural and Food Chemistry. 2006, 54, 59-64. 4 LORDAN S., Smyth T. J., Soler-Vila A., Stanton C. y Ross R. P. The α-amylase and α-glucosidase inhibitory effects of Irish seaweed extracts. Food Chemistry 141 (2013) 2170–2176.

39) **FoxO1 is necessary for the cytoprotective effect induced by TGF-β1 in cardiac fibroblasts**

Vivar, R[1]., Chiong, M[2].,Lavandero, S[2].,Díaz-Araya, G[2].,[1]Química Toxicológica y Farmacológica, Ciencias Químicas y Farmaceúticas, Universidad de Chile.[2]Advanced Center for Chronic Diseases Universidad De Chile. (Sponsored by Beca Postdoctoral 3130657 (RV); Fondecyt 1130300 (GDA); ACCDis (SL, MCh))

Background. The main function of cardiac fibroblasts (CF) is to maintain extracellular matrix (ECM) homeostasis. Whereas in cardiac diseases such as, myocardial infarction, they differentiate into cardiac myofibroblasts (CMF) increasing their activity to promote tissue healing. In normal conditions, CMF disappear from the injury zone through apoptosis, presumably by the oxidative environment that exist in damaged area. However it is currently unknown, why in pathological conditions, CMF do not die and they even persist in the damaged zone to promote the excessive deposit of ECM components and tissue fibrosis. TGF-β1 is a cytokine which induces CF differentiation and it is crucial in cardiac fibrosis. With this evidence, we suggest that TGF-β1 is responsible for cytoprotection against oxidative conditions, which favors both CMF survival and cardiac fibrosis. Furthermore, FoxO1 is a transcription factor involved in antioxidant defense, through regulation of catalase and SOD2 expression. In addition, we have demonstrated that TGF-β1 promotes FoxO1 activation in CF. Therefore, we hypothesized that FoxO1 is important to TGF-β1 promotes antioxidant protection in CF, controlling catalase and SOD2 expression. **Methodology.** Neonatal CF obtained from Sprague-Dawley rats was used. To induce oxidative stress, we used hydrogen peroxide (H_2O_2) 15 µM for 24 h. TGF-β1 was used at 10 ng/mL. To evaluate apoptosis, we used flow cytometry and we evaluated caspase 3 and 9 fragmentation by western blotting. The protein levels (FoxO1, catalase, SOD2, GAPDH) were assessed by western blotting. Cell death was determined by cell count. siRNA was used to silencer FoxO1, catalase and SOD2. Adenovirus was used to induce FoxO1 overexpression. **Results.** TGF-β1 inhibited both cell death, apoptosis and caspases 3 and 9 fragmentation induced by H_2O_2 in CF. In addition, TGF-β1 increased protein levels of antioxidant enzymes, catalase and SOD2, whereas the silencing of both antioxidants proteins inhibited the cytoprotective effect of TGF-β1. FoxO1 is a crucial regulator of catalase and SOD2 expression. In fact, FoxO1 overexpression increased catalase and SOD2 protein levels, both in the presence and absence of TGF-β1. FoxO1 overexpression increased the cytoprotective effect of TGF-β1 in CF subjected to oxidative stress. In contrast, FoxO1 silence prevented protein levels of the antioxidant enzymes catalase and SOD2 induced by TGF-β1, whereas abolished the cytoprotective effect of TGF-β1 in CF stimulated with H_2O_2. **Conclusions.** Our results suggest that: i) TGF-β1 inhibits the deleterious effects of oxidative stress in CF and ii) TGF-β1 requires FoxO1 to increase catalase and SOD2 protein level and to promote antioxidant effect in CF.



40) **Role of EPA/DHA over later stages of liver ischemia-reperfusion: antioxidant and antifibrotic effects**

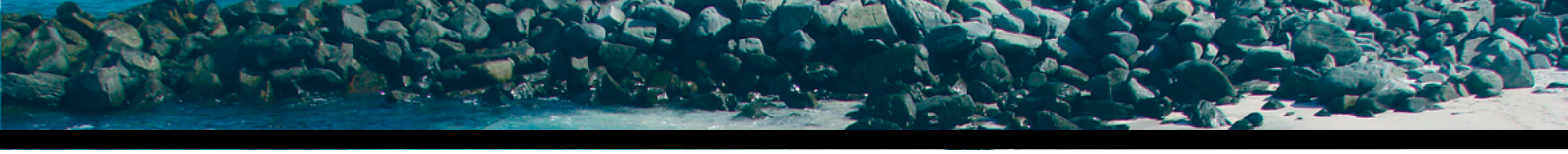
Zuñiga, Jessica[1]., Céspedes, Nicole[1].,Tamayo, Andrea[1].,Arenas, Hector[1].,[1]Laboratorio de Investigaciones Médicas, Escuela de Medicina, Universidad De Talca. (Sponsored by This Study Was Supported By The Internal Project I002974 (Dirección De Investigación, Universidad De Talca).)

The chronic administration of omega-3, in particular docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have preventive effects related to cardiac injury, brain stroke and hepatic ischemia-reperfusion (IR), action associated to their protective effects against induced hypoxia and ischemia/reperfusion damage observed in these tissues. The beneficial of EPA and DHA are associated to their antioxidant and anti-inflammatory capability, mediated by changes in membrane lipids and differential eicosanoids production. Previous studies have been demonstrated that the liver injury induced by IR is mediated by the inflammatory response stimulated by an early increase (3 h post ischemia) of NF-κB activity and late releases of pro-inflammatory cytokines (IL-1β, IL-6 y TNF-α). Our main objective was to study the antioxidant and anti-fibrogenic potential induced by the oral supplementation of EPA plus DHA in later stages of liver ischemia-reperfusion injury. Male Sprague-Dawley rats were pre-treated by ten days with diary oral doses of EPA/DHA (375mg/Kg/day), after that time the animals were subjected to 1 h of ischemia followed by 3-20-24 and 48 h of reperfusion. The supplemented EPA/DHA animals shown less liver damage (measured by transaminase level and histological analysis) than the non-supplemented rats, and all the values were normalized after the 24 h. In addition the non-supplemented animals display a sustained increased of collagen deposit (measure by masson-goldner trichrome staining), action that was prevented in animals subjected to IR and pre-treated with EPA/DHA, with not deposit of connective tissue observed in the first 24 hours and only a minor accumulation at 48 h post-ischemia was detected. The results seen in this study could be related with a potential antifibrotic capability of omega-3 fatty acids, contributing to the knowledge of the mechanism of Hepatoprotection.

41) **Neurochemical caracterization in cortico-striatal-thalamo-cortical circuit of Eaat3 heterozygous mice: role in neuropsychiatric disoders**

Gonzalez, Luis[2,3,1]., Espinosa, Pedro[1].,Sotomayor-Zárate, Ramon[1].,Moya, Pablo[2,3].,[1]Laboratorio de Neuroquímica y Neurofarmacología, Centro de Neurobiología y Plasticidad Cerebral, Instituto de Fisiología, Ciencias, Universidad De Valparaíso.[2]Laboratorio de Neurogenética, Instituto de Fisiología, Centro de Neurobiología y Plasticidad Cerebral, Ciencias, Universidad De Valparaíso.[3]Millennium Nucleus in Biology of Neuropsychiatric Disorders NU-MIND Universidad De Valparaíso.
(Sponsored by This Work Was Funded By Millennium Nucleus NU-MIND NC 130011 (PRM) And FONDECYT Grants 1141272 (PRM) And 11121205 (RS-Z).)

Obsessive-compulsive disorder (OCD) is one of the most prevalent neuropsychiatric disorders, affecting between 1-3 % of the world population. Although the exact causes of OCD are unknown, many lines of evidence suggest that OCD has a genetic basis. Recently, it has gained strength the idea of a dysfunction in glutamatergic neurotransmission in OCD. Several evidences of this dysfunction in OCD include certain polymorphisms in the NMDA receptor gene, elevated glutamate levels in the brain-spinal fluid of drug naïve patients, correlations between symptoms severity and the levels of glutamate metabolites and some drugs that modulate glutamatergic neurotransmission are currently being tested for OCD treatment. In addition, linkage, candidate gene as well as meta-analysis studies support an association of *SLC1A1* gene and OCD. *SLC1A1* encodes the Excitatory Amino-Acid Transporters 3 (EAAT3) that regulates the clearance of glutamate in synaptic cleft. At neurocircuitry level, the cortico-striatal-thalamo-cortical (CSTC) circuit has been involved in OCD through neuroimaging, animals and pharmacological studies. In this circuit, glutamatergic neurotransmission is key in the communication between cortex and striatum making synapses with medium spiny neurons (MSNs) that participate in Direct and Indirect pathway. In OCD patients, an overactivation of CSTC loop, reflecting likely a predominance of Direct pathway over Indirect pathway has been observed, suggesting a link to obsessions and compulsions. In this work, we decided study the neurochemical regulation in limbic and CSTC areas in heterozygous mice for Eaat33. Wild type (WT) and Eaat3 heterozygous (HET) littermate mice were sacrificed and their brain were removed in ice. Cortex, striatum, thalamus and hippocampus were microdissected at 4°C, homogenized in perchloric acid and centrifuged at 12000 x *g.* The cleaned supernatant was injected into a HPLC coupled electrochemical detection to detect dopamine (DA), 3,4-dihydroxy-phenyl acetic acid (DOPAC), serotonin (5-HT) and 5-hydroxy-indole acetic acid (5-HIAA). The results show increase in striatal and hippocampal DA content in Eaat3 HETmice without affect 5-HT content in the same brain areas. On the other hand, it was shown a reduction in cortical 5-HT and DA content and only a reduction in thalamus 5-HT content. Interestingly, these results show a monoaminergic cortical hypofunction that could be involved with alterations in decision-making observed in OCD patients.



42) **Generation of a novel genetic mouse model for Obsessive-Compulsive Disorder**

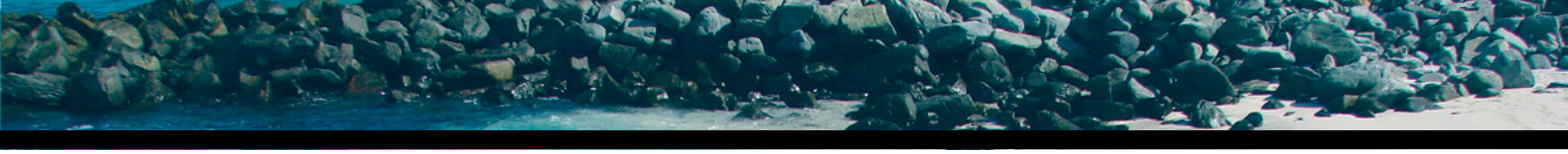
Moya, Pablo[1,2]., Utreras, Elias[3].,Martinez, Jonathan[1,2].,[1]Laboratorio de Neurogenética, Instituto de Fisiología, Centro de Neurobiología y Plasticidad Cerebral, Ciencias, Universidad De Valparaíso.[2]Millennium Nucleus in Biology of Neuropsychiatric Disorders NU-MIND Universidad De Valparaíso.[3]Biología, Ciencias, Universidad De Chile. (Sponsored by This Work Was Funded By Millennium Nucleus NU-MIND NC 130011 (PRM) And FONDECYT Grants 1141272 (PRM))

*SLC1A1 (*neuronal glutamate transporter gene, EAAC1, EAAT3) is an attractive candidate gene implicated in Obsessive-Compulsive Disorder (OCD). EAAT3 regulates –among other glutamate transporters- extracellular levels of glutamate in cortico-striato-thalamo-cortical (CSTC) circuit implicated in OCD. *SLC1A1* is the most evident brain-related gene of interest located in the chromosomalregion 9p24, the region identified in the firstgenome-wide linkage study of mixed large andsmall families with OCD. Also, in the first case-control study of this gene in OCD we found that *SLC1A1* was associated with OCD. The strongest evidence from this study indicated that a certain haplotype was almost two times more frequent in OCD patients than controls (OR = 1.89); two of three SNPs of this haplotype were found to be expression Quantitative-Trait Loci (eQTLs). These findings, together with family linkage studies as well as by a recent meta-analysis point all toward *SLC1A1* as the most solidly established gene identified in OCD. This project aims to generate transgenic mouse models for Cre-dependent conditional Eaat3 knockout and Eaat3 overexpression, and characterize their phenotypes using pharmacological, biochemical, anatomical and behavioral techniques. Such animal models are expected to provide seminal information regarding the mechanism of SLC1A1 dysfunction at the gene regulatory, neurochemical, and anatomical levels during various stages of development. In addition, mice with conditional Slc1a1 expression alterations may offer exciting possibilities for generating new animal models of psychiatric and/or neurodegenerative disorders and also help in the development of drugs that target glutamate neurotransmitter system for effective OCD treatment. Here, we describe the current status of validation and characterization of recently generated Cre-mediated Eaat3 altered expression mouse models. We present the proof-of-principle for Cre-dependent altered Eaat3 expression, using the CamKII-Cre driver mouse line and compared to wild-type littermates, studied at mRNA and protein levels in frontal cortex, hippocampus, striatum, thalamus, hypothalamus and pons. We also present data of ongoing phenotypical assessments evaluating anxiety-like (open field test, elevated-plus maze), and OCD-like (grooming index, marble-burying) behaviors.

43) **Evaluation of the mGluR plasticity processes in the transgenic mice APPswe/PS1δE9 and in the natural model of Alzheimer Disease *Octodon degus*.**

Valdivia, Gonzalo[1]., Salazar, Claudia[1].,Kirkwood, Alfredo[2].,Palacios, Adrián[2].,[1]Centro Interdisciplinario de neurociencia de Valparaíso Universidad De Valparaíso.[2]Mind/Brain Institute Johns Hopkins University. (Sponsored by Financial Support: Millennium Institute ICM-P09-022-F)

Alzheimer Disease (AD) is the most common form of dementia among older people that causes a slow decline in memory thinking and reasoning skills. These failures are related, among other things, to the impairment of synaptic plasticity mechanisms like LTP and LTD that depend on NMDA receptors in memory areas of the brain like the hippocampus. Here, we report LTP and LTD forms that depend on metabotropic glutamate receptor (MGLUR) are also impaired in CA1 of hippocampus of two rodent models of AD: the transgenic AD mouse APPswe/PS1δE9 and Octodon degus (degu). For this we combined electrophysiology of field potentials to evaluate plasticity and western blots, to quantify mGluR5 content. Our results show that the mGluR processes of LTD and LTP are totally disrupted in the old transgenic mice and are different to the old Wild Type Mice. On the other side in degu this processes are disrupted when the animal present memory deficits in the behavioral memory test Radial arm maze (RAM). This suggest a relation between this two animal models and the loss of mGluR plasticity in the hippocampus during the aging in the Alzheimer disease, which posits a significant target in the study of this pathology.



44) **Polymorphisms in *ABCB1* and *ABCC2* genes in patients with Drug-Resistant Epilepsy at Van Buren Hospital in Valparaíso, Chile**

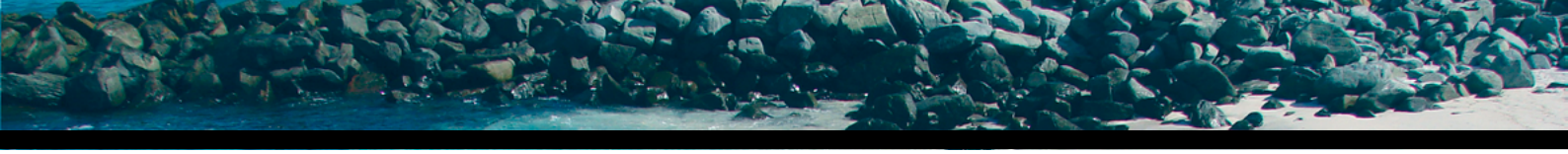
Martinez, Jonathan[1,2]., Riquelme, Julio[1,2].,Saldias, Cristina[1].,Rodriguez, Luciana[1].,Moya, Pablo[1,2].,[1]Laboratorio de Neurogenética, Instituto de Fisiología, Centro de Neurobiología y Plasticidad Cerebral, Ciencias, Universidad De Valparaíso.[2]Millennium Nucleus in Biology of Neuropsychiatric Disorders NU-MIND Universidad De Valparaíso. (Sponsored by Funded By Millennium Nucleus NU-MIND NC 130011 (PRM))

Epilepsy is a disorder affecting 1-2% of population worldwide. Despite treatment, about 25% of patients develop Drug-Resistant Epilepsy (DRE). Polymorphisms of Multidrug pumps in the Blood Brain Barrier (ABC Family) have been related to DRE, in particular on *ABCB1* and *ABCC2* genes. The aim of this study is to search for association between *ABCB1* and *ABCC2* polymorphisms and DRE in Chilean patients. This is a non-experimental correlational transversal study, approved by the Scientific and Ethics committee of Van Buren Hospital, Valparaiso, Chile. Patients (n=140) diagnosed with Epilepsy according to ILAE who attend the Neurology Clinic were prospectively recruited. We classified patients in two groups; those who qualified within DRE diagnosis (two or more trials of adequately chosen and tolerated drugs without seizure freedom within one year) and drug responsive patients. All patients were interviewed to recollect clinical and epidemiological data. Genomic DNA was extracted by standard lysis buffer procedure from saliva samples. Determination of *ABCB1* C3435T and *ABCC2* c.-24C>T polymorphisms was performed by PCR-RFLP, as previously reported in literature. We initially replicated the SNP calling methodology described using commercial human DNA panels (Coriell, USA). Allelic distribution of *ABCB1* and *ABCC2* polymorphisms do not significantly vary from those reported in literature and UCSC Genome browser. Patient recruitment is ongoing, and determination of SNP frequency is currently underway. To date, our data indicate that *ABCB1* C3435T and *ABCC2* c.-24C>T have both similar allelic distribution in Chilean Epilepsy patients to those reported in literature. We are currently determining putative differences in allele frequencies in Drug-Resistant vs Responders Epilepsy patients.

45) **Amyloid-β oligomers induce redistribution of neuronal pSer727-STAT3 in rat primary hippocampal cultured**

Muñoz, Yorka[1].,Paula-Lima , Andrea[2].,**Nuñez, Marco Tulio[2]**., [1]Department of Biology, Faculty of Sciences, Universidad De Chile.[2]Institute for Research in Dental Sciences, Faculty of Dentistry, Universidad De Chile. (Sponsored by CONICYT Ph.D. Fellowship 21130445 (YM), Fondecyt 1150756 (APL) And Project ACT-1114 From PIA, CONICYT (MTN).)

Amyloid-β oligomers (AβOs) induce the production of reactive oxygen species (ROS), oxidative damage and changes in mitochondrial dynamics in cultured neurons, block long term potentiation in hippocampal slices, AβOs and impair memory in animal models. Signal transducer and activator of transcription 3 (Stat3) is a crucial transcription factor in the CNS that regulates the expression of survival, antioxidant and anti-inflammatory genes. Stat3 is activated by ROS via Jak2. Phosphorylation of serine 727 (pSer727Stat3) modulates its transcriptional activity in many different cell types in a context-depending form. Recently, pSer727Stat3 was found associated to the mitochondrion, where it up-regulates mitochondrial activity and inhibits the MPTP formation. Here, we explored whether neuronal pSerStat3 expression and distribution is affected by AβOs treatment. Primary hippocampal neuron cultures were used. Immunocytochemistry and Western blot were employed to detect changes in the distribution and protein levels of pSerStat3, respectively. Treatment of mixed neuron-astrocyte cultures with 0.5 and 1 µM AβOs (24 h) invoked a change in the distribution, decreasing the nuclear and mitochondrial content of pSer727Stat3 and an increasing it in the cytoplasm. Also, the presence of pSer727Stat3 decrease in the mitochondria.. However, in astrocyte-depleted neuron cultures, AβOs treatment did not cause pSerStat3 redistribution but increased astrocyte reactivity, as determined by GFAP immunostaining. The data is consistent with the notion that AβOs activate astrocytes to release an undetermined signal(s) that induce nuclear and mitochondrial depletion of pSer727Stat3 in neurons.



46) **Early handling promotes resilience during childhood in prenatally stressed rats**

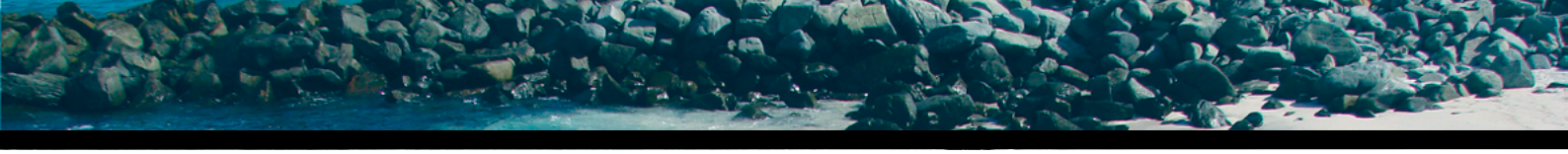
Pavez-Fox, Melissa[1]., Dagnino-Subiabre, Alexies[1].,[1]Laboratory of Behavioral Neurobiology, Center for Neurobiology and Brain Plasticity, Institute of Physiology, Faculty of Sciences, Universidad De Valparaíso. (Sponsored by This Work Was Funded By FONDECYT Grant N° 1141276 To AD-S. Labsite: Www.stress.cl)

Prenatal stress is a risk factor for several psychiatric disorders during childhood and adolescence. Conversely, short stress periods on early life (i.e. early handling), induces resilience to further stressors. So, the aim of this study was to determine whether early handling induces resilience in prenatally stressed rats. Male prenatally stressed rats were subjected to an early handling protocol, while animals of control group were not subjected to prenatal stress. After that all animals reached to childhood (postnatal day 24), resilient behaviors were evaluated in the open field and elevated plus maze tests (anxiety-like behaviors), as well by social interaction and forced swim test (depressive-like behaviors). Rats that were subjected to prenatal stress and early handling showed lower levels of anxiety like-behaviors compared to prenatally stressed rats. Furthermore, prenatally stressed rats, which were subjected to early handling, showed higher escape behaviors and sociability. These results suggest that early handling induces resilience in prenatally stressed rats. Thus, we have developed an animal model of childhood resilience, which could be compared with well-established animals model of prenatal stress. So, we can contribute to understanding of the neurobiological basis of resilience and chronic stress.

47) **Is the excitatory amino acid transporter 3 implicated in schizophrenia dysfunction?**

Pérez, Miguel[1,2].,Morales, Camila[1,2].,Arriagada, Jorge[1,2].,Moya, Pablo[2,3].,Fuenzalida, Marco[1,2].,[1]Laboratorio de Plasticidad Neural, Instituto de Fisiología, Facultad de Ciencias, Universidad De Valparaíso.[2]Millennium Nucleus in Neuropsychiatric Disorders NU-MIND, Instituto de Fisiología, Facultad de Ciencias, Universidad De Valparaíso.[3]Laboratorio de Neurogenetica, Instituto de Fisiología, Facultad de Ciencias, Universidad De Valparaíso. (Sponsored by This Work Was Supported By Grants From Millennium Nucleus NU-MIND NC-130011 (M.F And P.R.M.), FONDECYT 1130614 (M.F.) And 1141272(P.R.M.).)

Although the exact causes of schizophrenia (SZ) remain unknown, emerging evidence over several susceptibility genes is beginning to shed light on the mechanisms underlying the pathophysiology of SZ. One of these genes, *SLC1A1*, encodes the neuronal excitatory amino-acid transporter-3 (EAAT3), a member of the high affinity glutamate transporter family that co-localizes with glutamic acid decarboxylase 65 (GAD65) at axon terminals of GABAergic interneurons, which contributes to the synthesis of GABA; indeed, the blockage of EAAT3 results in a rapid reduction of GABA levels, which may alters the precise balance between excitation and inhibition (E/I). In our lab, we have found that Eaat3 haploinsufficient (HET) mice have dopaminergic alterations resembling some animal models of SZ. However, it is unknown whether in Eaat3 HET mice, the inhibitory synaptic transmission is impaired. Therefore, to determine if EAAT3 contribute to deregulation of GABAergic neurotransmission in SZ, we evaluated the inhibitory GABAergic transmission over pyramidal neurons in layer II/III of medial prefrontal cortex (mPFC), in a Eaat3 HET mice and conditional Eaat3 over-expressing (OE) mice compared to a pharmacological model of SZ induced by ketamine (Ket, 30mg/kg). Through electrophysiological assays we analyzed the paired-pulse ratio (PPR) of evoked inhibitory postsynaptic current (eIPSC), the frequency, amplitude of spontaneous (sIPSC) and miniature IPSCs (mIPSCs). We found that Eaat3 HET and ket-mPFC slices had lower GABA release probability compared to WT mice or vehicle, while Eaat3 OE had increased GABA release probability. Furthermore, conditional Eaat3 HET and ket-mPFC slices showed a lower GABAergic synaptic efficacy compared to WT slices, while in Eaat3 OE the frequency and amplitude of sIPSC and mIPSC were increased. These results demonstrate a relationship between SZ, genetic alterations on Eaat3 expression and changes on GABAergic synaptic transmission and point to a new potential presynaptic therapeutic target at the GABAergic neurotransmission in SZ.



48) **Dietary n-6 PUFAs induces depressive-like behavior while n-3 has antidepressant effect in a rat model of depression.**

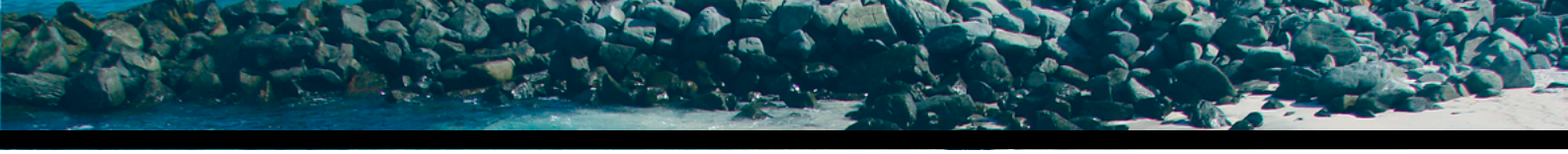
Pérez, Catherine[1]., Dagnino, Alexies[1].,[1]Laboratory of Behavioral Neurobiology, Center for Neurobiology and Brain Plasticity, Institute of Physiology, Faculty of Sciences, Universidad De Valparaíso. (Sponsored by This Work Was Funded By FONDECYT Grant N° 1141276 To AD-S. Labsite: Www.stress.cl)

In humans, n-3:n-6 dietary polyunsaturated fatty acid (PUFA) ratio is associated with longitudinal changes in depressive symptoms, with a higher ratio linked to a slower increase in depressive symptoms over time. The aim of this study was to evaluate the effects of n-3 and n-6 PUFAs supplementation on the development of depressive-like behaviors. Male Sprague-Dawley rats were subjected to chronic unpredictable stress (CUS), an animal model of depression. Afterward, animals were supplemented with n-3 (fish oil), n-6 (primrose oil) or water. Anhedonia (loss of pleasure) and hopeless are the core symptoms of major depression, both were evaluated in the rats as depressive-like behaviors by saccharin consumption and forced swim tests (FST), respectively. Rats that were exposed to CUS showed reduced saccharin intake and floating behavior in the FST, while supplementation with n-3 PUFA prevented these alterations. Conversely, supplementation with n-6 PUFA by itself induced depressive-like behaviors, while n-6 had synergic effects with CUS on saccharin consumption. We speculate that the etiology of depressive-like behaviors in the brain is related in part with alterations on the PUFA brain metabolism.

49) **Large scale integration mechanisms are differentially altered in conscious disorders.**

Rivera-Lillo, Gonzalo[1,2]., Egaña, Jose[3].,Díaz, Violeta[4].,Maldonado, Pedro[5].,[1]Kinesiología, Medicina, Universidad De Chile.[2]Laboratorio de Neurosistemas, Medicina, Universidad de Chile.[3]Anestesiología, Medicina, Universidad De Chile.[4]Neurología, Medicina, Universidad De Chile.[5]Fisiología, Medicina, Universidad De Chile. (Sponsored by Supported By ICM P10-001-F, P09-015-F Biomedical Neuroscience Institute And CONICYT For GR)

The vegetative state is a conscious disorder characterized by lack of a sustained, reproducible or voluntary behavioral responses to sensory stimulation. The minimally conscious state is a condition of severely altered consciousness characterized by minimal but definite behavioural evidence of self or environmental awareness. Previous reports have showed that subjects in this condition form a heterogeneous group, presenting different evoked and oscillatory response to different complexity stimuli. This remnant activity could be associated with different processing abilities and can be related to the indemnity of some brain regions, or to remaining connectivity between cortico-cortical and cortico-thalamic structures. Different mechanisms had been proposed to explain the conscious disorders, the most of them share the idea about the lack of large scale integration such as the main aspect related with lost of consciousness. Some of this mechanisms of large scale integration could be observed through both, changes in oscillatory activity at different frequency bands and temporal correlations between the activity at different brain locations. We propose that transitory changes in oscillatory activity amplitude and phase synchrony at low frequency bands, depend on the different complexity stimuli and reflect how different large scale integration mechanisms are affected in people with conscious disorders. We recorded data from ten subjects in vegetative states, two subjects in minimally conscious state and ten control subjects. We measured the evoked and oscillatory activity (24-Channel EEG) to auditory stimuli using a three classical oddball paradigm where the high complexity deviant stimulus was the patient's own name pronounced by a family member (emotional valence), middle complexity stimuli was a tone formed by three frequencies and low complexity stimuli was a tone formed by one frequency. We found significant changes in alpha/theta band power spectrum in response to stimuli presentation in the most of subjects associated with high complex stimuli (activation neural network), but without a selective response when we compare between own name with other first names (lack of selective response of neural network). For the other side, more specific large scale integration mechanisms like phase synchrony (measured through Weighted Phase Lag Index) was affected mainly in theta band independent of stimulus complexity. These results contribute to the model that proposes a lack of cortical integration due to loss of functional connectivity between different cortical areas, and to emphasize that different mechanisms are not affected in a similar form.



50) **Enhanced tracer coupling between striatal medium spiny neurons in a mouse model of Huntington's disease**

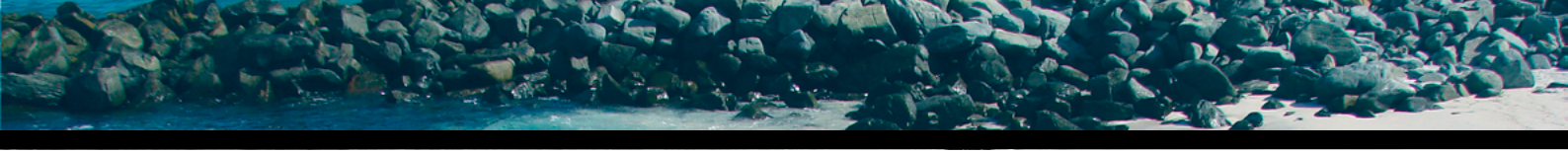
Rozas, Carlos[1]., Kadriu, Bashkim[2].,Chacon, Marcelo[2].,Shlomo, Dellal[2].,Faber, Donald[2].,[1]Biology, Chemistry and Biology, University of Santiago de Chile.[2]Neuroscience Albert Einstein College of Medicine, Yeshiva Univ.. (Sponsored by FONDECYT 11140430, CHDI Foundation A-5376)

Electrical transmission between neurons via gap junctions is present throughout the brain. Gap junctions permit the diffusion of small signaling molecules and metabolites between connected cells. Specifically, the neurotransmitter dopamine has been demonstrated to both weaken and enhance coupling between cells in the retina through its actions at D1 and D2 receptors, respectively. Huntington's disease (HD) is a hereditary neurodegenerative disease characterized by severe motor impairment that involves the loss of striatal medium spiny neurons (MSNs) and cortical projection neurons. Dopamine (DA) is a critical factor for the normal operation of the basal ganglia, and during the early stages of HD in humans, DA levels are increased and DA receptor expression is decreased. We were interested in whether this dopaminergic dysfunction in the striatum in HD leads to aberrant tracer-coupling/electrical connectivity between MSNs. To begin to address this question, we performed whole-cell patch-clamp recordings on MSNs with the diffusible morphological tracer neurobiotin (NB), which is small enough to cross-gap junctions. We then performed immunohistochemistry (IHC) on these brain slices using a fluorophore-tagged streptavidin, collected z-stacks via confocal laser-scanning microscopy, and then counted the number of tracer coupled cells. We found that on average, the number of coupled cells was threefold greater for MSNs from R6/2 mice, a rapidly progressing HD model, as compared to their wild type (WT) littermates. This difference was observed at both the early and late time points of the disease (5 and 12 weeks). We also found that when brain slices were treated with the gap junction blocker meclofenamic acid (100 µM), there was significantly less tracer coupling and there was no longer a difference in tracer coupling between WT and HD, indicating that the tracer coupling was due primarily to passage of the NB from the recorded cell across gap junctions. In agreement with this observation, we found that there was increased expression of connexin 36 in HD striatum (R6/2, 5 weeks), but not in cortex, as assessed by Western blot analysis. These results complement prior findings that HD pathology is characterized by significant changes in both chemical and electrical transmission in the striatum. Future work will aim to determine whether the increases in cell coupling in striatum result from a dysregulated dopaminergic system and whether it contributes to the motor symptoms of HD.

51) **Beta-amyloid clearence on Alzheimer's Disease: Role of APEH on brain and cerebral-vascular function**

Sandoval, Rodrigo[1]., Moraga, Josefina[2].,Valenzuela, Javier[1].,Lamas, Guillermo[1].,Moraga, Fernando[1].,Pancetti, Floria[1].,[1]Ciencias Biomédicas, Medicina, Universidad Catolica del Norte.[2]Pedagogía Universidad Metropolitana De Ciencias De La Educación.

The accumulation of amyloid beta 1-42 in the brain is a characteristic of Alzheimer's disease. An accumulation of amyloid beta (AB) 1-40 in the brain vasculature promotes cerebral amyloid angiopathy. Then, the mechanisms of degradation of AB would help prevent disease progression. The most studied enzyme (neprilysin and insulin degrading enzyme) have other substrates, in this context acetyl-peptide hydrolase (APEH), capable of degrading in vitro 1-40 AB becomes relevant. So it is important to determine whether inhibition of this enzyme alters the amount of AB in the brain and vasculature. We hipotesized that APEH inhibition result in increased AB 1-40, altering 1-42/1-40 ratio, producing pathophysiological changes in cerebral and vascular characteristics of Alzheimer's disease.By using techniques of biochemical, electrophysiological and behavioral, using older rats injected with DDVP APEH inhibitor, we quantified AB levels, AB degrading enzymes activity and content, synaptic plasticity and cerebral-vascular electrophisiology and spatial learning. We found that APEH inhibition by DDVP increases synaptic levels of AB 1-40 and decreases levels 1-42, coincident with increases in the activity of neprilysin in synapses of injected rats ($P < 0.001$). Also, APEH inhibition by DDVP is associated with a decrease of cerebral vascular contractility ($p < 0.001$). In addition, AB 1-40 decreases NMDA activity and increases alpha7-NAChR activity in hippocampal synapses. finally, APEH inhibition exhibit spatial memory impairment. These resultas suggests that changes in 1-42/1-40 ratio due to alterin AB clearence mechanisms induces pathophysiological features characteristic of Alzheimer's disease, and positions APEH as a putative target for parmacological tratment of the disease.



52) **ATP-mediated astroglial hyperexcitability in a rat model of chronic epilepsy.**

Wellmann, Mario[1]., Álvarez-Ferradas, Carla[1].,Sáez, Juan Carlos[2].,Bonansco, Christian[1].,[1]Instituto de Fisiología, CNPC, Facultad de Ciencias, Universidad De Valparaíso.[2]Departamento de Ciencias Fisiológicas, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile.

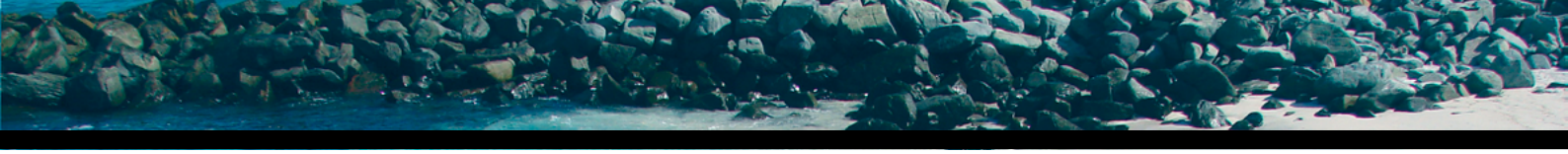
It is widely accepted that astrocytes play an active role in a wide range of neuropathologies. In spite of that, how astroglial function changes in pathological conditions remains unclear. Previously, we showed that hippocampal astrocytes from chronic epileptic rats exhibit an augmented incidence of spontaneous ATP-dependent slow Ca^{2+} transients (ST), which upregulates glutamate Ca^{2+}-dependent gliotransmission and therefore the strength of excitatory CA3-CA1 synapses. Because ATP is the main gliotransmitter involved in astrocyte-to-astrocyte communication, we assessed if it plays a role in this astroglial Ca^{2+}-mediated hyperexcitability. In order to determine the participation of non-vesicular ATP release mediated by pannexin-1 (Px1-HC) and connexin-43(Cx43-HC) formed hemichannels, we recorded spontaneous astroglial Ca^{2+} transients in presence of the petidomimetics 10-Panx and Gap-26 in control and kindled hippocampal slices. PX1-HC blockade reduces the mean duration of astroglial Ca^{2+} transients in kindled hippocampal slices by diminishing the percentage and frequency of ST, with no effect in the number of oscillations per area. Similar effects were observed in presence of the P2Y1 receptor (P2Y1R) specific antagonist MRS2179. Remarkably, both antagonists had no effect in control condition, suggesting that non-vesicular ATP release via Px1-HC represents a pathophysiological feature of the epileptic tissue. Blockade of Cx43-HC doesn't change any of the measured parameters in control and kindled hippocampus. These evidences suggest that astrocyte-to-astrocyte signaling via PX1-HC and P2Y1R, likely mediated by ATP, causes the astroglial hyperexcitability observed in the epileptic hippocampus. Astroglial dysfunction could represent a new key in epilepsy physiopathology, likely contributing to the reduction of seizure threshold and epilepsy chronicity. Funding: 1130491 (CB) from FONDECYT, CID 1/2006 from DIPUV (CNPC), CONICYT 22120213 fellows (MW), UVA 0804 2010 (JM) and UVA 0603 MECESUP fellows (CAF).

53) **The transcription factor NF-kB is translocated to the nucleus in epilepsy-related excitotoxicity.**

Corvalan, Katherine[1].,Gómez, Teresa[1].,Varas-Godoy, Manuel[2].,**Wyneken, U[3].**, [1]Centro Investigaciones Biomédicas Universidad De Los Andes.[2]CIB Universidad De Los Andes.[3]CIB, Medicina, Universidad De Los Andes. (Sponsored by Fondecyt 1140108)

Neurodegenerative disorders are strongly associated to excitotoxicity, i.e. neuronal death by pathological stimulation of N-methyl-D-aspartate (NMDA) receptors in susceptible areas of the central nervous system. Neuronal death depends on NMDA-receptor mediated Ca^{2+} overload and activation of downstream effectors such as nitric oxide synthases. In primary cell cultures, we have described an experimental condition in which hippocampal neurons are differentially susceptible to an NMDA challenge (30 µM for 1 hour) when compared to cortical neurons, that are resistant to this insult. We found that a differential signaling pathway leading to nitric oxide production and particular protein S-nitrosylation patterns. Specifically, the p65 subunit of the NF-kB transcription factor is S-nitrosylated in cortical, but not in hippocampal neurons. To test whether this differential S-nitrosylation pattern is associated with activation and nuclear translocation of p65, we obtained nuclear extracts from cell cultures followed by Western blots. Immunocytochemical studies are in course, as well as experiments with luciferase-NF-kB reporter construct. We found that p65 translocates to the nucleus after NMDA stimulation in hippocampal, but not in cortical neurons. This is associated to no changes in the phosphorylation of the canonic NF-kB activation pathway.

A non-canonic pathway leading to NF-kB activation after an excitotoxic challenge may provide novel targets for prevention of cell death in neurodegenerative disorders.



54) **Plasma nanovesicles as potential biomarkers of stress-induced depressive behaviors**

Gomez, Cristobal[1].,Ramirez, Juan Pablo[1].,**Wyneken, U[1].**, [1]CIB, Medicina, Universidad De Los Andes. (Sponsored by Fondecyt 1140108)

Exosomes, i.e. extracellular nanovesicles that originate in multivesicular bodies of cells, are emerging as potential biomarkers of diseases due to their molecular cargo consisting of proteins and RNA species. Mayor depressive disorder (MDD) is a multifactorial disease with increasing evidence for the existence of sub-types. Animal models of MDD are based on exposure to chronic stress. Previous work in our laboratory demonstrated that chronic stress protocols based on movement reduction, either by restriction (RS) in small cages or immobilization (IS) in plastic bags, for 2 h during 10 days, were able to induce depressive-like behaviors that were selectively reverted by different types of antidepressant drugs. Moreover, the protein Aldolase C (AldoC) within exosomes increased in the CSF selectively after stress induced by RS, but not by IS.

In this work, we focused on the presence of putative brain-derived biomarkers in exosomes present in the plasma of animals subjected to chronic stress by RS or IS. By in utero electroporation of telecephalic astrocytes, we show that exosomes containing AldoC can be harvested from the serum. Moreover, endogenous AldoC was differentially detected after RS or IS. Furthermore, our results show that AldoC in exosomes is modified by sumoylation. By mass spectrometry and Western Blot we found that several putative brain-derived proteins are differentially present in serum exosomes of rats subjected to both stress protocols, including Aldolase A, GFAP, Synaptofysin and Reelin. Additionally, miR26a, highly expressed in forebrain astrocytes, is present in serum exosomes.

Our results show that serum nanovesicles provide a novel and useful tool to identify protein and miRNA markers present in stress models with differential pharmacological sensitivity, which could help to define markers for stress induced depressive disorder.

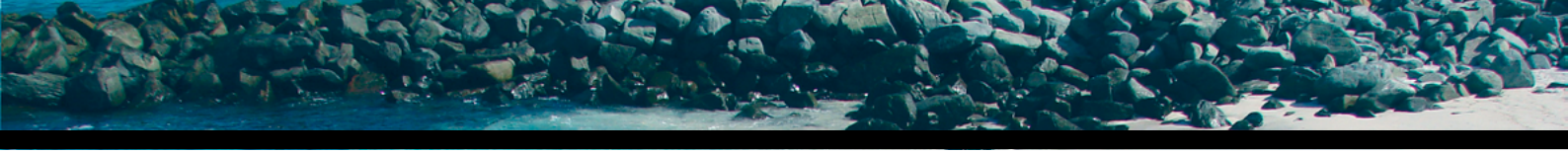
55) **Metabolic and gene expression changes underlying axonal regeneration during diapause.**

CANEO, Mauricio[1]., Alkema, Mark[2].,Calixto, Andrea[1].,[1]CENTRO DE GENOMICA, BIOLOGIA, Universidad Mayor.[2]NEUROBIOLOGY University of Massachusetts Medical School.

Food deprivation causes profound changes in physiology and behavior. Animals with the ability to enter dormancy in response to lack of food ensure survival by suppressing oxidative metabolism to minimize energy expenditure. Hypometabolism during dormancy is associated with dramatic changes in gene expression, protein repertoire and enzymatic activity. In response to starvation, the nematode *Caenorhabditis elegans* enters diapause forming the dauer larva, a state accompanied with dramatic changes in morphology and metabolism. We found that diapause formation protects and regenerate *C. elegans* sensory neurons that have been triggered to die by hyperactivation of the MEC-4d degenerin. The *mec-4d* mutation *(A713V)* causes constitutive opening of MEC-4 channel, sodium entry and increased intracellular Ca^{2+} causing neuronal death by necrosis.

One fundamental change that occurs in diapause is the downregulation of the insulin receptor DAF-2, which promotes DAF-16/FOXO activation and the consequent increase in cellular antioxidant capacity, known to be neuroprotective (Calixto et al., 2012). However, while *daf-2;mec-4d* double mutants show extensive neuronal protection they do not regenerate damaged neurons as dauers do, suggesting that other gene networks are involved in neuroregeneration during diapause. To understand the gene expression changes that underlie the increased regenerative capacity of dauers, we analyzed the expression levels during diapause of genes known to be crucial for regeneration. A key regulator of regeneration is the mitogen-activated protein kinase DLK-1, required for axon regrowth after axotomy (Hammarlund et al., 2009), and spontaneous breakage of axons (Hammarlund et al., 2007). DLK-1 promotes regeneration by simultaneous activation of *jnk* and p38 pathways. We found the p38 pathway to be upregulated in dauers as well as *ccpp-6, a* target of DLK-1. Additionally, negative regulators of regeneration such as *klp-7* and *efa-6*, involved in microtuble catastrophe are downregulated.

To test whether DLK-1 has a role in the regeneration observed in dauers, we generated a double mutant of *mec-4d* and *dlk-1. mec-4d;dlk-1* mutants fail to completely regenerate, showing that, as in axotomized axons, DLK-1 is also important for the regeneration of degenerin expressing axons. However, we observed that 50% of animals have wild type axons at time of dauer entry in contrast with 8-10% in non-dauers, highlighting an additional effect which is dauer dependent by independent of the activity of DLK-1. This suggests that the dauer state conveys a large number of factors that are neuroprotective and stimulate regeneration.



56) **EFFECTS OF VOLUNTARY EXERCISE ON SPATIAL AND OBJECT RECOGNITION MEMORY OF OCTODON DEGUS DURING AGING.**

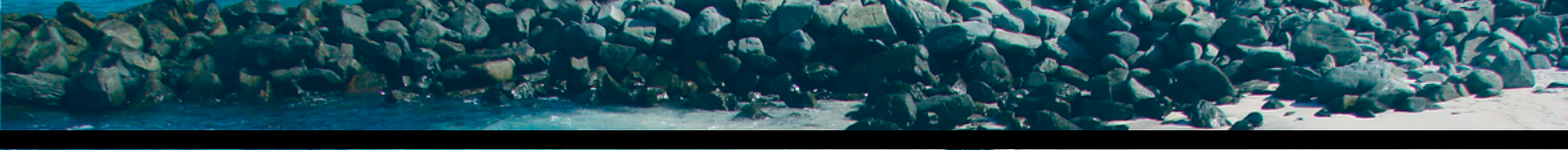
Salazar, Claudia[1]., Palacios, Adrian [1].,[1]Centro Interdisciplinario de Neurociencia de Valparaíso, Ciencias, Universidad De Valparaíso. (Sponsored by Millennium Institute ICM-P09-022-F)

The Octodon degus (degu) is a rodent that naturally develops the main signs of Alzheimer's disease (degu AD like) during aging, including the increase of soluble Aß, and Tau hyper-phosphorylation and failures associated with learning and memory (spatial and object recognition) capacity and alterations in synaptic plasticity (LTP and LTD). Several other studies suggest that exercise (free access to a wheel) can prevent or delay the deterioration of cognition during neurodegenerative processes. Here we have study in degu of different ages, the effect of voluntary long-term physical exercise on their cognitive capacities. We have tested there, locomotor activity, recognition memory and spatial memory through an open field test, object recognition and radial arm maze in young and old animals. Our results show that both young and old exercised degus have better cognitive performance compared to degu without (wheel) activity. For example, after 4 months of without wheel inactivity both age groups show deterioration in behavioral test. Thus, voluntary exercise could correspond to an effective therapeutic usage, reducing cognitive impairment caused by degu AD like.

57) **Rat olfactory sensory neurons cilia incorporate glucose and may take it from the mucus to use as complementary energy source for odor transduction**

BACIGALUPO, JUAN[1]., VILLAR, PABLO[1].,BLANCHARD, KRIS[1].,VILLALOBOS, DANIELA[1].,DELGADO, RICARDO[1].,VERGARA, CECILIA[2].,REYES, JUAN[3].,[1]BIOLOGIA, CIENCIAS, Universidad De Chile.[2]BIOLOGIA, CIENCIAS, Universidad Central De Chile.[3]INSTITUTO DE QUIMICA, CIENCIAS, Pontificia Universidad Católica De Valparaíso. (Sponsored by FONDECYT 1140520)

Olfactory cilia (~60 µm long, 0.2 µm diameter) project from the apical knob of the single dendrite of olfactory sensory neurons (OSNs). Odorants binding to G-protein coupled receptors trigger a cAMP-cascade in the cilia that leads to the opening of Ca^{2+}-conductive cyclic nucleotide-gated channels (CNGs); Ca^{2+} itself activates Cl^- channels. Both channels generate a depolarizing receptor potential. The cilia require ATP for the activity of the adenylyl cyclase, ATPases and kinases. The mitochondria in the knob are the closest to the cilia, which lack any inner membranes. Slow ATP diffusion from the knob and limited basal ATP availability in the cilia suggest the possible existence of additional ATP sources to sustain transduction during periods of intense odor stimulation. Immunohistochemistry previously revealed glucose transporters in the olfactory epithelium ciliary layer, populated with OSNs cilia and supporting cells (SCs) microvilli (Nuñez-Parra et al, Chem Senses, 2011). We hypothesized that glucose is released by SCs to the mucus and incorporated by the cilia to produce ATP by glycolysis. We detected the presence of glucose in the mucus covering the epithelia with a colorimetric assay. By immunocytochemistry on dissociated cells we confirmed the presence of the glucose transporter GLU-3 in OSN both in OSN cilia and SC microvilli. With a fluorescent glucose analog (2-NBDG), we observed that the cilia incorporated this sugar from the mucus. Glycolytic enzymes are present in ciliary membranes, as revealed by immunoblotting. Glycolysis as well as oxidative phosphorylation inhibitors partly abolished odor responses in field recordings from the olfactory epithelium and suction pipette recordings from isolated OSNs in the presence of external glucose (0.2 mM). OSNs underwent fatigue upon glucose removal from the extracellular solution. These results support the notion that the cilia take from the mucus glucose released by SCs and process it by glycolysis to supply ATP for chemotransduction, in addition to ATP supplied bythe knob.



58) **Chronic phenytoin treatment enhances rat petrosal ganglion responses to acetylcholine**

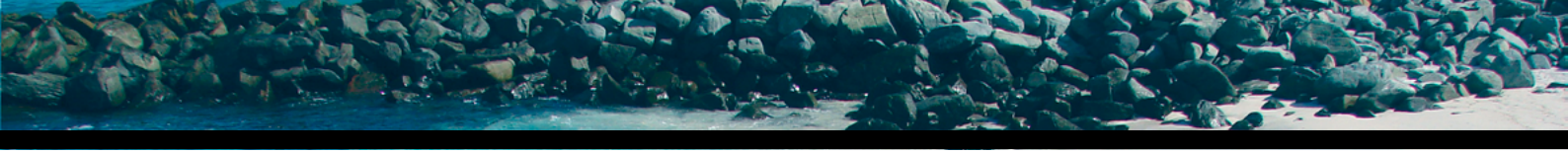
Ortiz, Ignacio[1]., Vera, Jorge[1].,Alcayaga, Julio[1].,[1]Biología, Ciencias, Universidad De Chile. (Sponsored by Supported By Grant FONDECYT 1130177)

The carotid body (CB), the main arterial chemoreceptor in the rat, transduces arterial gases and pH and synaptically drives the activity of afferent neurons which somata are located in the petrosal ganglion (PG). These neurons, driven by transmitters released by CB cells, present a persistent Na^+ current (I_{NaP}), which acute blockade by phenytoin reduces basal afferent discharges, and both increased afferent discharges and ventilatory responses to acute hypoxia. However, because chronic effects of phenytoin on the PG neurons have not been reported, we studied their electrophysiological properties and transmitter-induced responses in neurons from chronically phenytoin treated rats. Male Sprague-Dawley rats (180-200g), under isoflurane anesthesia, were implanted subcutaneously with an osmotic pump (Alzet 2ML4) that delivered a 10 mg/day dose (n = 7); control animals were implanted with pumps containing vehicle (n = 3). Both antibiotic and anti-inflammatory were injected at the end of surgery. After 1-4 weeks the animals were anesthetized with sodium pentobarbitone and the PG was obtained bilaterally. The tissue was placed in a chamber under constant flow with Hanks´ solution at 30°C. Conventional intracellular recordings were performed with 3M KCl-filled glass electrodes connected to a microelectrode amplifier (Axoclamp 900A); stimulus control and data acquisition was controlled by software (pClamp 10). Acetylcholine (ACh) was applied near the PG. Animals were sacrificed by an anesthetic overdose at the end of the experiment. Phenytoin treatment had no effect on resting membrane potential (control: -61.1 ± 7.5 mV; Treated: -56.8 ± 0.6 mV) or input resistance (control: 23.1 ± 4.3 MΩ; Treated: 27.1 ± 4.5 MΩ). Responses to a single dose of ACh (100 mM, 10 µL) produced a depolarization that was significantly larger ($P < 0.05$, Mann Whitney test) in treated rats ($\Delta V = 5.5 \pm 1.5$ mV) than in control ($\Delta V = 2.3 \pm 0.5$ mV). Thus, chronically administered phenytoin does not affect the measured electrical properties of PG neurons, while increasing their responses to exogenously applied ACh. It is suggested that these observed modifications may alter ventilatory responses after chronic phenytoin treatment.

59) **Role of TRPM8 channels in the altered sensitivity to cold of corneal mice primary sensory neurons caused by axonal damage.**

Piña, R[1,2]., Campos, Matías[1].,Ugarte, Gonzalo[1].,González, Alejandro[1].,Bacigalupo, Juan[2].,Madrid, Rodolfo[1].,[1]Departamento de Biología, Facultad de Química y Biología. Laboratorio de Neurociencias, Universidad de Santiago de Chile.[2]Departamento de Biología, Facultad de Ciencias. Laboratorio de Fisiología Celular, Universidad de Chile. (Sponsored by Grants CONICYT Anillo ACT-1113 (RM, GU), FONDECYT 1131064 (RM), 1140520 (JB) And CONICYT PhD Fellowship And Grant 21110327 (RP).)

Injury of corneal sensory fibers is followed by dysesthesias, altered tear production and changes in thermal and chemical sensitivity. The neural and molecular bases of these alterations are poorly understood. Both, cold-sensitivity and spontaneous firing of corneal cold-sensitive neurons (CCSNs) depend on the expression of the cold, voltage and menthol-activated channel TRPM8. In addition of detecting cold, CCSNs expressing TRPM8 are humidity detectors of the eye surface. The role of these neurons in the sensory alterations induced by axonal damage remains unclear. We used focal extracellular recording of CCSNs, in combination with calcium imaging and patch clamping of FM1-43 retrograde labeled corneal neurons from trigeminal ganglia, to investigate whether their thermal- and chemical-sensitivity were altered after controlled mechanical injury of corneal sensory axons. We found an increase in the percentage of CCSNs and their responses to cold and menthol, with no major differences in the mean temperature threshold compared to control animals. Immunohistochemistry of the ophthalmic region of trigeminal ganglia revealed an increase in the percentage of TRPM8-expressing neurons after injury. Using transgenic mice Thy-1 YFP, we evaluated the recovery of corneal sensory fibers after injury, and our results reveal partial reinnervation after 21 days. Spontaneous firing and menthol-sensitivity were higher in CCSNs from damaged corneas. Interestingly, the injured animals exhibited a more pronounced basal tear flow. These results unveil functional changes in CCSNs after injury, suggesting that sensory alterations induced by damage of corneal nerve fibers may be linked to an enhanced TRPM8 expression.



60) **Chronic phenytoin treatment reduces rat ventilatory responses to acute hypoxia**

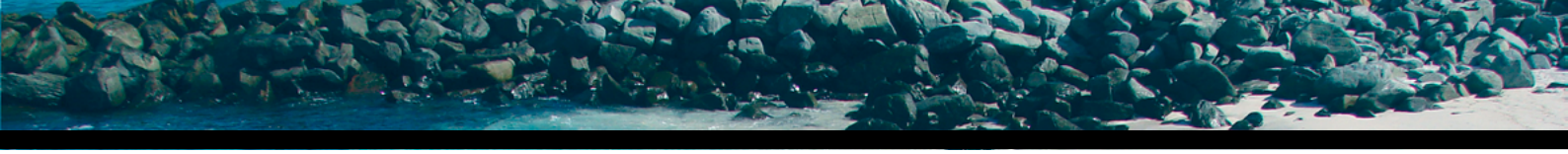
Pino, Gabriela[1]., Montero, Pablo[1].,Alcayaga, Julio[1].,[1]Laboratorio de Fisiología Celular, Ciencias, Universidad De Chile. (Sponsored by Supported By Grant FONDECYT 1130177)

Carotid body (CB) afferent activity drives ventilation from the periphery by changes in arterial gases and pH. Sensory neurons innervating the CB present a persistent Na^+ current (I_{NaP}); acute blockade of I_{NaP} with phenytoin reduces normoxic ventilation and responses to short hypoxic challenges. Because the effects of long-term phenytoin treatment on ventilation and ventilatory responses are unknown, we recorded normoxic ventilation and responses to a wide range of oxygen inspiratory fractions (F_IO_2) in rats chronically treated with phenytoin. Male Sprague-Dawley rats (192 ± 3 g), under isoflurane anesthesia, were subcutaneously implanted with an osmotic pump (Alzet, 2ML4) containing vehicle (control; n = 9) or phenytoin (10 mg / day; n = 20); the animals received both an antibiotic and an anti-inflammatory immediately after surgery. From 7 to 31 days after surgery the animals were anesthetized with sodium pentobarbitone (60 mg / Kg) and placed in a thermoregulated pad. The thraquea was cannulated and connected to a pneunotacograph, connected in turn to a differential pressure transducer to assess ventilatory flow (J_V). The left femoral artery was cannulated and connected to a pressure transducer (Statham P23D) to measure arterial pressure (Pa) and assess cardiac frequency (F_C), and a rectal probe used to monitor the temperature of the animal. Animals breathed air spontaneously and changes in F_IO_2 (0 – 100 % range) were applied for 30 s. All signals were digitally acquired at 2 KHz / channel and stored in a personal computer. Tidal volume (V_T) and ventilatory frequency (F_V), respectively, were calculated from J_V recordings, and minute volume (V_E) was calculated as the product V_T * F_V. The animals were sacrificed with an anesthetic overdose at the end of the acute experiment. Phenytoin treatment had no significant effect ($P > 0.05$, ANOVA) on normoxic ventilatory (V_T, F_V, V_E) or cardiovascular (Pa, F_C) variables. Phenytoin significantly reduced ($P < 0.05$, Bonferroni test after 2 Way ANOVA) the increases of V_T and V_E induced by acute hypoxic challenges after 13 and 29 days of treatment, without modifying F_V ($P > 0.05$, 2 Way ANOVA). No significant changes in cardiovascular responses to hypoxia were observed ($P > 0.05$, 2 Way ANOVA). Our results show that phenytoin treatment reduces ventilatory responses to hypoxia, without modifying the cardiovascular responses, suggesting a specific effect mediated by I_{NaP} blockade.

61) **Pannexin 1 modulates the function of the supporting cells of the Organ of Corti.**

Prado, Pavel[1]., Jara, Oscar[1].,Flores, Carolina[1].,Maripillán, Jaime[1].,Martínez, Agustín[1].,[1]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad De Valparaíso. (Sponsored by Work Supported By FONDECYT 3150442 To PP, FONDECYT 1130855 And ANILLO-ACT1104 To ADM. The Centro Interdisciplinario De Neurociencias De Valparaíso Is A Chilean Millennium Institute (P09-022-F))

Pannexin 1 (Panx1) is a trans-membrane protein that forms non-selective plasma membrane channels permeable to ATP. In the cochlea, this molecule is expressed in different cellular groups, including the supporting cells of the organ of Corti (OC). Lack of Panx1 in the cochlea results in sensorineural hearing loss (SNHL). We hypothesized that this type of deafness might arise from the disruption of the cochlear purinergic signaling pathway, which finally results in the abnormal maturation of the OC. To get insight about the role of Panx1 in hearing, cochleae of wild-type mice with different postnatal ages were collected, and physiological properties of supporting cells were characterized as a function of the age and the effect of pharmacological agents that specifically block Panx1 channels. It was found that whole cell voltage-dependent ionic currents of isolated supporting cells increase with the maturational stage of the animal. The magnitude of the ionic currents was importantly reduced by acute treatment with Panx1 blockers (probenecid and the mimetic peptide ^{10}Panx1), suggesting a critical role of Panx1 channels in the excitatory properties of cochlear supporting cell. Furthermore, the basal release of the ATP by the cochlear supporting cells was also reduced when organotypic cultures of the OC were incubated with Panx1 blockers. Although still preliminary, difference in the single channel activity were observed among animals of different age brackets These results support the idea of an age-dependent expression of Panx1 channels in the murine cochlea, which might be important for the correct functioning of the Organ of Corti and the developing of hearing.



62) **Direction seletivity in a network of non-homogeneous Starbust Amacrine Cells (SAC)**

Salgado, Simon[1]., Castro, Samy[1].,Escobar, María José[2].,Orio, Patricio[1].,[1]Centro Interdisciplinario de Neurociencia de Valparaiso, Facultad de Ciencias, Universidad De Valparaíso.[2]Departamento de Electrónica Universidad Técnica Federico Santa María.
(Sponsored by Supported By Grants Fondecyt 1130862, 1140403, ACT-1104, ACT-1113, FB0008. The Centro Interdisciplinario De Neurociencia De Valparaíso (CINV) Is A Millennium Institute Supported By The Millennium Scientific Initiative Of The Ministerio De Economía (Chile))

Direction-Selective Retinal Ganglion Cells (DSRGC) respond selectively to stimuli moving towards one direction, while a stimulus moving in the opposite direction produces little or no response. This activity is proposed to originate in the Starburst Amacrine Cells (SACs), which release GABA which inhibit DSRGCs when stimulus moves contrary to the preferred direction. Intracellular calcium studies in rabbit\\\'s SACs have shown an asymmetrical response to a bar moving through opposite dendrites. Also, biophysical modelling suggests that SAC dendritic structures are intrinsically selective to direction because of passive cable properties. SACs highly overlap with each other, and interact through GABAergic inhibitory synapses, but the role of this SAC-SAC inhibition is not well understood. We are working in a novel conductance-based model of the SACs network considering SAC-SAC inhibitory interaction under the PyNEURON simulation environment. We optimized the parameters of the GABAergic synapses in a SAC network stimulated by glutamatergic bipolar inputs, and studied the effect of GABAergic and glutamatergic conductance levels. In our model, different SACs show slight different velocity tuning curves, which corresponds to the variability found in biological experiments. As a result, a non-homogeneous population of SACs can respond to a wider range of stimulus velocities. The reciprocal GABAergic inhibition greatly enhances the direction selectivity measured, providing evidence that a network performs better than isolated SACs. Future work will be focused in integrating a DSRGC to the model as well as separate ON and OFF bipolar/SAC layers.

63) **Effects of cannabinoid receptor activation in OFF bipolar cells activity**

Vielma, Alex[1,2]., Schmachtenberg, Oliver[2].,Chávez, Andrés[2].,Fuenzalida, Marco[1].,[1]Centro de Neurobiología y Plasticidad Cerebral, Facultad de Ciencias, Universidad De Valparaíso.[2]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad De Valparaíso. (Sponsored by Postdoctoral FONDECYT #3140599 (AV), Regular FONDECYT #1120513 (OS), #1130614 (MF), And #1151091 (AEC). Millennium Institute CINV (OS, AEC) And Millennium Nucleus NU-MIND NC-130011 (AEC, MF).)

The cannabinoid receptor type 1 (CB1R), one of the most abundant G protein-coupled receptors in the brain, has been shown to mediated a number of physiological actions such as inhibition of excitatory and/or inhibitory synaptic transmission through inhibition of voltage-gated Ca^{2+} channels. In the mammalian retina, CB1Rs are widely distributed in the outer and inner plexiform layers (IPL), strongly suggests that this receptor may play an important neuromodulatory role in regulating retinal information processing. However, the physiological significance of CB1R activation in the mammalian retina remains unclear. Using whole-cell recording techniques in light-adapted rat retinal slices, we investigated whether activation of CB1R might directly modulate OFF bipolar cell function. Exogenous application of the specific CB1R agonist WIN 55212-2 (WIN; 1 µM) by localized perfusion of the IPL, generated membrane hyperpolarization due to an outward current, recorded at 0 mV to isolate inhibitory Cl^- currents. On the other hand, the specific CB1R antagonist AM251 (5 µM) caused initial membrane depolarization followed by a prolonged hyperpolarization, product of a sustained outward current observed at 0 mV. No currents were induced by WIN or AM251 at -60 mV, the Cl^- reversal potential in our recording conditions. These results suggest that CB1R might directly modulate the intrinsic activity of OFF bipolar cells. The potential contribution of eCBs and the role of CB1R in regulating inhibitory synaptic inputs onto OFF bipolar cells is currently under investigation.

64) **Sodium Potassium Chloride co-transporter 1 (NKCC1) is responsible of high excitability in chronic epilepsy in adult rats**

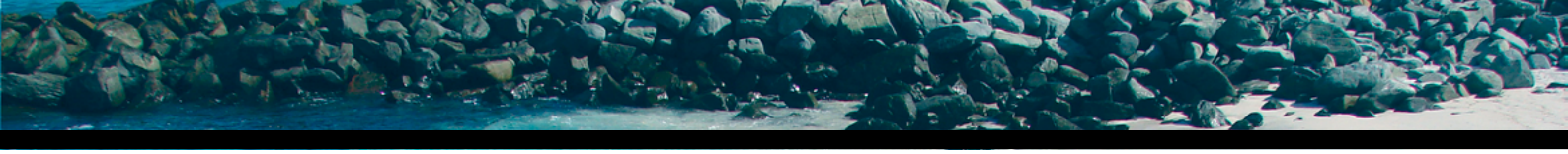
Lara, Marcelo[1]., Lorca, Enrique[1].,Rojas, Patricio[1].,[1]Biología, Química y Biología, Universidad De Santiago De Chile.

The increase in hippocampal excitability is one of the cardinal symptoms of temporal lobe epilepsy (TLE), the most common epilepsy type. In epileptic patients as well as animal models have been observed an increase in Dentate Gyrus excitability, which is thought to be responsible of hippocampal foci in TLE. An increase in intracellular chloride and protein level of the chloride co-transporter 1 NKCC1, which drives the electroneutral uptake of this anion, have been observed in human an animal models suggesting an excitatory GABA (aminobutyric acid) effect. Being similar to what observed in immature neurons during early development. In order to evaluate the contribution of NKCC1 to the elevated circuit excitability in chronic epilepsy, we evaluated Dentate Gyrus excitability by extracellular field recordings in acute brain slice of chronic epileptic animals. This allow to not perturb the intracellular chloride concentration. Input-output relationships of synaptic and population action potentials needed less stimulation to reach saturated responses, consistent with a facilitation of synaptic transmission. The GABAergic component is also shifted toward less stimulation. Inhibition of NKCC1 by Bumetanide decreases 50 % of the amplitude of GABAergic component in epileptic slices, while only 10 % in controls. This elevated NKCC1 activity results in an excitatory GABA effect in epileptic tissue. Finally, Bumetanide was able to decrease the frequency of epileptiform-like firing in slices from epileptic but not control animals. In overall, these results show that NKCC1 is expressed and active in dentate gyrus of chronic epileptic animals, and its blockade is able to produce anti-epileptic effects.

65) **The structure of the axon initial segment correlates with basal firing rate in substantia nigra dopaminergic neurons**

Meza, R[1]., Oñate, Alejandro[2].,Henny, Pablo[2].,[1]Anatomia Normal, Medicina, Pontificia Universidad Católica De Chile.[2]Anatomia, Medicina, Pontificia Universidad Católica De Chile. (Sponsored by Funded By FONDECYT 1171140 And ANILLO ACT-1109 Grants To P.H. And CONICYT Graduate Scholarship To R.M.)

In substantia nigra compacta (SNc) dopaminergic neurons the axon usually emerges from a primary or higher order dendrite. Actions potentials are generated at the most proximal region of the axon, the axon initial segment (AIS). This unmyelinated region is enriched in sodium and other ionic channels, as well as scaffolding proteins such as Ankyrin-G (Ank-G). As shown in other central neurons, the molecular composition, size and location of the AIS is known to influence the neuron's activity. In order to describe the mechanisms that influence firing in SNc dopaminergic neurons, we carried out a project to examine whether the structural characteristics of the AIS relate to the spontaneous tonic firing pattern showed by these neurons *in vivo*. Adult male mice SNc neurons were recorded under urethane anaesthesia. Neurons were recorded during spontaneous activity at least 15 minutes before neurobiotin labelling using the juxtacellular method, after which animals were perfused and their brains removed and serially sectioned. Neurons were revealed using streptavidin-Cy3 and identified as dopaminergic using immunofluorescence for tyrosine hydroxylase. To determine the shape and localisation of the AIS, entire individual neurons were traced and 3D reconstructed from labeled fragments acquired with a confocal microscope. The localisation of the AIS was confirmed using further immunofluorescence staining for Ank-G. Structural analysis show variable dendritic origin and size of the AIS in SNc dopaminergic neurons. The two variables nonetheless relate in that length of AIS diminishes with distance from the soma. Notably, electrophysiological analyses show that AIS length/localisation predicts spontaneous basal firing rate, in that neurons with large/proximal AIS fire faster than neurons with small/distal AIS.



66) **Serotonin induces inhibitory long-lasting depression by activation of presynaptic 5-HT1 receptors**

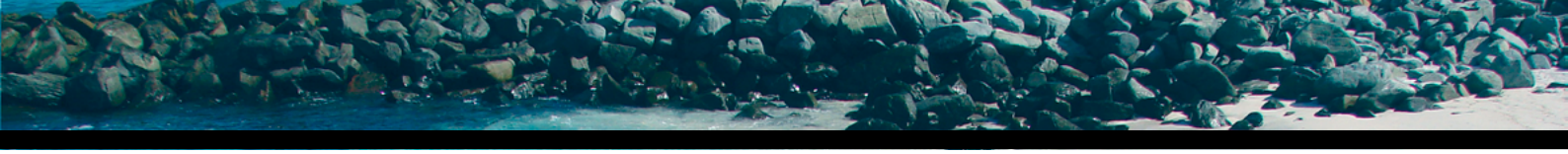
Morales, Koyam[1,2]., Moya, Pablo[3,2].,Fuenzalida, Marco[1,2].,[1]Laboratorio de plasticidad neural, Facultad de Ciencias, Universidad De Valparaíso.[2]Millennium Nucleus in Neuropsychiatric Disorders NU-MIND, Facultad de Ciencias, Universidad De Valparaíso.[3]Laboratorio de Neurogenética, Facultad de Ciencias, Universidad De Valparaíso. (Sponsored by This Work Was Supported By Grants From Millennium Nucleus NU-MIND NC-130011 (M.F And P.R.M.), FONDECYT 1130614 (M.F.) And 1141272 (P.R.M.).)

GABAergic inhibitory synapses are essential for maintaining the excitation and inhibition (E/I) ratio, whose imbalance underlies various brain diseases. Changes in the efficacy of synaptic transmission in the prefrontal cortex (PFC) have been proposed as the neural substrate of several cognitive processes such as learning and memory. The neurotransmitter serotonin (5-HT) exerts a powerful control of PFC synaptic transmission. Given the widespread innervation of the brain, it is not surprising that the 5-HT system is the target of many drugs used to treat brain diseases. However, the action of serotonergic signalling on inhibitory synapses efficacy is still unknown in PFC. Through electrophysiological patch-clamp recordings, we studied the changes in the inhibitory plasticity generated by 5HT. Our results showed that activation of 5-HT1 receptors by 5-HT (50 µM) or by the agonist5-carboxamidotryptamine(5-CT, 100 nM) induced a long-lasting depression of evoked inhibitory post-synaptic current (eIPSC) in PFC pyramidal neurons of Layer 2/3. Also, we observed that activation of interneuron serotonergic receptors might induce an increase of paired pulse ratio (PPR) and a decrease of spontaneous activity (sIPSC) frequency, suggesting that 5-HT reduces the release probability of GABAergic interneurons. Thus, our results suggest that 5-HT-dependent changes in the GABAergic efficacy of PFC could be an important functional target to the treatment of different neuropsychiatric diseases such as anxiety, obsessive-compulsive disorder and schizophrenia.

67) **The effect of a reduced sAHP-conductance on the glutamatergic synaptic plasticity of kindled rats**

Morales, Juan[1,2]., Fuenzalida, Marco[1,2,3].,Bonansco, Christian[1,2].,[1]Instituto de Fisiología, Facultad de Ciencias, Universidad De Valparaíso.[2]Centro de neurobiología y plasticidad cerebral, Facultad de Ciencias, Universidad De Valparaíso.[3]Millennium Nucleus NU-MIND, Facultad de Ciencias, Universidad De Valparaíso. (Sponsored by Funding: 1130491 (CB) And 1130614 (MF) From FONDECYT, Millennium Nucleus NU-MIND NC-130011 (M.F.), CID 1/2006 From DIPUV (CNPC), UVA 0804 2010 (JM) MECESUP.)

The slow after-hiperpolarization (sAHP) is one of the three main Ca^{2+}-dependent K^+ conductances activated post bursts of action potentials (APs), which reduces neuronal excitability, regulates dendritic integration and restricts the temporal coincidence between pre and post-synaptic activity requiered for induction of long term plasticity (LTP) through shunting of post-synaptic EPSPs. In epilepsy, it has been reported a diminished sAHP, directly enhancing neuronal excitability; however the effect of a pathologically reduced sAHP on synaptic plasticity is unknown. In this work we investigate if a pathologically reduced sAHP could facilitate the potentiation of glutamatergic synapses in the epileptic hippocampus. Using whole-cell patch-clamp configuration we recorded the sAHP in pyramidal neurons in CA1 region of the hippocampus from adult (p60) control and kindled (KD) rats, determining its effect on synaptic plasticity induced by a low frequency spike timing dependent plasticity protocol (STDP; pairing between EPSP and a back propagated AP). In KD group, neurons showed a redistribution of the sAHP conductance: lower than 2.0 nS (classified as L-sAHP, 26.9% of the neurons recorded) and over 8.0 nS (H-sAHP, 23.0%) without significant changes in AP firing, while the control group rarely presented the L-sAHP phenotype (<8.3%). In both groups, L-sAHP did not significantly modified the EPSP time course nor its amplitude (no shunting), which could increase the temporal window for pairings and thus facilitate synaptic potentiation. Both groups exhibited similar levels of sAHP activation during STDP, but only KD neurons with sAHP lower than 4.0 nS showed potentiation, whereas those with higher sAHP showed depression, similar to control synapses. In KD neurons, STDP protocol applied under constant sAHP activation resulted in a transient initial potentiation (123.5±8.2% until 20 minutes after STDP) followed by long term depression (LTD, 79.0±1.5% after 40 minutes), nevertheless control neurons only showed LTD (79.7±7.7% from 15 minutes on). These results indicate that KD L-sAHP neurons allow the induction of transient initial synaptic potentiation followed by LTD. This abnormal form of short term potentiation exhibited by this neuronal network in epileptic hippocampus could contribute to the disruption of synaptic plasticity that is required for epilepsy progression.



68) **The neurovascular coupling-initiated astrocyte Ca^{2+} signal is mediated by sequential glutamate metabotropic and NMDA receptor activation**

Muñoz, Manuel[1]., Puebla, Mariela[1].,Figueroa, Xavier[1].,[1]Departamento de Fisiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile.

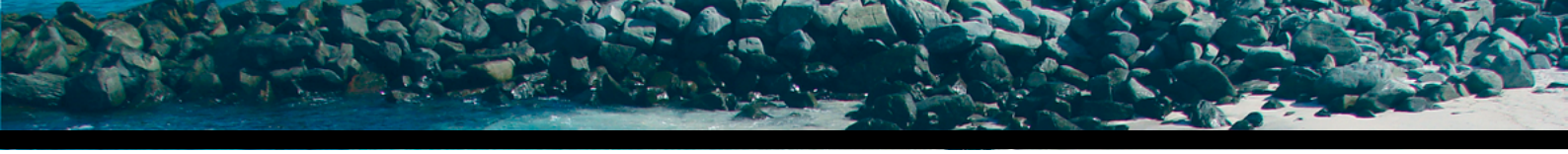
Brain functions depend on fine regulation of cerebral blood flow by a mechanism known as neurovascular coupling. Neurons and cerebral arterioles are functionally communicated through the astrocytes located between these two cell types and an increase in synaptic activity is rapidly transduced into release of vasodilator signals by the astrocytic-end feet that are encasing the cerebral blood vessels. The astrocyte-dependent neurovascular coupling is initiated by the neurotransmitters released during an increase in synaptic activity, which activate an intracellular Ca^{2+} signal that propagates to the astrocytic-end feet. Then, the main neurotransmitter involved in neurovascular coupling is glutamate through the activation of glutamate metabotropic receptors on astrocytes. However, the activation of glutamate metabotropic receptors leads to D-serine and glutamate release by astrocytes, which may contribute to the Ca^{2+} signaling by the stimulation of ionotropic NMDA glutamate receptors (NMDAR). Therefore, we used primary cultures of astrocytes to evaluate the participation of NMDAR in the increase in intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ initiated by the activation of glutamate metabotropic receptors in astrocytes. Expression of NMDA receptor NR1 subunit was analyzed by inmmunofluorescence and Western Blot and the changes in $[Ca^{2+}]_i$ were recorded by loading the astrocytes with the Ca^{2+} indicator, Fluo-4. Stimulation with glutamate (30 µM) or the glutamate metabotropic receptor agonist, t-ACPD (150 µM), evoked an increase in $[Ca^{2+}]_i$ that shows an onset at ~20 s and a peak at ~60 s. In addition, NMDA (50 µM) or NMDA (50 µM) plus D-serine (100 µM) induced a Ca^{2+} response of a similar magnitude, but in this case, the peak was observed at ~20 s. Interestingly, the increase in $[Ca^{2+}]_i$ elicited by glutamate and t-ACPD was blocked by the NMDAR antagonist, DL-AP5 (50 µM). NMDAR were found to be expressed in astrocytes. Then, these results strongly suggest that activation of NMDAR is involved in the Ca^{2+} signal initiated by the stimulation of glutamate metabotropic receptors in the neurovascular coupling.

FONDECYT 1150530

69) **Hipercarbic acidosis induce ATP release from brainstem astrocytes in culture**

Olivares, María José[1]., Donoso, María Veronica[1].,Huidobro-Toro, Juan Pablo[1].,Llona, Isabel[1].,Eugenín, Jaime[1].,[1]Departamento de Biología, Facultad de Química y Biología, Universidad De Santiago De Chile. (Sponsored by Fondecyt Regular #1130874 Y #1141132)

Homeostatic regulation of breathing is achieved through feedback information provided by peripheral and central respiratory chemoreceptors. Peripheral chemoreceptors sense $O_2/CO_2/H^+$ in the arterial blood and central chemoreceptors CO_2/H^+ in the cerebrospinal fluid. In the brainstem, respiratory chemoreceptors are found, among other sites, in the retrotrapezoid nucleus (RTN), ventral respiratory group, solitary tract nucleus, ventral lateral medulla, medullary raphe, and prebötzinger complex. Gourine et al, 2010, found that ATP plays a fundamental role like gliotransmitter released by pH/CO_2 sensitive astrocytes in response to acidosis at the RTN. The increase in RTN neurons activity is attenuated by the presence of P2 receptors antagonists or apyrase. In the present work, we evaluated whether astrocytes from the brainstem are able of releasing other ATP derivatives together with ATP, like ADP, AMP and Adenosine (ADO). Release of purines was evaluated from brainstem and brain cortex astrocytes in dissociated cell cultures, when these were stimulated with hypercarbic acidosis (10% CO_2). Two days old CF1 mouse neonates were anesthetized (3% isofluorane), decapitated and their brains were extracted, disaggregated, and cultured in DMEM-F12 medium equilibrated with air containing 5% CO_2 at 37°C for 2 weeks. During stimulation, DMEM-F12 medium was replaced by artificial cerebro spinal fluid (aCSF) 3mM KCl. Astrocytes cultures were exposed at basal condition (5% CO_2) for 45 min, or at hypercarbic acidosis (10% CO_2) for 45 min at 37° C. Samples were collected at 5, 15, 30, and 45 min for the two conditions. The concentration of ATP and derivatives were measured using high-performance liquid chromatography (HPLC) technique. ATP released from brainstem astrocytes increased 3-fold during hypercarbic acidosis compared to the basal condition. ADP and AMP concentration increased during hypercarbic acidosis to the basal condition, but ADO concentration increased after the maximum release of ATP from brainstem astrocytes. In cortex astrocytes, it was not observed an increase of ATP or ADP induced by hypercarbia. AMP and ADO concentration are higher than ATP and ADP concentration in both conditions. AMP and ADO maximum concentration not appear to respond to hypercarbic acidosis, because it behaves the same way as the basal condition. Our results indicate that brainstem astrocytes, but not cortical astrocytes are able of release ATP as the main purinergic gliotransmitter in response to hypercarbia.



70) **The endothelial nitric oxide synthase isoform is present in neuronal synapses and lipid rafts.**

Caviedes, Ariel[2].,Nualart, Francisco[1].,**Wyneken, U[2].**, [1]Fisiología, Medicina, Universidad De Concepción.[2]CIB, Medicina, Universidad De Los Andes. (Sponsored by Fondecyt 1140108)

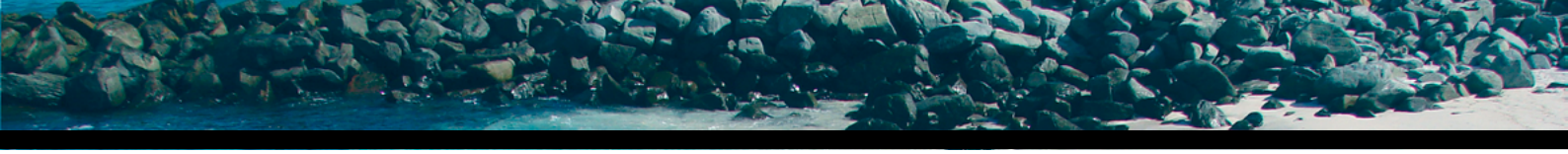
Nitric oxide (NO) modulates several processes in the central nervous system while its increase in oxidative stress conditions is associated to neuronal damage. Its synthesis in the brain has been most commonly been ascribed to the neuronal nitric oxide synthase (nNOS) isoform although the endothelial isoform (eNOS) has been implicated as a retrograde messenger in cellular plasticity. In endothelial cells, eNOS is associated to plasma membrane lipid rafts, specialized domains rich in cholesterol and sphingolipids that contain specific proteins like Thy1 and caveolin and provides a platform for multiprotein complex formation and signaling. A better characterization of the possible localization of eNOS in neurons would help to propose functional roles of the enzyme in the central nervous system. This should be performed in a cellular system devoid of endothelial cells. We thus studied the localization of eNOS by immunocytochemistry of primary hippocampal and cortical neurons and by Western Blots of subcellular fractions. Finally, cell viability was assessed with the Tripan blue test.

We show by confocal and super-resolution microscopy that eNOS co-distributes with post-synaptic markers (Shank2 and PSD95), but not with pre-synaptic markers, and is localized in dendritic spines. We also found that eNOS co-distributed with lipid raft markers. Moreover, eNOS is enriched in synaptic membranes and in postsynaptic densities isolated from neuronal cultures and from the rat forebrain. eNOS inhibition in cortical cells has a negative impact on cell survival after excitotoxic stimulation with NMDA. In turn, hippocampal neuronal death depends on nNOS-dependent NO synthesis and eNOS inhibition does not affect neuronal viability. Our results show that eNOS is located at excitatory synapses and in lipid rafts where it could represent a major source for NO production able to modulate synaptic function and neuronal survival.

71) **Transfer of Aldolase C containing exosomes from astrocyte to neurons induces morphological rearrengements in neurons.**

Luarte, Alejandro[1].,Ramirez, Juan Pablo[1].,Masalleras, Matias[1].,Villalobos, Isabel[1].,Varas, Manuel[1].,**Wyneken, U[1].**, [1]CIB, Medicina, Universidad De Los Andes.

Astrocytes are the most abundant glial cells in the central nervous system and they release several soluble factors (gliotransmitters) that modulate neuronal network connectivity. Furthemore, vesicles secreted from astrocytes into the extracellular space, such as exosomes, may also regulate synaptic activity by transferring lipids, proteins and microRNAs to neurons. We have found that the astrocyte specific glicolytic enzyme Aldolase C is released from astrocytes within exosomes that can be internalized by neurons. Endogenous Aldolase C and Aldolase C-GFP containing exosomes induced a dose-dependent decrease in neuritic arborization selectively in hippocampal cultured neurons. This effects could be explained, in part, by the effect of micro RNA miR-26a, that is highly enriched in exosomes and possibly associated to Aldolase C, as revealed by immunoprecipitation of Aldolase C followed by RT-qPCR. Morphological efects of exosomes on neurons are reverted by incubation with miR-26a antago (inhibitor) and partially replicated by miR-26a mimic. Our results show that astrocyte-to-neuron communication can be mediated by exosomes, affecting morphology, while the mechanisms involved in the regulation of neurotransmission are currently under study.



72) **Change in the position of the action potential initiation site in Granule Cells of the Dentate Gyrus during repetitive firing**

Palma-Espinosa, Javier[1]., Orio, Patricio[2].,Rojas, Patricio[1].,[1]Laboratorio de Neurociencias, Facultad de Química y Biología, Universidad De Santiago De Chile.[2]Centro Interdisciplinario de Neurociencia de Valparaiso Universidad De Valparaíso.

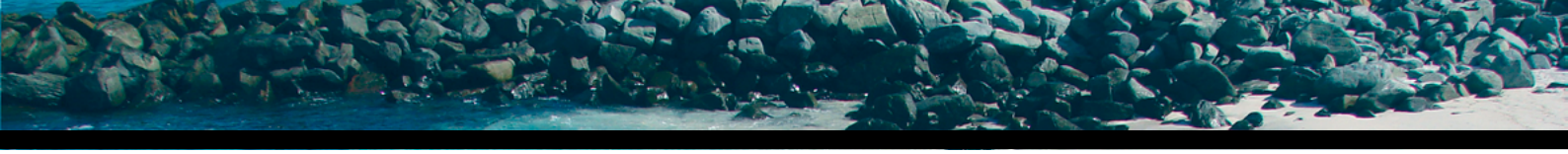
In Granule cells of the dentate gyrus, action potentials are generated in the Axon Initial Segment (AIS), which is located 20-40 µm from the soma, closer compared to other neurons. In layer V pyramidal neurons, where AIS is located 60-100 µm from the soma, two peaks appear in the second derivative of V_m, where the first peak correspond to action potential initiated at the AIS, and the second corresponds to the somatic action potential. Because of a closer initiation site in granule cells, the second derivative of single action potential does not show the usual two separate peaks corresponding to axonal and somatic initiations. However, in the latest action potentials within a successive train, two peaks appears in the second derivative, which is more evident as "hump" by inspecting phase plots. The apparition of the hump in the second derivative could be due to a movement of the initiation site away from the soma due to inactivation of voltage dependent sodium channels in the proximal AIS. In order to investigate this behavior, we used a computational model for granule cells that can reproduce features of single action potential (Schmidt-Hieber C. and Bischofberger J. J Neurosci. 30:10233-42), and modify it in order to reproduce features of spike trains. This model allowed us to explore several combination of values and position in the axon for several conductances, in order to explain the hump in the second derivative under repetitive firing.Our results suggest that action potential initiation in granule cells is a dynamic process that can account for plastic changes in firing properties by moving position and length of initiation site, as has been proposed for other brain structures.

73) **Control of neurovascular coupling by S-nitrosylation of astrocytic calcium homeostasis modulator 1 channel.**

Puebla, M[1]., Muñoz, MF[1].,Figueroa, XF[1].,[1]Physiology Pontificia Universidad Católica De Chile. (Sponsored by Fondecyt 1150530)

Brain function relies on the coordination of neuronal activity with cerebral blood flow, which is known as neurovascular coupling. Thereby, the increase in metabolic demand associated to an increment in synaptic activity is satisfied by the vasodilation of local parenchymal arterioles. The intercellular signaling that functionally connects neuronal activity with arteriolar vasodilation is mediated by an increase in astrocyte intracellular Ca^{2+} concentration, which propagates to the astrocytic end-feet and induces the release of vasodilator signals. Gap junction channels and hemichannels formed by connexins 30 (Cx30) and 43 (Cx43) coordinate this signaling process. As astrocytes express the Ca^{2+}-dependent nitric oxide (NO)-synthetizing enzymes eNOS and nNOS, and NO activates hemichannels, we evaluated if NO is involved in the control of neurovascular coupling. Hemichannel activity, changes in intracellular Ca^{2+} concentration and ATP release were evaluated in primary cultures of astrocytes and vasomotor activity of cortex arterioles was recorded in rat brain slices. Stimulation with glutamate or the glutamate metabotropic receptor agonist,1-aminocyclopentane-trans-1,3-dicarboxylic acid (t-ACPD), induced hemichannel activation, an increase in astrocytic Ca^{2+} concentration, ATP release and vasodilation of parenchymal arterioles. Blockade of hemichannels with the connexin blocking peptide $^{37, 43}$Gap27inhibited the increase in Ca^{2+} and vasodilation, but not ATP release, which was abolished by the calcium homeostasis modulator 1 (CALHM1) channel blocker, ruthenium red. Connexin hemichannel activity was also blocked by pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS), a purinergic receptor antagonist. Astrocytes related to parenchymal arterioles were found to express both eNOS and nNOS, and blockade of NO production with N^G-nitro-L- arginine (L-NA) abolished the glutamate- or t-ACPD-initiated astrocytic Ca^{2+} signaling, vasodilation and ATP release. In addition, glutamate also evoked the S-nitrosylation of CALHM1 channels. These results suggest that NO production plays a central role in the control of astrocyte-mediated neurovascular coupling through activation of CALHM1 channels by S-nitrosylation and the further opening of Cx43 hemichannel via ATP release and purinergic receptor activation.

FONDECYT 1150530



74) **Pannexin 1 is equally expressed in neurons, microglias and astrocytes of the lamina I-II of the spinal cord in normal and neuropathic rats**

Bravo, D[1,2]., Maturana, Carola[3].,Hernández, Alejandro[1].,Juan, Saez[3,4].,Constandil, Luis[1].,[1]Departamento de Biología, Facultad de Química y Biología, Universidad De Santiago De Chile.[2]Kinesiología, Facultad de Salud, Deporte y Recreación, Universidad Bernardo O`higgins.[3]Departamento de Fisiología Pontificia Universidad Católica De Chile.[4]Instituto Milenio, Centro Interdisciplinario de Neurociencias de Valparaíso Universidad de Valparaíso . (Sponsored by This Work Was Supported By Grant FB0807 From CEDENNA (LC And DB) And Grants From ICM-Economía P09-022-F (JCS And CJM).)

Pannexin 1 (Panx1) is a membrane glycoprotein, vastly expressed in the central nervous system (CNS) of mammals. It forms high conductance, ATP release channels related with several CNS diseases, such as stress, epilepsy, ischemia, neuroinflammation and, recently described, chronic pain. Although the participation of these channels in the signaling of chronic pain in the spinal cord have been demonstrated, and the presence of the Panx1 in total spinal cord has been reported, it remains unclear whether Panx1 is present in spinal areas related with pain transmission. These areas include the dorsal horn of the spinal cord, and specifically the Laminae I and II of Rexed, where nociceptive information from the peripheral nociceptive neurons is integrated to the CNS. This site is where the most important pathological neuroplasticity and glial activation are observed in chronic pain. Hence, we performed western blot analysis of Panx1 in homogenates of the posterior quadrant of the lumbar spinal cord, ipsilateral to a sural nerve lesion (NP rats, a pain model), and compared them to control rats. Furthermore, we performed immunofluorescence in spinal cord slices to locate Panx1 in neurons, microglia and astrocytes in Laminae I and II of Rexed of NP rats. Similar levels of Panx1 were detected in NP animals. Panx1 was localized in laminae I and II, in all tested cells of control rats, and its reactivity in neurons, microglia and astrocytes was similar in tissue of NP rats. Hence, Panx1 is constitutively expressed in the dorsal horn of the spinal cord in areas related with spinal signaling of pain, probably in acute and in chronic states. This constitutes the first evidence that Panx1 is expressed in the dorsal horn areas related with pain, opening new frontiers to pharmacological research in this topic.

75) **EXPRESSION OF UNCOUPLING PROTEIN 2 AND 3 DURING RAT SPERMATOGENESIS**

Paillamanque, Joaquin[3].,Carmona, Emerson[3].,Osses, Nelson[3]., Santibañez, Cristián[3].,Moreno, Ricardo[1].,Pino, Jose[3].,Bernales, Sebastian[2].,Gomez, Francisco[2].,**Reyes, Juan[3]**., [1]Departamento de Fisiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile.[2]Translational Research Group Fundación Ciencia y Vida.[3]Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica De Valparaíso. (Financed By Fondecyt 1140758)

One of the mechanisms associated to the regulation of spermatogenesis is an apoptotic process occurring in the first wave of spermatogenesis, where pachytene spermatocytes are removed by apoptosis, apparently to maintain an adequate relation of germ and Sertoli cells. Fas, mitochondria, pHi, $[Ca^{2+}]i$ and ROS play important roles in this process. Besides the classical mechanisms associated to mitochondria-mediated apoptotic events, uncoupling proteins (UCP) and their activities can also link mitochondria, ROS and cell apoptosis both by uncoupling oxidative phosphorylation and regulating ROS production. In this research, we explored the presence of UCP2 and UCP3 in rat testis and spermatogenic cells at different stages of postnatal development using q-PCR determinations of UCP2 and 3 mRNA, and UCP3 protein expression by Western blot, immunocytochemistry, and the arachidonic acid-induced and GDP inhibition of mitochondrial membrane potential decrease. Our results show differential kinetics of testis UCP2 and 3 levels during postnatal development. UCP2 mRNA increases up to 25 days of age, decreasing afterwards, while UCP3 only can be detected at 20 days increasing at least up to 55 days of age. Consistent with mRNA expression, UCP3 proteins can be detected in 25 and 60 days old rats, with some differences between puber and adult rats. In adult rat spermatids, arachidonic acid was able to induce a GDP-sensitive decrease of mitochondrial membrane potential, consistent with the functional expression of UCP2 and/or 3 in these cells.

76) **Role of pannexin hemichannels in calcium mobilization from intracellular stores of mice sperm during ATP induced acrosome reaction.**

Torres-Fuentes, Jorge[1]., Tomes, Claudia[2].,Saez, Juan[1].,Darszon, Alberto[3].,Treviño, Claudia[3].,Moreno, Ricardo[1].,[1]Fisiología, Ciencias Biológicas, Pontificia Universidad Católica de Chile.[2]Instituto de Histología y Embriología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo.[3]Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México. (Sponsored by FONDECYT 1150352 (RD.M))

During transit through the female genital tract, spermatozoa must undergo several biochemical and functional changes before they are ready fertilize the oocyte. These events prepare the sperm to undergo the reaction of acrosome (AR), an exocytic event that release of enzymes, necessary for fertilization. The AR could be induced by ATP or Progesterone and involves mobilization of extracellular and internal stores calcium. Pannexins (1, 2 or 3) are proteins that form hemichannels with the ability to be permeable to ions, involved both extracellular and internal stores calcium regulation. These proteins are present in mice spermatozoa, but their role in AR is unknown. The objective of this work was to characterize in vitro the participation of pannexin hemichannels in AR induced by ATP. Using spermatozoa obtained from wild-type and Panx1 knock-out mice, presence and localization were studied by Inmunofluorescence and Western Blotting. The AR was determined by coomassie blue staining, hemichannels functionality by ethidium uptake, calcium mobilization by single cell imaging with FLUO-3AM, and calcium store participation was studied with SLO toxin permeabilized sperm samples and cyclopiazonic acid. Results showed that ATP induces the AR along with a rapid calcium increase which was partially modulated by Panx inhibitors. This calcium increase is related with calcium mobilization from internal stores. These results show that pannexins are involved in calcium signaling from internal stores in AR induced by ATP in mouse sperm.

77) **Analysis of pachytene spermatocytes transcriptome treated with arachidonic and cyclopiazonic acid**

VASQUEZ, CLAUDIA[1]., ACEVEDO, CRISTIAN[2].,MORENO, RICARDO[3].,OSSES, NELSON[3].,REYES, JUAN[1].,[1]INSTITUTO DE QUIMICA, CIENCIAS, Pontificia Universidad Católica De Valparaíso.[2]CENTRO DE BIOTECNOLOGIA Universidad Técnica Federico Santa María.[3]CIENCIAS FISIOLÓGICAS, CIENCIAS BIOLOGICAS, Pontificia Universidad Católica De Chile. (Supported By FONDECYT 1140758 And FSM 12041 Mecesup Research Assistantship Scholarship.)

During mammalian spermatogenesis the presence of various stages of germ cells is correlated with different patterns of gene expression. Factors derived from Sertoli cells could be partly responsible for this coordinated gene expression in spermatogenic cells. One of the derivatives of the Sertoli cell, arachidonic acid (AA), stimulates an increase in $[Ca^{2+}]_i$ in rat and mouse spermatogenic cells *in vitro*. The consequences of AA and changes in $[Ca^{2+}]i$ on transcription in the germ cells is unknown. The goal of this study was to analyze the gene expression of mouse spermatocytes subjected to the effects of AA or cyclopiazonic acid (CPA), an ICaS release agent. Pachytene spermatocytes cells isolated and incubated with CPA or AA, both 4 µM, for 3 h. Subsequently, the cell samples were used to synthesize cRNA, which was hybridized to Illumina platform MouseRef-8 containing a total of 25697 sequences reference tests of mouse genome. A total of four independent cell preparations and experiments were analyzed. The data were normalized and expressed as fold changes (FC). We applied first, a filter of those sequences that were below the background level of the microplate reader, leaving 10532 valid sequences. Afterward, two separate data files, AA treatment and CPA treatment, were generated. Then the Log_2 of FC was applied to these sets of data and a t test was performed, selecting those sequences that had a p-value <0.05, leaving a total of 650 sequences for treatment with AA and 731 for CPA. Common sequences between these two treatments were 121. The latter were analyzed using the software MeV MultiExperimental Viewer, forming two major groups of sequences. The 121 genes were searched in the database Gene Ontology for biological processes in which these sequences would be classified. Thus, AA and changes in $[Ca^{2+}]i$ can regulate genes that are important for many processes associated to cellular component organization or biogenesis, localization, reproduction and metabolism of rodent spermatogenic cells.

78) **Role of glucose transporters GLUT1 and GLUT8 in proliferation and lactogenesis in murine mammary gland**

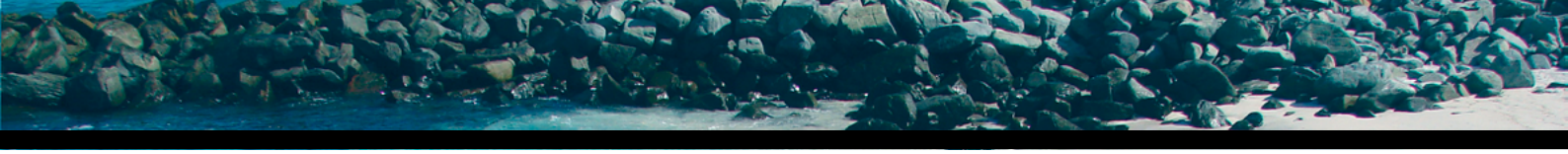
Villagran, Marcelo[1]., Muñoz, Katia[1].,Muñoz, Mirna[1].,Del Pozo, Reginald[1].,Mardones, Lorena[1].,[1]Ciencias Básicas, Medicina, Universidad Católica De La Santísima Concepción. (Sponsored by FONDECYT 11121367)

The mammary gland increases its energetic requirements during pregnancy and lactation to support the high rate of proliferation and the synthesis of milk nutrients. We studied the role of glucose transporters GLUT1 and GLUT8 in both processes in Balb/C mice. The temporal changes in the expression of both transporters were analyzed by western-blot, PCR, semi-quantitative immunoperoxidase and compared with temporal expression of proliferative marker Ki67 and lactalbumin (LALB), the regulatory subunit of lactose synthetase. GLUT1 was expressed weakly in over the 50% of the glands in all the period, although in early pregnancy more of the 60% of the alveolar cells were stained. Similar expression pattern for GLUT1 was found in whole protein extracts, reaching an increase of 4 fold in early pregnancy. For GLUT8, the percentage of stained gland increased from 20 to 80% during progression from pregnancy to lactation and an increase in intensity of GLUT8 stain was also observed. At mRNA level and in whole protein extracts, the increase reached 10 and 3 fold, respectively. Ki67 increased the expression in alveolar cells with the progress of the pregnancy and the lactation, from 20 to 100%, accompanied with an increase in the intensity of the staining, and LALB, that was not expressed in virgin mice, showed a similar pattern that GLUT8 in pregnancy and lactation, increasing in percentage of stained gland (20 to 60%), intensity of the staining, and expression by western-blot (3 fold). GLUT8, GLUT1 and LALB showed a granular intracellular expression and co-localized by immunofluorescence, meanwhile Ki67 stained showed only nuclear expression. The intracellular location of GLUT8 and the temporal expression of GLUT1 discard their participation in alveolar cells proliferation. The temporal expression of GLUT8 and its co-location with LALB suggest that GLUT8 but not GLUT1, is the transporter responsible of glucose entrance into Golgi supporting the lactose synthesis. The lactogenesis and the proliferation of mammary alveolar cell share a common pattern in this murine model.

79) **Pseudogenes and heat shock response for Rnf19a, an E3 ubiquitin ligase in spermatogenesis.**

Párraga, Mario[1]., Rejas, Carolina[1].,San Martín, Sebastián[1].,Villena, Juan[1].,Del Mazo, Jesus[2].,[1]Centro de Investigaciones Biomédicas, Medicina, Universidad De Valparaíso.[2]Laboratorio de Biología Molecular de la Gametogénesis Centro de Investigaciones Biológicas (CSIC). (Sponsored by Centro De Investiagaciones Biomédicas. CID 05/06)

Spermatogenesis is a complex terminal differentiation process, which occur at lower temperatures than other processes in the body. Regulation of spermatogenesis relies on molecular mechanisms, which monitor the progression of germ cell development. These are associated to degradation mechanisms, which eliminates aberrant spermatogenic and sperm cells. Such mechanisms involve evolutionary conserved proteolytic pathways, as it is the ubiquitination system. Alterations of these pathways or any of their components may lead to spermatogenic impairment and infertility as a consequence. One protein expressed during spermatogenesis and related to the conserved ubiquitination system is the E3 ubiquitin ligase Rnf19a. The objective of this work is to characterize *Rnf19a* genomic structure including the functionality of its promoter. To address this, we carried out computational sequence comparison analysis using different gene databases. These analyses showed that *Rnf19a* is present in organisms as diverse as worm mouse and human, accounting for its evolutionary conservation. This gene is located on chromosome 15 in the mouse. *Rnf19a* genomic structure is divided into 10 exons and 9 introns. Further analysis on the mouse genome showed different *Rnf19a* homologous sequences in a distinct chromosome that turned out to be pseudogenes. Other species analyzed also showed pseudogenes for *Rnf19a*. These were rat (*Rattus norvegicus*) and mosquito (*Anopheles gambiae*). However, no *Rnf19a* pseudogenes were found in neither human nor *C. elegans*. Specifically, we found three mouse pseudogenes in chromosome 9. These contain no introns and have several mutations along their sequence that disrupt the open reading frame. According to these characteristics we infer that they are processed pseudogenes. Analyzing upstream sequences from the gene and pseudogenes we identified their respective putative promoters. Within the promoter we found regulatory sequences such as TATA box, GC box and others. We also found some interspersed HSE sequences in those promoters. HSE sequences are characteristic of heat shock responding genes. Heat shock proteins are part of the conserved molecular pathways that in spermatogenesis function with the ubiquitination system as it has been described. We cloned these promoters for the gene and pseudogenes in EGFP containing plasmids and the function of these promoters were tested under normal and heat stressed conditions. Our results demonstrate that, *in vitro*, these promoters drive EGFP overexpression responding to heat shock. Further experiments analyzing both the response to heat shock and the expression of the gene and pseudogenes *in vivo* should be fulfilled.



80) **Development of a lentiviral vector system to study Andes virus entry and neutralization**

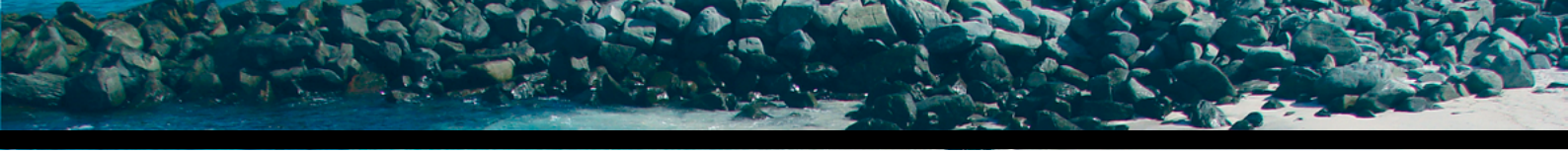
Varas, Nicolás[1]., Starck, Maria Francisca[1].,Beltrán, Camila[1].,Fernandez, Yaiza[1].,Sánchez , Oliberto[1].,[1]Laboratorio de Biofármacos recombinantes, Facultad de Ciencias Biológicas, Universidad De Concepción.

Andes virus (ANDV) is a Hantavirus that causes an acute disease called Hantavirus Pulmonary Syndrome (HPS). HPS is a highly pathogenic disease with a case-fatality rate of 40%. ANDV is endemic in Argentina and Chile and a total of 786 cases of HPS occurred during 1995–2012 in our country. Moreover, ANDV is the only Hantavirus reported to spread directly from human-to-human. For the study and the diagnostic of this virus is usually necessary to work with the highly infectious virus particles, what can only be done in high biosafety-level facilities. In the present study we develop a pseudovirion system based on Human Immunodeficiency Virus (HIV) vector pseudotyped with the ADNV-Gn/Gc envelope glycoproteins. This was done by replacing the gen for the G protein of Vesicular Stomatitis Virus (VSVG), present in a commercial lentiviral expression kit, for the gen of the Glycoprotein precursor protein (GPC) that is posttranslationally cleaved to form Gn and Gc. It is widely reported that Gn/Gc are anchored to the Golgi apparatus and that is where Hantavirus supposedly bud. On the other hand lentiviruses bud in the cell membrane. Here we present an indirect immunofluorescence assay that show that Gn and Gc can also be found in the cell membrane and thus allowing the pseudotyping of lentiviruses. The incorporation of Gn and Gc onto HIV-derived vector particles was assessed by western blot. In addition, we test it infectivity with various cell lines, including a HEK 293 constitutively overexpressing beta-3 integrin (HEK 293-ib3), a known receptor for Hantavirus. The pseudotyped lentivirus was able to infect a variety of cell lines, but in less amount that the common lentivirus vector. The use of HEK 293-Ib3 cause a 2-fold increase in its infectivity. Finally, sera of ANDV infected humans were able to block cell entry of the psedotyped lentivirus. The Gn/Gc pseudotyped HIV vector has several advantages, high titer vector production and easy quantification of cell infection by monitoring GFP reporter gene expression by flow cytometry. Such pseudotyped lentiviral vectors can be used to develop a quantitative and high-throughput pseudovirion assay to study early steps of ANDV cell infection, neutralization and screen for potential hantavirus cell entry inhibitors, all of this in biosafety level 2 facilities (BSL-2). This work was financed by grants FONDECYT 1110925 and VIUR 120015

81) **Study of cellular internalization mechanism of polyamidoamine dendrimers drug nanocarriers**

Vidal, F[1]., Vásquez, P[1].,Soto, R[1].,Díaz, C[2].,Alderete, J[2].,Guzman, L[1].,[1]Department of Physiology, Faculty of Biological Sciences, Universidad De Concepción.[2]Department of Organic Chemistry, Faculty of Chemical Sciences, Universidad De Concepción.
(Sponsored by FONDECYT 1131004)

In many cases pharmacological targets are intracellular components, which means that drugs must have the ability to enter the cell. However, they do not always have the property to cross the plasma membrane, a process that could be improved. Dendrimeric drug nanocarriers are chemical structures of diverse nature that contain, transport and deliver the desired drug in biological systems. One of the dendrimers that has been most successfully used is based on polyamidoamine (PAMAM). Their versatile architecture and easily modifiable surface allow to use them in different applications and make them the most promising nanocarrier systems. In general, their ability to enter the cell has been described and it has associated to endocytic mechanisms. Endocytosis is the process in which cell are able to internalize large molecules by forming vesicles from plasma membrane. Different endocytosis mechanism has been described, such as clathrin-mediated endocytosis, caveolae-mediated endocytosis and pinocytosis. In this work we are focus in determine the endocytic pathway that PAMAM dendrimers are internalized, understanding that their entry kinetics, intracellular distribution, association to organelles and drug release depend on this process. To study their cellular internalization, confocal microscopy technics were used and PAMAM dendrimers were marked with the fluorescent dye fluorescein isothiocyanate (FITC) which was linked to the amino groups of their surface. Their internalization was studied in two different cell types, HeLa cells and mouse hippocampal neurons. To determine the internalization pathway of PAMAM dendrimers co-localization analysis was performed with anti-clathrin, anti-caveolin-1 and anti-Rab-5 antibodies. Rab-5 is a small GTPase which is crucial for the early endosomal dynamics and is used as endocytosis marker. The co-localization observed with anti-Rab-5 in both cellular types suggest that PAMAM dendrimers are internalized by a classical endocytosis process. These results demonstrate that PAMAM dendrimers internalization involved endocytic pathways, but more studies are needed to determine specific details. Also, the studies would be centered on the neuronal cells because the relevance of the potential applications on pharmacology of the central nervous system.



82) **The behavioral effects induced by expression of a mutation for PINK and its relationship with dopaminergic neurons in *Drosophila melanogaster***

Molina-Mateo, Daniela[1]., Fuenzalida-Uribe, Nicolás[2].,Molina-Fernandez, Claudia[2].,Figueroa, Reinaldo[3].,Tevy, Florencia[3].,Campusano, Jorge[2].,[1]biologia celular y molecular, ciencias biológicas, Pontificia Universidad Católica De Chile.[2]biología celular y molecular Pontificia Universidad Católica De Chile.[3]Centro de Genómica y Bioinformática Universidad Mayor. (Sponsored by Fondecyt 1141233.)

Parkinson disease (PD) is a degenerative disorder associated with several motor symptomsincluding shaking, rigidity, slowness of movement and difficulty with walking, and is related to the death of dopaminergic neurons. More than 96% of PD patients also present olfactory dysfunction. Although the molecular mechanisms responsible for this disease are not clear it has been described that mutations in specific genes, including the PTEN-induced putative kinase 1 (PINK1), would be responsible for some cases of familial PD. In this work we describe the behavioral effects induced by expression of a mutation for PINK in *Drosophila* and its relationship with dopaminergic neurons.

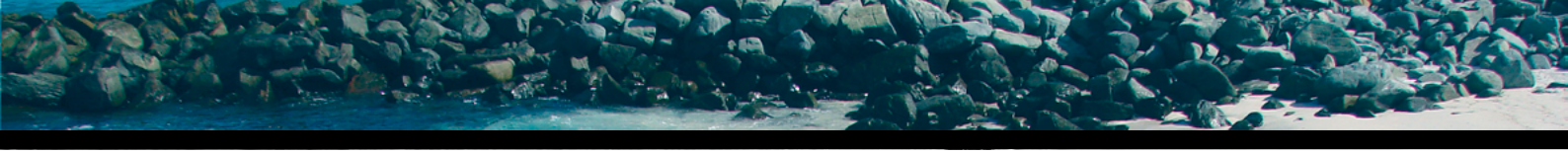
We used flies expressing a mutation for PINK1 and flies of the Canton-S strain as controls. In behavioral experiments single male flies were evaluated at different ages (0-3; 7-10; 14-17; 21-24 and 28-31 days old). Flies were placed in a circular arena with two cottons in opposite sides. Behavior of each fly was recorded for 3 min in absence of any stimulus. Afterwards, cottons were soaked with benzaldehyde 1% or distilled water and fly behavior was recorded for additional 3 minutes. Using the Buridan Tracker software we traced the position of flies. Olfactory discrimination (OD) was evaluated as odor Preference Index (PI). Several motor parameters were also measured. Additionally, we used immunofluorescent techniques to visualize dopaminergic neurons in young and old male flies.

It is possible to observe differences in PI between PINK and controls flies early on. In contrast, changes in different motor parameters are only evident as flies age, as expected. We are evaluating whether the number of dopaminergic neurons change in mutants for PINK, although preliminary results show no change in young versus old animals.

83) **Muscarinic Acetylcholine Receptors Contribute to Aversive Olfactory Learning in Drosophila**

Molina-Fernández, Claudia[1]., Silva, Bryon[1].,Ugalde, María Beatriz[1].,Campusano, Jorge M[1].,[1]Departamento de Biología celular y molecular, Facultad de ciencias biológicas, Pontificia Universidad Católica De Chile. (Sponsored by Supported By Fondecyt 1141233)

The most studied form of associative learning in *Drosophila* consists in pairing an odorant, the conditioned stimulus (CS), with an unconditioned stimulus (US). The timely arrival of the CS and US information to a specific *Drosophila* brain association region, the mushroom bodies (MB), can induce new olfactory memories. Thus, the MB is considered a coincidence detector. It has been shown that olfactory information is conveyed to the MB through cholinergic inputs that activate acetylcholine (ACh) receptors, while the US is encoded by biogenic amine (BA) systems. In recent years, we have witnessed an important advance in our understanding on the specific neural BA pathways and receptors involved in olfactory learning and memory in flies. However, little information exists on the contribution of cholinergic receptors to this process. Here we evaluate for the first time the proposition that, as in mammals, muscarinic Ach receptors (mAChRs) contribute to memory formation in *Drosophila*. Expression studies show that mAChRs are expressed in the MB. Our behavioral data show that pharmacological and genetic blockade of mAChRs in MB disrupts the formation and retrieval of olfactory aversive memory in larvae. This effect is not explained by an alteration in the ability of animals to respond to odorants or to execute motor programs. These results show that mAChRs expressed in MB contribute to the generation of olfactory memories in *Drosophila* larvae.



84) **N-3 PUFAs supplementation increases hippocampal neurogenesis and improves memory of stressed rats**

Peñaloza Sancho, Valentín[1]., Dagnino-Subiabre, Alexies[1].,[1]Laboratory of Behavioral Neurobiology, Center for Neurobiology and Brain Plasticity, Institute of Physiology, Faculty of Sciences, Faculty of Sciences, Universidad de Valparaíso. (Sponsored by FONDECYT Grant N° 1141276 To AD-S Labsite: Www.stress.cl)

Introduction: Neurogenesis in the hippocampus is key for learning and memory. Chronic stress reduces hippocampal neurogenesis and impairs memory, while omega-3 polyunsaturated fatty acids (n-3 PUFAs) enhances learning in rats.

Objective: The aim of this study was to evaluate, in the same stressed rats, the effects of n-3 PUFAs supplementation on hippocampal neurogenesis, learning and memory consolidation.

Methods: Male *Sprague–Dawley* rats were randomly assigned to unstressed and stressed (chronic restraint stress) experimental groups. Afterward, animals were supplemented with n-3 PUFAs (DHA and EPA mix) or water. Neurogenesis was evaluated with 5-bromo-2 desoxiuridina (BrdU) and quantified by immunohistochemistry. Learning and memory were analyzed by the Morris water maze.

Results: Stressed rats that were supplemented with n-3 PUFAs showed higher levels of neurogenesis in the dentate gyrus of the hippocampus. This result was correlated with an improvement in the memory consolidation respect to non-supplemented animals.

Conclusions: We speculate that n-3 PUFAs supplementation could be used in the treatment of stress-related psychiatric disorders where patients have affected the hippocampus, such as major depression.

Support: This work was funded by FONDECYT Grant N° 1141276 to AD-S. Labsite: www.stress.cl.

85) **Interactions between adrenergic activity and glucocorticoids in the Insular Cortex modulate arousal-induced taste Neophobia**

Mendez, Luis[1]., Jeréz Baraona, Juan Manuel [1].,Rojas, Sebastián[1].,Díaz-Galarce, Raul[1].,Quintana-Donoso, Daisy[1].,Moraga-Amaro, Rodrigo[1].,Stehberg, Jimmy[1].,[1]Laboratorio de Neurobiología, Centro de Investigaciones Biomédicas, Universidad Andrés Bello.

Glucocorticoids (GCs) are steroidal stress hormones released from the adrenal glands in response to the activation of the hypothalamic-pituitary-adrenal axis. Previous studies in humans have shown that systemic GCs may have paradoxical effects, acting as both anxiolytic and anxiogenic, but no mechanism or brain area has been suggested to mediate such paradoxical effects. Here we studied the role of glucocorticoids at the Insula in the reluctance to novel tastes (taste Neophobia), which can be exacerbated by stress and high arousal contexts (HA), and is used as a measure of anxiety. The present results suggest that glucocorticoids in the insular cortex modulate taste neophobia and arousal-induced increases in taste neophobia. Interestingly, intra-insular corticosterone injections induced increases in neophobia at low doses and decreases in neophobia at high doses. Furthermore, intra-insular glucocorticoids restored the behavioral effects of systemic blockage of GC synthesis, and showed anxiogenic or anxiolytic effects depending on concentration and on previous adrenergic activation. The effects of intra-insular corticosterone showed to be paradoxical not only in taste neophobia, but also in the elevated plus maze, suggesting that the Insula is an important site mediating the effects of GCs in anxiety, and that GCs may have anxiolytic or anxiogenic effects when acting at the Insula.

Funded by Proyecto FONDECYT N° 1130724

86) **Dopamine Receptor type 5 knockout mice (D5RKO) show memory impairments but normal affective behavior.**

Moraga, Rodrigo[1]., González, Hugo [3].,Rojas , Patricio [2].,Ugalde , Valentina[3].,Pacheco, Rodrigo[3].,Stehberg, Jimmy[1].,[1]Laboratorio de Neurobiología, Centro de Investigaciones Biomédicas, Universidad Andrés Bello.[2]Laboratorio de Neurociencias. Departamento de Biología, Facultad de de Química y Biología Universidad De Santiago De Chile.[3]Laboratorio of Neuroinmunología Fundación Ciencia & Vida.

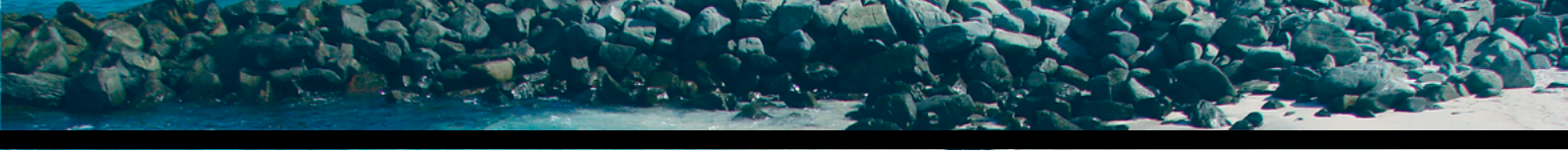
The Dopamine D1 receptor family which includes D1 and D5 receptors (D1R and D5R) has been shown to be important for learning and memory in a variety of learning tasks, brain areas and animal species. Further analyses using genetic approaches have corroborated the relative contribution of D1R in these cognitive tasks, but the relative contribution of D5R in learning and memory remains unclear due to a lack of studies using genetic approaches and the unavailability of drugs that can discriminate between D1R and D5R. A few studies have reported limited evidence suggesting that D1R but not D5 receptors may be important for memory, but studies testing D5R knockout mice (D5RKO) on memory paradigms are lacking. The present study was designed to determine whether the D5R is involved in memory by testing D5RKO mice in a battery of behavioral tests. D5RKO mice showed unaffected affective behavior, showing no depressive-like symptoms. They also showed significant impairments in spatial memory using the Morris watermaze, but normal working memory and unaffected object recognition memory. Electrophysiological analyses performed in D5RKO mice hippocampal slices showed significant deficits in long-term-potentiation. Further analyses at the molecular level showed that genetic deficiency of D5R results in a significant down-regulation of the hippocampal NMDA receptor subunit NR2B. These findings demonstrate a role for D5R in memory and suggest a functional interaction between D5R and hippocampal glutamatergic pathways involved in synaptic plasticity.

Funded by Proyecto FONDECYT n° 1130724

87) **Adrenergic transmission in the modulation of arousal-induced reluctance to try novel tastes by the insula in the rat**

Rojas, Sebastian[1]., Diaz Galarce, Raul[1].,Jerez Baraona, Juan Manuel[1].,Quintana Donoso, Daisy[1].,Moraga Amaro, Rodrigo[1].,Stehberg, Jimmy[1].,[1]Laboratorio de Neurobiologia , Centro de investigaciones Biomédicas, Universidad Andrés Bello.

Reluctance to try novel tastes (taste neophobia) is a common adaptive behavior that ensures a cautious response to a novel taste until its safety has been ascertained. However, neophobia is significantly increased when the novel taste is presented in a high arousal context (HA), compared to when the novel it is presented in a low arousal context (LA). This increased reluctance to try novel tastes induced by arousing contexts is used to measure anxiety in rodents and depends on the adrenergic system. To determine whether adrenergic activity at the insula regulates arousal-induced increases in reluctance, a combination of systemic and intra-insular manipulations of adrenergic activity was performed before the presentation of saccharin 0.1% as a choice to water, either in a HA or LA context. Bilateral intra-insular microinjections of norepinephrine or the non-selective beta blocker propranolol were found to modulate the effects of arousing contexts on reluctance to try novel tastes. Moreover, systemic effects of oral propranolol were blocked by intra-insular administration of norepinephrine, while intra-insular propranolol blocked epinephrine- induced reluctance to novel tastes. In conclusion, these results suggest a critical role for adrenergic activity at the Insula in regulating the effects of arousal in the reluctance to try novel tastes. Funded by Proyecto FONDECYT N° 1130724



88) **Phenylalanine variability as a determinant factor during neurodevelopment. Outcomes in higher cognitive functions**

Santander, Daniela[2]., De La Parra, Alicia[1].,Castro , Gabriela[1].,Arias, Carolina[1].,Cabello, Francisco[1].,Cornejo, Veronica[1].,Ossandón , Tomás[2].,[1]Laboratorio de Genética y Enfermedades Metabólicas, I.N.T.A., Universidad De Chile.[2]Departamento de Psiquiatría, Facultad de Medicina y Centro Interdisciplinario de Neurociencia, Pontificia Universidad Católica De Chile.

Phenylketonuria (PKU) patients who have been correctly treated display mild deficits in cognitive functions like attention and working memory, which emulate the symptoms observed in attention deficit hyperactivity disorder (ADHD). In both PKU and ADHD the underlying cause of cognitive symptoms may relate to a deficit in catecholamines, which are products of tyrosine metabolism. We compared the cognitive performance between PKU and ADHD children, and established a quantitative relationship between Phe means and variability levels and high-order cognitive functions in PKU patients. Clinical data from 129 early-treated PKU patients with no concomitant ilnesses were analyzed (age range: 6 months - 15 years), sixty ADHD patients and sixty controls. As result, indicators of psychomotor, mental development, and IQ data are negatively correlated with mean phenylalaninemia levels and variability, especially starting from the 24th month since birth. Although age and IQ are uncorrelated, the IQ drops with age. Both groups show worse verbal IQ results than performance IQ, and a low score in a digit retention subtest. We concluded that even though phenylalaninemia levels are universally accepted as the most relevant indicator to assess the success of PKU treatment, our results highlight the importance to maintain stable levels throughout as much at the neurodevelopment stage as at the rest of a patient's life.

89) **Mechanisms of autonomic regulation during social cognition task**

Varas, G[1]., Maldonado, Pedro[2].,[1]Fisiología, Medicina, Universida de Chile.[2]Fisiologia Universidad De Chile.

The perception, interpretation and generation of responses to the intention and behaviors of others are known as social cognition (3). The recognition of facial expressions and the ability to infer the likely mental states of other people are an important feature of social cognition, this ability is called Theory of Mind. The emotions that humans experience while interacting with their environment are associated with varying degrees of physiological arousal (2). A key system involved in the generation of this physiological arousal is the autonomic nervous system (ANS). Heart rate variability (HRV) analysis is emerging as an objective measure of regulated emotional responding, and functions related to social cognition and Theory of mind. The polyvagal theory and the Neuro visceral integration model proposes that the ANS, through vagal tone activity and activity of the prefrontal cortex, improves the interactions of a subject with their environments through an inhibitory effect on the sino-atrial node (pacemarker) (1). There is evidence that, at rest, subjects with spinal cord injury (SCI) have a predominance of sympathetic autonomic activity which correlates with low HRV (4). Our hypothesis proposes that this type of basal activity of the ANS decrease autonomic flexibility that has been described as favorable for social cognition tasks. We measured HRV, as autonomic marker, in 18 healthy subjects and 10 subjects with SCI, diagnosed with paraplegia, who were pursuing a period of adaptation and socio-labor integration. A 5 min. quiet sitting period at the beginning of the assessment was used to collect baseline HRV. Than HRV was measured during performance of the The Reading the Mind in the Eyes Test (RMET), which assesses the affective component of the theory of mind. Based on our results it was observed that the group of subjects with SCI had a worse performance in the test ($p=0.001$), a significantly lower level of security on responses compared with the group of healthy people ($p=0.002$), lower HRV at rest($p=0.005$), and a smaller increase in the HRV during the task relative to the baseline condition (0.007). These results suggest that there be alterations in social cognition, in subjects with SCI, diagnosed with paraplegia, who were pursuing a period of adaptation and socio-labor integration. Our results also confirm a positive correlation between limitations in autonomic flexibility and worse performance in social cognition tasks.

90) **Dopamine D4 receptor of the prelimbic cortex is important to the expression of innate fears in rat**

Vergara, M[1]., Gysling, Katia[2].,Fuentealba, José[3].,[1]Biología Celular y Molecular, Ciencias Biológicas, Pontificia Universidad Católica De Chile.[2]Biología Celular y Molecular, Biología Celular y Molecular, Pontificia Universidad Católica De Chile, Biología Celular y Molecular, Ciencias Biológicas .[3]Quimica y Farmacia, Quimica y Farmacia, Pontificia Universidad Católica De Chile.

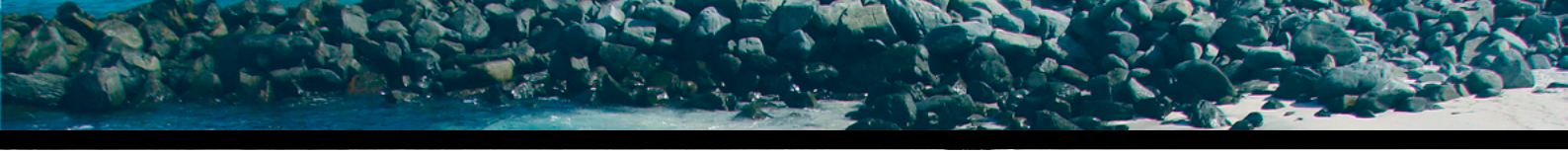
The aversive memories are a type of memory very important in the generation of appropriates behaviors and the decision making of an individual. Dopamine plays a key role in the regulation of aversive memories in the medial prefrontal cortex (mPFC). The facing of innate aversive stimuli (footshock or predator odor) increases dopamine release in PFC. The possible mediator in this response is the dopamine D4 receptor (D4R), which is highly expressed in the medial prefrontal cortex and several evidence suggests that this receptor would be the molecular target by which dopamine exerts itsactions related to aversive memories. In the present study we characterize the role of the D4R in the prelimbic cortex (area of the mPFC related whit the expression of emotional memories). For this propose, we perform a bilateral infusion in the prelimbic cortex of L-745,870, a selective antagonist of the D4R, using the cat odor paradigm. The infusion of L-745,870 significantly decreased the innate fear behaviors (number and time that rats expressed freezing behavior), associated to the presence of cat odor. Also, CamKII shows, in preliminary data, an increase in phosphorylation associated with the infusion of the L-745,870.

Our data show that D4R in prelimbic area of the mPFC plays a role in the expression of innate fear behavior.

91) **Visual sensory response is differentially affected by the representational format of self-generated thoughts**

Villena-González, Mario[1]., López, Vladimir[1].,Rodríguez, Eugenio[1].,[1]Psicología, Ciencias Sociales, Pontificia Universidad Católica De Chile. (Sponsored by This Project Was Supported By A PhD Fellowship From CONICYT-PCHA/Doctorado Nacional/2014-21140290)

Mind wandering has been studied mostly as a unitary “off task” attentional state, giving little importance to its phenomenological content. Nevertheless, differences in representational format of thoughts, such as visual imagery or inner speech, might affect the sensory processing of external stimuli. We recorded the brain activity of 20 participants (12 women) while they were exposed to a probe visual stimulus in three different conditions: executing a task on the visual probe, generating inner speech, and performing visual imagery. Event-related potentials results showed that the amplitude of early P1/N1, related with sensory response, was significantly attenuated during the visual imagery condition. Additionally, spectral analyses showed that alpha’s power in visual areas was higher when participants engaged in visual imagery than in the other two conditions. Furthermore, an N400-like negativity, usually related with language processing, was clearly larger during the inner speech condition. Our results show, for the first time to our knowledge, that cortical resources allocation to external stimuli during self-generated thoughts is differentially affected by the representational format of the ongoing train of thoughts.



92) **Do I switch tasks better when I feel well?**

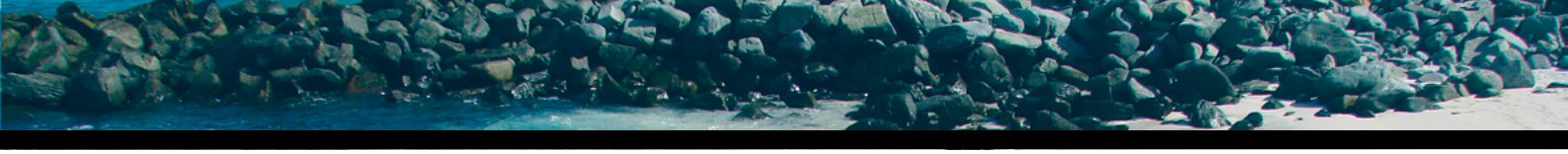
Vasquez-Rosati, Alejandra[1].,López, Vladimir[1].,Cosmelli, Diego[1].,[1]Escuela de Psicología Pontificia Universidad Católica De Chile.
(Sponsored by CONICYT 21120514)

Imagine a scenario where you are writing a mail and as someone comes into the office and starts talking to you, or the phone begins to ring. You would have to stop what you are doing and start responding to the changing demands, switching between different stimuli, operations and mental sets. This ability is known as cognitive flexibility. Now, add to this scenario a strong emotional atmosphere that invades you. How you would behave? Recent findings suggest that emotional states do modulate cognitive flexibility, but these findings are still controversial. Moreover, there is no evidence of the underlying brain processes. The purpose of the present study was therefore to examine such interaction while monitoring changes in ongoing cortical activity using EEG. We hypothesized that positive emotional states that promote a general feeling of openness (open stance) would facilitate cognitive flexibility. Conversely, negative emotional states that promote a general feeling of retreating inwardly (closed stance) would hinder it. Such effects should be detected as a change in switch cost as measured by reaction time (RT) and error rates, when comparing repetition versus switch trials. In order to answer this question, we used two musical stimuli to induce emotional states (positive/high arousal/open stance and negative/high arousal/closed stance). Fourteen participants performed first 2 blocks of the Madrid Card Sorting Task (MCST) in a neutral silence condition and then 4 blocks while listening to the counterbalanced musical stimuli. Our results show, first, a training effect that is observable already during the silent condition. Switch costs of the second block were smaller than those of the first block. Second, in the first block of emotional conditions, we found that compared to the positive stimuli, negative stimuli decrease RT and errors for the first shift signal. Our data shows also that the valence of the first emotional block is determinant in the RTs of the subsequent blocks. When the stimulus valence of the first block is negative, the error rate increases in the subsequent blocks as compared to the positive stimuli. Our results suggest that the first impression made by negative emotions helps participants focus on the task initially. However, in the long term, it promotes impulsivity when compared with positive emotions. This findings show that the interaction between emotion and cognitive flexibility is more complex than previously thought and points a new way of understanding these processes.

93) **Effect of color on long term memory for faces.**

Osorio, Marcela[1]., Yañez, Rodrigo[2].,Toledo, Pedro[3].,Rodríguez, Eugenio[1].,[1]Escuela de Psicología, Facultad de Ciencias Sociales, Pontificia Universidad Católica De Chile.[2]Instituto de Economía, Facultad de Ciencias Económicas y Administrativas, Pontificia Universidad Católica De Chile.[3]Departamento de Electrónica Universidad Técnica Federico Santa María. (Sponsored by Acknowledgements To Vicerrectoría De Investigación Pontificia Universidad Católica, Concurso De Investigación Pregrado Invierno 2015.)

Memory is a cognitive function involving the encoding of new information, which is modulated by various factors influencing the process of encoding and subsequent recall of information. We are conducting an online experiment of facial recognition to assess the effect of color filters in the encoding process. Using facial recognition allows us to control for most of the confounding factors of the recognition task, such as own-age bias (OAB), distinctiveness and hometown effect, letting us to isolate the effect of the color in the encoding process. The experiment is divided in two consecutive day sessions of 4 minutes. During the first session, the subjects are exposed to 36 images of faces for 5 seconds each one, which are separated by a black transition of 1 second. All the images have been previously edited in gray scale, but one third of them is presented with a red filter, one third is presented with blue filter, and the remaining third is presented without color filter. The next day, in a second session, we present 36 images, all in gray scale. One third of the images were presented the first day, meanwhile the remaining two thirds are new images. The task consists in recognize the faces that were effectively presented the first session. We will analyze the effect of color filters in the recognition of faces after a second presentation of the pictures in gray scale using the hit and false alarm difference in means test for the different filters, and by multiple binomial regressions. We expect that the results will reveal an improvement tendency to the recognition of faces initially presented with the red filter.



94) **Chronic stress impairs decision-making and attention in adolescent rats**

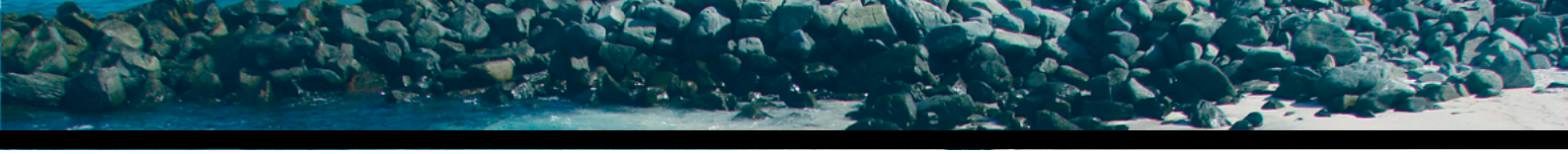
Ovando, Marcela[1]., Dagnino-Subiabre, Alexies[1].,[1]Laboratory of Behavioral Neurobiology, Center for Neurobiology and Brain Plasticity, Institute of Physiology, Facultad de Ciencias, Universidad De Valparaíso. (Sponsored by This Work Was Funded By FONDECYT Grant N° 1141276 To AD-S. Labsite: Www.stress.cl)

Medial prefrontal cortex (mPFC) regulates decision-making and attention. This brain area is highly sensitive to chronic stress during adolescence. Therefore, the aim of this study was to determine whether chronic stress affects decision-making and auditory attention in adolescent rats. Both cognitive functions were quantified by two-alternative choice task (2-ACT), a behavioral paradigm to study auditory attention in rats. Trained animals, that reached a performance over 80% of correct trials in the 2-ACT during adelescence, were randomly assigned to control and restraint stress experimental groups. To analyze the effects of restraint stress (7 days/3 hrs) on decisión-making and auditory attention, trained rats of both groups were subjected to 50 2-ACT trials one day before and one day after of the stress period. A different score was determined by substracting time of intertrial interval (ITI) and the number of correct trials (CT) after from those before the stress protocol. Locomotor activity (open field, OF) and anxiety (elevated plus maze, EPM) were evaluated in all animals. Chronic stress did not affect locomotor activity, while the number of CT in the 2-ACT was lower in the stressed rats than that of control animals. On the other hand, ITI and anxiety were higher in the stressed rats compared to the performance of control animals. Our results suggest that other cognitive functions regulated by the limbic system, such as cognitive flexibility, could be affected by the stress-induced impairment on decision-making and auditory attention.

95) **Changes on growh hormone expression in gills, liver, kidney, intestine and pituitary during smoltification in *Salmo salar***

Morera, FJ[1]., Vargas-Lagos, C[2].,Pontigo, JP[2].,Oyarzun, R[2].,Yañez, A[3].,Vargas-Chacoff, L[2].,[1]Instituto de Farmacologia, Ciencias Veterinarias, Universidad Austral De Chile.[2]Instituto de Ciencias Marinas y Limnologicas, Ciencias , Universidad Austral De Chile.[3]Instituto de Bioquimica y Microbiologia, Ciencias , Universidad Austral De Chile. (Sponsored by Funding By INNOVA-Corfo 13IDL2-23565 (to LVC, FJM And AY); Fondap-Incar, No. 15110027 (to AY), FONDECYT De INICIACION 11130308 (to FJM) And DID-UACH (to FJM))

The smoltification is a critical point in the Salmon Aquaculture. During this process, also called parr-smolt transformation, fish suffer many changes under endocrine control. Growh hormone (GH) is a hormone considered of the same family of Prolactin (PRL) and Somatolactin (SL), because of their structural similarities. The aim of this work is to describe the Growh hormone expression during the process of smoltification (induced by photoperiod). We took samples of tissues as gills, kidney, liver, intestine (3 portions: anterior, mid and posterior) and pituitary of *Salmo salar* in 2 points of smoltification process: 1) parr, 2) smolt. We analysed the expression of GH gene by RT-Q-PCR. In gills, kidney, posterior intestine and pituitary the gene expression of GH significantly increased during smoltification process, while anterior and mid intestine do not presented statistical changes during this process. We found that the levels of GH increased during smoltification process in several tissue, although the intestine in two portion do not presented changes, being the posterior portion more important in this process. These data suggest that GH plays a relevant role in the osmoregulatory tissues during the smoltification process in *S. salar*, but further functional studies are necessary.



96) **Regulation of the expression of the (pro)renin receptor by angiotensin II in renal collecting duct cells.**

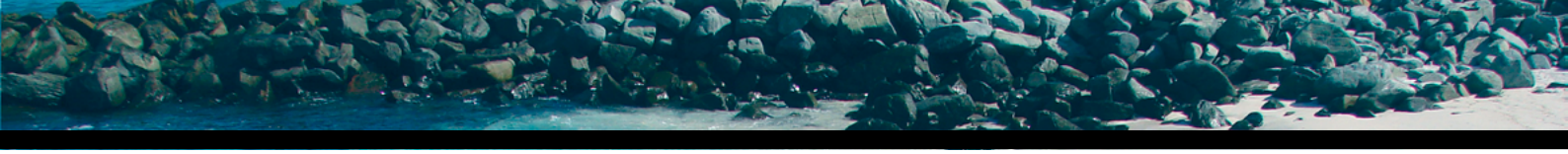
Reyes-Martínez, Cristian[1]., Salinas-Parra, Nicolás[1].,Gonzalez, Alexis A[1].,[1]Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica De Valparaíso. (Sponsored by FONDECYT 11121217)

The (pro)renin receptor (PRR) is an ATPase H^+-transporting lysosomal accessory protein (*ATP6AP2*) that also acts as a receptor of inactive prorenin and renin activating prorenin and increasing renin activity. Besides, it is involved in a series of different processes, such as Wnt-signaling and pH homeostasis, its activation has been related to renal injury in hypertension and diabetes. Binding of prorenin and renin to the PRR activates mitogen-activated protein kinases (MAPK) and induces transforming growth factor (TGF-β). It has been suggested that the expression of renal PRR is upregulated by cGMP-PKG signaling pathway under low-sodium conditions *in vivo.* We have shown that PRR is expressed in collecting duct (CD) cell line M-1 and that angiotensin II (Ang II) increases the expression of PRR in this cells. However, the molecular mechanism by which Ang II modulates the expression of PRR in CD cells remains unknown. We hypothesized that Ang II-mediated increase in PRR is mediated by the activation of protein kinase A (PKA) and/or protein kinase C (PKC). In order to prove it, we treated CD cells with Ang II plus PKA or PKC inhibition using H89 and calphostin C, respectively. Our results showed that the increase in PRR protein levels mediated by Ang II are prevented by PKC and H89 independently. Furthermore, forskolin (cAMP enhancer) augments PRR however PKC inhibition prevented this effect in forskolin treated cells. Our results suggest that the action of Ang II is modulated by PKA and PKC.

97) **Activation of E-Prostanoid Receptor EP1 and EP4 regulates renin expression in renal collecting duct cells**

Salinas-Parra, Nicolás[1]., Gonzalez-Vergara, Alex[1].,Figueroa, Steffany [1].,Gonzalez, Alexis A[1].,[1]Instituto de Quimica, Facultad de Ciencias, Pontificia Universidad Católica De Valparaíso. (Sponsored by FONDECYT 11121217)

Prostaglandin E2 is the major metabolite produced by cyclooxygenase-2 in the kidney and exerts its effects via G protein-coupled receptors EP1 and EP4 in the renal collecting duct (CD) cells where it has been demonstrated all components of the renin-angiotensin axis are expressed. In this study we evaluate the effect of the activation of prostaglandin E2 receptor EP1 and EP4 on renin expression and molecular pathways involved using a CD cell line M-1. Our results demonstrate that the EP1 receptor is found in greater extent in M-1 cells, while EP4 is less abundant (0.667 arbitrary units (AU) v s. 0.304 AU). A dose response curve with different concentrations of prostaglandin E2, showed an increase in the expression of renin in the nanomolar range (132 ± 43% over the control). EP1 is a protein kinase C coupled receptor and EP4 is coupled to Gs protein, both receptors may activate PKC and / or PKA. To establish the role of PKA activation by cyclic AMP (cAMP) and its possible dependence of PKC, we increased cAMP levels with Forskolin (10-6 Molar) in the presence or absence of Calphostin C (10-7 Molar), a PKC inhibitor. The Western blot analysis showed that forskolin increases the expression of renin (135 ± 18% compare to control, P<0,05) and prorenin (136 ± 22% compare to control, P<0.05) protein levels while forskolin plus PKC inhibition suppresses this effect. These results suggest that increased renin and prorenin protein levels mediated by the activation of cAMP/PKA pathway is dependent on PKC activity.



98) **Altered cortical actin polymerization in dysferlin-deficient skeletal myocytes**

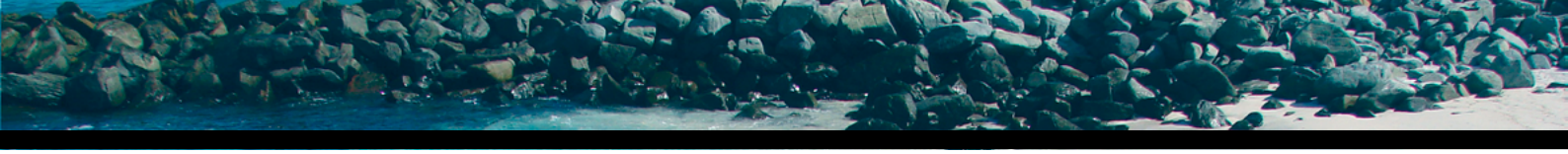
Báez, Ximena[1]., Gonzalez, Arlek[1].,Cea, Luis[2].,Bevilacqua, Jorge A[3].,Mouly, Vincent[4].,Caviedes, Pablo[5].,Cárdenas, Ana María[1].,[1]Centro Interdisciplinario de Neurociencias Universidad De Valparaíso.[2]Programa de Anatomía y Biología del Desarrollo, Instituto de Ciencias Biomédicas Universidad De Chile.[3]Programa de Anatomía y Biología del Desarrollo, Instituto de Ciencias Biomédicas. Universidad De Chile.[4]UM76 - UPMC Univ. Paris 6 / U974 - Inserm / UMR72 47, bld de l´Hôpital - G.H. Pitié-Salpétrière Paris Universidad Paris .[5]Programa de Farmacología Molecular y Clínica, ICBM. Universidad De Chile. (Sponsored by This Work Has Been Supported By Grants Anillo ACT-1121.)

Dysferlinopathies are a group of muscular dystrophies caused by mutations affecting the expression of dysferlin (Dysf), a protein highly expressed in skeletal muscle and essential for sarcolemma repair. Reportedly, animal models of dysferlinopathies display a deregulated expression of proteins involved in actin cytoskeleton dynamics. We therefore hypothesize that Dysf participates in the remodeling of the actin network. To address this possibility, we evaluated whether dysferlinopathy-associated mutations modify actin remodeling in muscle cells. With this aim, we used myoblasts of cell lines derived from skeletal muscles of patients harboring Dysf mutations (ER, AB320, 107 and 379 cells). As previously reported, all these mutations severely reduce the expression of dysferlin. In parallel, we used RCMH myoblasts, a cell line derived from skeletal muscle of a normal human patient as a control. Actin polymerization was determined by permeabilizing cells with digitonin in the presence of 2 mM ATP-Mg^{2+}, 10 mM free Ca^{2+} and 0.3 mM Alexa Fluor 488 G-actin conjugate. Since only actin polymers exhibit fluorescence, the fluorescence intensity reflects the formation of new actin filaments. The expression and overall organization of F-actin was evaluated by staining the cells with phalloidin rhodamine-B. We found, compared to control RCMH cells, that dysferlin-deficient myoblasts (ER, AB320, 107, 379) display a significantly reduced F-actin polymerization and expression. To further evaluate the role of Dysf in F-actin polymerization, we expressed a dysferlin-Venus construct in all dysferlin-deficient cell lines. We found that the expression of this construct restores actin polymerization in either the mutant cell lines. Altogether, these results suggest that Dysf is involved in F-actin polymerization in skeletal myoblasts, and that this process is impaired in dysferlinopahies.

99) **Effect of metformin during gestation in obese rats on reproductive and metabolic parameters in offspring**

Olguín, Sofía[1]., Alvarez, Daniela[1].,Ceballo, Karina[1].,Cerda, Tania[1].,Cruz, Gonzalo[1].,[1]Laboratorio de Alteraciones Reproductivas y Metabólicas, Instituto de Fisiología, Facultad de Ciencias, Universidad De Valparaíso. (Sponsored by FONDECYT INICIACION 11130707 (GC) And Centro De Neurobiología Y Plasticidad Cerebral (CNPC) Of Universidad De Valparaíso.)

Obesity epidemic is one of the major concerns in the world. Among OECD countries, Chile is the fourth country with higher levels of obesity. Regarding sex distribution, obesity is more prevalent in women (31%) than in men (19%). In fact, 50% of pregnant women in Chile have either overweight or obesity. These conditions lead to different abnormalities in pregnancy and delivery. In addition, recent studies show that the offspring of obese mothers has an increased probability to suffer cardiovascular, metabolic and reproductive diseases. Our group and others demonstrated that exposure to a high fat diet is related to obesity, increased liver weight, advanced puberty and increased estradiol levels in the progeny. We aimed to determine if metformin prevents this developmental reprogramming produced by a high fat diet exposure. Sprague Dawley rats were distributed in 3 groups: Control Diet (13%Kcal in fat); High Fat Diet (HF) (60% Kcal in fat, Research Diet, USA) and HF+ Metformin (60% Kcal in fat, Research Diet, USA + metformin 150-200mg/Kg in tap water). Diet was administered for 1 month previous to pregnancy, during pregnancy and nursing. Metformin was administered from 1 week previous to pregnancy until weaning of the offspring. Metformin did not affect the weight gain during pregnancy and fail in prevent increased weight in offspring of obese mothers. Regarding hepatic weight, maternal obesity increases hepatic weight in the offspring, but metformin do not prevent this increase. At postnatal day 14 there is an increase in estradiol in offspring of obese mothers, but metformin does not prevent this increase. In conclusion, the dose of metformin is insufficient to prevent alterations triggered by maternal obesity on the offspring.



100) **Angiotensin-(1-7) prevents skeletal muscle wasting induced by lipopolysaccharide decreasing proteasomal degradation and autophagy**

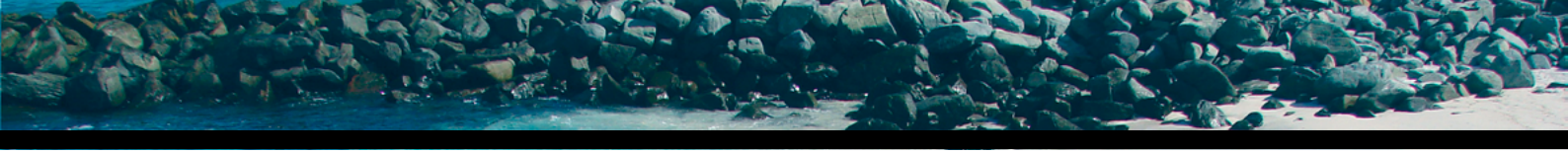
Rivera, Juan Carlos[1,2]., Morales, María Gabriela[1,2].,Abrigo, Johanna[1,2].,Simon, Felipe[3,4].,Cabello-Verrugio, Claudio[1,2].,[1]Laboratorio de Biologia y Fisiopatología Molecular, Depto. Ciencias Biológicas, Facultad de Ciencias biológicas, Universidad Andrés Bello.[2]Laboratorio de Biologia y Fisiopatología Molecular Millennium Institute on Immunology and Immunotherapy.[3]Laboratorio de Fisiología Integrativa, Depto. Ciencias Biológicas, Facultad de Ciencias biológicas, Universidad Andrés Bello.[4]Laboratorio de Fisiología Integrativa Millennium Institute on Immunology and Immunotherapy. (Sponsored by Funding: Association-Francaise Contre Les Myopathies AFM #16670; FONDECYT #1120380, 1121078; IMII #P09-016-F; UNAB DI-741-15/N.)

The skeletal muscle atrophy can be induced by lipopolysaccharide (LPS), causing a fast and severe loss of mass and muscle strength. Among the mechanisms involved is the proteasomal degradation of myofibrillar proteins and autophagy. Angiotensin-(1-7) [Ang-(1-7)], a peptide of the non-classical axis of Renin Angiotensin System, has beneficial effects in skeletal muscle via its receptor Mas. We evaluated the effect of Ang-(1-7), and the mechanisms involved, on muscle wasting induced by LPS. Culture of C_2C_{12} myotubes or C57BL/10J mice were exposed to LPS in absence or presence of Ang-(1-7). Muscle strength, fiber diameter, myosin levels and markers of proteosomal system (atrogin-1 and MuRF-1) and autophagy (LC3II, Bnip, BnipL, GABARAP and Atg7) were determined. Our results shown that Ang-(1-7) recovers the decreased muscle strength, fiber diameter and MHC levels in tibialis anterior of mice. In addition, we observed that Ang-(1-7) prevents the increment of atrogin-1, MuRF-1, LC3II and BnipL induced by LPS in mice. Studies in vitro using C_2C_{12} cells, shown similar effects of Ang-(1-7) potentiating the observation that it is a peptide with anti-atrophic properties in skeletal muscle. The mechanism through Ang-(1-7) produces it anti-atrophic effect involves the inhibition of autophagy and proteasomal degradation of myofibrillar proteins.

101) **P2X receptor evolution: sequence and structural comparisons from unicellular green algae to the human receptor subtypes.**

Latapiat, Verónica[1]., Huidobro-Toro, J Pablo[1].,[1]Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile. (Sponsored by Funded Fondecyt 1141132, PFB 0807.)

P2X receptors are trimeric ATP-activated ion channels permeable to Na+, K+ and Ca2+; these receptors are involved in multiple physiological responses. The seven P2X receptor (P2XR) clones have 40–50% identical amino acid sequence between them, with a similar variability within vertebrate subtypes.Each subunit has two transmembrane domains connected by an extracellular loop of ~280 amino acids. The only crystallographic structure available for P2XR is the zebrafish P2X4R (zfP2X4R); the evolutionary tree of this family of receptors is unkown. The unicellular green alga *Ostreococcus tauri* is the smallest free-living eukaryote described; a P2XR (otP2XR) was identified in this alga which is over 1,000 million years old. Therefore, the question arrises as to the evolution and putative phylogeny of the P2XR family, which is now addressed. To examine an evolutionary pattern of P2XR in the tree of life, we used bioinformatics tools to determine structural differences between P2XR in several organisms from unicelular algae to multicelular red, green and brown algae and humans. We also used molecular modeling based on the zfP2X4R template (PDB: 4DW1), to generate the otP2XR tridimensional structure. Moreover, molecular dynamics simulation techniques were applied to establish an evolutive analysis of these P2XRs. Blast alignment program (tblastn) were used to identify possible P2X sequences in *Chlamydomonas* and *Ulva compressa* genomes, as well as other representative red and brown algae, using all P2XR sequences presents in NCBI database. Comparative homology modelling and multiple sequence aligment showed that the otP2XR contains 4/5 conserved disulfide bonds characteristic of vertebrate P2XR; it lacks C217, C227 of the dorsal fin. Key functional residues, such as conserved K68 and K309 are likewise conserved, summing a 45% sequence identity with zfP2XR4. Similar analysis were undertaken to study the conserved homology of the otP2XR with multicelular algae P2XRs. Preliminary data is consisted with P2X-like aminoacidic sequences in the genomeof *Chlamydomonas*, as well as multicellular algae; further analysis have been undertaken to precise the validity of the sequence homologies found. The sequence identity of OtP2XR and zfP2X4R allows proposing convergence evolutionary mechanism. The conserved structural and functional characteristics of P2X-like receptors in different genomes will illustrate evolutionary relationships among organisms through the phylogenetic tree. This study also offers new insights into the molecular pharmacology of P2XRs; future in-silico docking assays will assess the ATP orthosteric site and compare the pharmacophore.



102) **Mechanisms controlling Nur77 expression and activity: implications in neuronal plasticity**

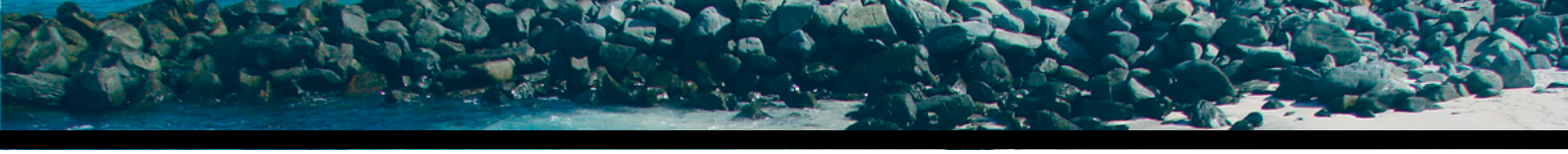
Olivares, Montserrat[1]., Parada, Guillermo[1].,Andrés, María Estela[1].,[1]Biología Celular y Molecular, Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by Fondecyt Project N° 1150200)

Nur77 is a transcription factor and orphan member of the nuclear receptor superfamily encoded by an early gene. Nur77 expression in brain is regulated by dopamine and glutamate in nuclei of the motivated circuit. In the striatum, nucleus Accumbens and prefrontal cortex, Nur77 expression is induced by stress and exposure to psychostimulants. Even though Nur77 has been highly implicated in pathological processes as addiction and Parkinson disease, its role in the brain or the mechanisms regulating its expression have not been elucidated. Here, we used bioinformatics, chromatin immunoprecipitation (ChIP) and reporter assays to learn about mechanisms controlling Nur77 expression. The analysis of available bioinformatics data showed that Nur77 binding sites are present in strong enhancers and active promoters. In promoters, Nur77 binding is abundant near to TSS (± 500 bp) and the majority of Nur77 binding sites lack of NBRE elements. Interestingly, the data showed that Nur77 targets several genes of neuronal plasticity. Recently, it was shown that lysine-specific histone demethylase 1 (LSD1, also known as KDM1a) plays a significant role in plasticity and neuronal excitability. Here, we show evidence suggesting that LSD1 regulates Nur77 expression. Reporter gene assays, surprisingly and contrary to what was expected, showed that LSD1 and the neuronal variant LSD1-8a increase luciferase reporter activity driven by the human Nur77 promoter. In addition, preliminary data from ChIP assays carried out with mouse striatum chromatin, showed that LSD1 is present in Nur77 promoter. A result supported by bioinformatics analyses of reported ChIP data assays showing that the human Nur77 promoter binds HDAC2 and CoREST1, both partners of LSD1. Taken together, the evidence suggests that LSD1 may be involved in modulating plastic genes through regulating the expression of Nur77

103) **Unravelling the coordination of zinc in ionotropic receptors: from structural to allosteric modulator sites.**

Peralta, Francisco[1]., Huidobro-Toro, Juan[1].,[1]Departamento de Biología, Facultad de Química y Biología, Universidad De Santiago De Chile. (Sponsored by Funded By Fondecyt Grant 114-1132, CONICYT Basal Grant 087 (CEDENNA) And CONICYT 21140407 Doctoral Fellowship.)

Zinc is a transition metal selectively stored in synaptic vesicles together with neurotransmitters, from where it is co-released. The extracellular synaptic space contains variable amounts of the metal, which may reach micromolar concentrations. Zinc coordinates with structural protein motifs as in zinc fingers and/or in the catalytic site of enzymes. The metal is an allosteric regulator of almost all ionotropic receptors, either as a positive or a negative modulator. Moreover, a zinc-activated channel was described, an indication that this metal may also play a role as a ligand acting on a receptor channel particularly sensitive to zinc. We hypothesized that the coordination of the metal is different for each of these three functions. To assess how zinc interacts with ligand-gated receptors, we examined how zinc coordinates to proteins, differentiating between structural and catalytic roles. To characterize the zinc protein coordination geometries and investigate the preferred ligands in these functions, we compared the zinc ligands found in proteins accomplishing the three identified functions. To this aim, we searched in the databank of proteins crystallized with zinc and in papers describing zinc coordination motifs. Based on this double scrutiny, we discovered that the zinc coordination to ionotropic receptors is similar to that found in enzymes catalytic motifs but different to the zinc motifs of structural proteins. Our search consistently showed that in the coordination of structural proteins, the frequency of Cys as a metal ligand was 75%, His (20%) and Asp/Glu (3%). In contrast, in catalytic or allosteric proteins, the most frequent ligand for zinc was His (46 and 66%, respectively), followed by carboxylic acid (35 and 18%, respectively) with Cys being the least common ligand (20 and 6%). We also showed that the zinc coordination ligands for positive and negative allosteric modulators are different; whereas in the positive modulator sites we found 11% of Cys, none was found in the negative modulators (0%). Moreover, the presence of Lys and Gln was found 4-times more frequently in the negative modulators compared to the positive modulators (19 versus 5%). Finally, we compared the zinc coordinating spheres in P2X4 and P2X2 receptors, two receptors positively modulated by zinc) and conclude that these receptors have two different zinc allosteric modulation pockets in relative close regions of the extracellular domain, consistent with the findings at other positive modulator sites. This study provides novel structural determinants of zinc metal coordination in allosteric regulatory mechanisms of receptor channels.



104) **The expression of scFv anti-LDL oxidized is affected negatively for low temperatures in cultures of yeast *Pichia pastoris.***

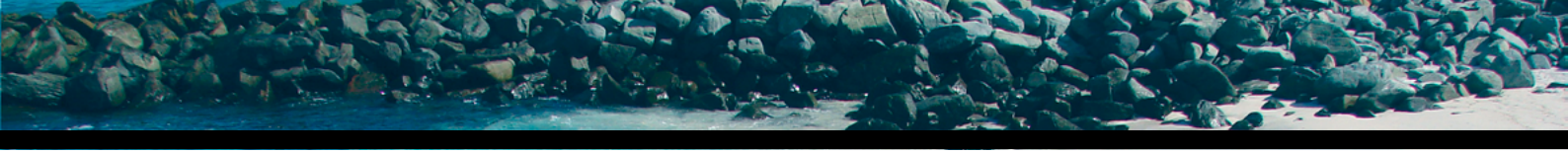
Zepeda, A[1,2]., Figueroa, C[1,2].,Figueroa, E[1].,Pessoa, A[2].,Farías, J[1].,[1]Departamento de Ingeniería Química, Facultad de Ingeniería y Ciencias, Universidad De La Frontera.[2]Departamento de Ciencias Bioquímicas y Farmacéuticas, Facultad de Ciencias Farmacéuticas, Universidade de Sao Paulo. (Sponsored by Acknowledgments: Scholarships For PhD In Chile, CONICYT. Coordination Of Superior Level Staff Improvement(CAPES, Brazil), National Council For Scientific And Technological Development (CNPq, Brazil) Y São Paulo Research Foundation (FAPESP, Brazil).)

Modified forms of low density lipoprotein (LDL) are important to the formation of foam cells and mediators involved in the progression of atherosclerosis immuno-inflammatory process. Recombinant antibody fragments (scFv) anti-LDL oxidized are a promising biotechnology product for the prophylaxis, treatment and diagnosis of cardiovascular diseases. In methylotrophic yeasts, which are able to be used as sole carbon source as methanol, *Pichia pastoris* is an excellent model for the expression of heterologous proteins, largely because this organism carries out post-translational modifications, such as glycosylation, very similar to human, which is reflected in the large number of proteins that have been obtained in the biological model. For this reason, the aim of this study is to determine the most optimal growing condition for *P. pastoris* recombinant allows to obtain glycosylated and high concentration of recombinant protein. To conduct the study, we evaluated three critical factors for temperature (14ºC, 18ºC, 22ºC) and methanol concentration (1%, 1.5%, 2%).The purification of the recombinant protein was performed using ionic chromatography, the quantification was using the Bradford Assay and to analyze the purity and forms obtained was used the polyacrylamide gel electrophoresis. The results show that after 72h of induction with methanol the cultures to 18°C+1,5%M and 22°C+2%M exhibit the most highest concentration with respect the others cultures with a media of 27,25 mg/L and 23,39 mg/L, respectively. Subsequent in this period is observed that the only ones cultures that produce 100% of glycosylated protein are the cultures performed to 22°C, independently of the methanol concentration. In conclusion, the most favorable culture conditions to produce an optimal antibody fragment are those with a temperature of 22°C,but the more economically profitable production is a 22°C+2%.

105) **Transforming growth factor-β1 increases Cdk5 activity and TRPV1-dependent Ca^{2+} influx in trigeminal neurons**

Lazcano, Pablo[2].,Wilson, Carlos[1].,Gonzalez-Billault, Christian[1].,**Utreras, Elias[2]**., [1]Laboratory of Cellular and Neuronal Dynamic, Department of Biology, Faculty of Science, Universidad de Chile.[2]Laboratory of Cellular and Molecular Mechanisms of Pain, Department of Biology, Faculty of Science, Universidad de Chile. (Sponsored by Supported By Grants ACT1114 And Fondecyt 1140325 To C.G.-B And Fondecyt 11110136, 1151043 And PAI-79100009 To E.U.)

Inflammation is positively correlated with several molecular mechanisms involved in orofacial pain. Increased levels of Transforming growth factor-β1 (TGF-β1) have been reported in fluids of migraine patients, suggesting its role during orofacial pain. We reported earlier that Cyclin-dependent kinase 5 (Cdk5) plays a crucial role in pain signaling. Cdk5 is a kinase active in neurons, where its activator p35 is predominantly expressed. We also reported that peripheral inflammation promotes Cdk5 activity by enhancing transcription of p35. Additionally, we described that Cdk5 phosphorylates the TRPV1 channel at threonine 407, increasing Ca^{2+} influx in nociceptive neurons. Interestingly, we demonstrated that TGF-β1 increases Cdk5 activity in DRG neurons and odontoblast-like cells. However, this regulation has not been studied in the trigeminal ganglion (TG). Considering this data, we evaluated the contribution of TGF-β1 in the regulation of Cdk5 activity which in turn affects TRPV1 phosphorylation and function in an ex vivo model of TG as well as cultured TG neurons from mice. To test our hypothesis, we induced a local inflammation in the mouse vibrissae pad through injection of l-carrageenan during 3 h and then we evaluated TGF-β1 levels, and activation of canonical pathway and non-canonical pathways (phospho-Smad2 and phospho-ERK1/2, respectively). Moreover, we evaluated immunodetection of Cdk5, p35 and Egr1 (transcription factor for p35 expression), and phospho-T407-TRPV1 in mouse TG after inflammation. Alternatively, cultured TG neurons were treated with recombinant TGF-β1 for 24 h in absence or presence of SB431542 (the inhibitor of TGF-β1 type 1 receptor), to evaluate the expression of the already mentioned proteins. Our results showed that orofacial inflammation increased TGF-β1 levels and activated its signaling pathways locally in TG. In addition, inflammation increased Cdk5, p35, Egr1 inmmunodetection as well as phospho-T407-TRPV1 in TG compared to control mice. Remarkably, the treatment with TGF-β1 increased both p35 and phospho-T407-TRPV1 in cultured TG neurons, which was blocked by SB431542 pre-treatment. Finally, TGF-β1 treatment increased Ca^{2+} influx mediated by TRPV1, suggesting a functional regulation of the channel in an inflammatory context. Taken together, our results shown that TGF-β1 increases the expression of p35 and Cdk5 activity during an inflammatory process, affecting the function of TRPV1 channel. These results suggest a molecular mechanism involving TGF-β1, Cdk5 and TRPV1 between inflammation and the increased pain sensation in the orofacial region.



106) **Study of protein interaction sites of Gβγ and Glycine Receptor by peptides**

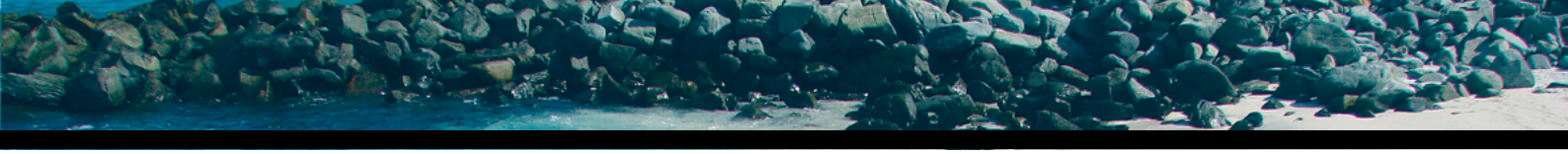
Neira, Luis[1]., González, Daniela[1].,Nova, Daniela[1].,Guzmán, José[1].,[1]Physiology, Biological Sciences, Universidad De Concepción.
(Sponsored by This Work Has Been Funded By The FONDECYT Proyect 1131004, IDeA-FONDEF Proyect CA12I10280 And CMA Bío-Bío)

One of the most commonly used drugs worldwide is ethanol, and its abuse has led to social, economic and health problems. It has been determined that one of its main molecular targets of ethanol channels in the central nervous system are ligand-gated ion, being glycine receptor (GlyR) one of their representative members of this family. Several studies have revealed that ethanol regulates the function of this receptor increasing Cl^- conductance, which causes hyperpolarization of the plasma membrane increasing the inhibitory activity of this ion channel. It has been demonstrated that ethanol could be acting by an indirect mechanism involving the βγ dimer of the G protein, which would interact with the intracellular domain of GlyR in regions with basic amino acids (aa), such as lysine and arginine. In our laboratory, we have designed peptides derived from the N-terminal region (316-320aa) of the intracellular domain of the GlyR capable of inhibit the interaction with βγ dimer. Therefore, continuing with this line of research, we performed experiments using new peptides based on intermediate and C-terminal regions of the cytoplasmic domain of GlyR such as P6 (HKDDEGG), P9 (RFNFSAY), P10 (FSAYGMG), P13 (CLQAKDG), P24 (KLFIQRA), and P28 (RAKKIDK). Hence, a bioinformatic study of protein-peptide docking was done using tools like PyMol and Schrödinger to model and predict the interaction sites and most favorable complexes between the peptides and Gβγ proteins. The online software FastContact was used to calculate the energies of electrostatic interaction between the surface amino acid residues of the dimer and the peptides: Gβγ-P6 (-25.5 kcal/mol), P9 (-10.9 kcal/mol), P10 (-8.6 kcal/mol), P13 (-11.8 kcal/mol), P24 (-19.6 kcal/mol) and P28 (-33.4 kcal/mol). Subsequently, *in vitro* analyses were carried out using immunocolocalization in HEK293 cells to assess the entrance of the peptides into the cell and their interaction with Gβγ. Colocalization was evaluated by measuring the Manders ratio (%). We found that P28 peptide was one of the most favorable to interact with Gβγ (36.6 ± 1.4%) and their locations were analyzed by three-dimensional (3D) reconstructions. Furthermore, through electrophysiological assays we found that two peptides intracellularly applied (P24 and P28) were able to reduce ethanol potentiation of GlyRα1. Using 100 mM ethanol, P24 and P28 (200 uM) inhibited the potentiation of ethanol from 43.8 ± 5.2% to 35.7 ± 9.9% and 34.2 ± 3.9% respectively. Finally, the specificity of the peptides was studied in the PLCβ2 pathway using fluorescent measurements and the data demonstrated that these peptides have no effect in this pathway.

107) **The gasotransmitter hydrogen sulphide (H_2S) modified ciliary activity in mouse tracheal epithelial cell cultures.**

Ríos, M[1]., Droguett, Karla[1].,Althaus, Mike[2].,Villalon, Manuel[1].,[1]Department of Physiological Science, Faculty of Biological Science, Pontificia Universidad Católica De Chile.[2]Institute of Animal Physiology Justus-Liebig University of Giessen. (Sponsored by Fondecyt Postdoctorado 3150652)

In airway mucociliary epithelium, ciliary activity is determinant for the effectiveness of mucociliary clearance (MCC), the basic defense mechanism that contribute to eliminate noxious contaminant from the inhaled air. Several chemical and mechanical signals regulate ciliary beat frequency (CBF) therefore affecting MCC velocity. ATP is locally release by the airway epithelium and increases CBF by a mechanism dependent of changes in $[Ca^{2+}]_i$. Hydrogen sulfide (H_2S), an endogenous gas, with the characteristic odour of rotten eggs is a recently known gasotransmitter that influences a variety of cellular and organ function. In respiratory system, H_2S has significant vasoactive and anti-inflammatory effects, with raised levels observed in serum and in sputum supernatants of asthmatics patients. H_2S decreases transepithelial Na^+ transport, implicated in the regulation of mucociliary epithelial lining fluid, however it is unknown whether H_2S has an effect on other ciliary epithelial function. Using mouse tracheal epithelial cell cultures (MTEC) we studied the effect of H_2S on basal ciliary activity, measuring CBF by videomicroscopy (Sisson Ammons Video Analysis), eATP measured by luminometric assay using luciferin/luciferase; $[Ca^{2+}]_i$ measured using FURA-2AM and the dye uptake rate using bromure ethidium. The same parameters were measured in cultures stimulated by ATP in the presence of H_2S. We evaluated cultures exposed to different concentrations of sodium hydrosulphide (NaHS), a H_2S-donating drug. NaHS in high concentrations (100 and 300 µM) increased transiently CBF (20%) during 5 min effect that temporarily correlate with the increase in $[Ca^{2+}]_i$. Low concentrations (1 and 10 µM) reduced CBF by 10-15% but no changes in $[Ca^{2+}]_i$ were observed. Low concentrations of NaHS (10 µM) increased the rate of dye uptake during 2 min (0.4 ± 0.1 Vh vs 0.9 ± 0.3 NaHS (n=4 and 5 respectively)), however we did not observe changes in eATP levels following NaHS (10 µM) addition. Furthermore, NaHS (10 µM) reduced the CBF increase in response to a chemical stimulus of ATP (1 µM) (62%, $p<0.05$), and the change of the dye uptake rate (120.6 ± 32.4 ATP/Vh vs 83.6 ± 18.6 ATP/H2S, n=5). These results showed that H_2S has a dual effect on ciliary activity, at high concentrations, H_2S increased CBF and $[Ca^{2+}]_i$ and at low concentration, H_2S decreased CBF and the increment of CBF and dye uptake rate induced by ATP. These findings demostrate that exogenous H_2S affect ciliary activity, and provide evidence that this gasotransmitter has physiological and pathophysiological implications in the regulation of mucociliary transport in the airways.



108) **Interaction between CRF-BP and CRF2αR increases CRF2αR in the plasma membrane**

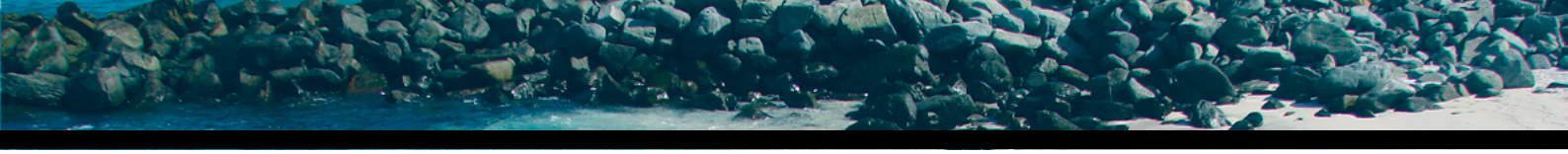
Slater, Paula[1]., Cerda, Cledi[1].,Andrés, María[1].,Gysling, Katia[1].,[1]Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by Funded By FONDECYT Grant Nº 1110392 And 1150244, MSI Grant N° P10/063-F And CONICYT Grant For PhD Thesis Research To S.P.G.)

The family of corticotrophin releasing factor (CRF) links stress and addiction. Intra ventral tegmental area (VTA) injections of CRF as well as stressful stimuli induce relapse to drug seeking in cocaine-experienced rats, due to the potentiation of glutamatergic synapses onto VTA dopaminergic neurons. These potentiation observed in cocaine-experienced rats depends on CRF binding protein (CRF-BP) and on type-2 CRF receptor ($CRF_{2\alpha}R$). However, the molecular mechanism underlying this functional interaction is presently unknown. We have observed that CRF-BP and $CRF_{2\alpha}R$ are able to physically interact. We hypothesize that this interaction affects the sub-cellular localization of the proteins. Thus, we studied the subcellular localization of CRF-BP and $CRF_{2\alpha}R$ in transfected PC12 cells. We analyzed co-localization of CRF-BP or $CRF_{2\alpha}R$ with different organelle markers, using Van Steensel co-localization analysis. We also analyzed the presence of $CRF_{2\alpha}R$ in the plasma membrane as fluorescence intensities of $CRF_{2\alpha}R$ membrane/total. The results showed that CRF-BP is located primarily in secretory granules and $CRF_{2\alpha}R$ in endoplasmic reticulum. Interestingly, when CRF-BP and $CRF_{2\alpha}R$ are co-expressed a high degree of co-localization in the endoplasmic reticulum is observed, evidencing that $CRF_{2\alpha}R$ retains CRF-BP in endoplasmic reticulum. Furthermore, the presence of $CRF_{2\alpha}R$ in the plasma membrane increases when is co-expressed with CRF-BP. The interaction and sub-cellular localization changes between CRF-BP and $CRF_{2\alpha}R$ are specific, since they are not observed when the $CRF_{2\beta}R$ isoform is used. In summary, our results show that CRF-BP and $CRF_{2\alpha}R$ form a protein complex allowing modification of the subcellular localization of both CRF-BP and $CRF_{2\alpha}R$.

109) **Traffic of dopamine D2L receptor: basic mechanisms and kappa opioid receptor control**

Ureta, Roxana[1]., Escobar, Angélica[1].,Andres, Maria[1].,[1]Biologia Celular y Molecular, Facultad de Ciencia Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT Project # 1150200)

Dopamine (DA) in the Nucleus Accumbens (NAc) is a key neurotransmitter controlling motivated behavior. Dopamine signaling is carried out mainly by activation of D2 and D1 receptors in the NAc. Two spliced variants of D2 receptors (D2R) are expressed in the NAc. D2 short variant receptors are presynaptically located in dopaminergic terminals where their activation decrease DA extracellular levels, while D2 long variant receptors (D2L) are postsynaptically located in medium spiny GABA neurons where their activation favors motivated behavior. DA neurotransmission in the NAc is modulated by Kappa opioid receptors (KOR). KOR are located mostly on dopaminergic terminals or in close apposition to them. Acute activation of KOR decreases DA extracellular levels in the NAc and reduce motivated behaviors. Our previous work showed that repeated activation of KOR decreases the inhibitory function of D2R on DA release in the NAc. Interestingly, co-activation of KOR potentiates locomotor sensitization induced by repeated administration of quinpirole (QNP), a D2R agonist. Together these data indicate that KOR and D2R functionally interact, both pre and postynaptically. We hypothesized that KOR activation modulates levels of D2R in plasma membrane, controlling the internalization and traffic of this receptor. To evaluate this hypothesis, we designed a vector SepHluorin-D2L-mCherry to follow D2L trafficking, since green fluorescence of SepHluorin protein is emitted only at pH 7. Thus, if D2R is endocyted green fluorescence decreases. HEK 293T cells were transiently transfected with SepHluorin-D2L-mCherry and treated with DA (5 µM, 10 µM, 50 µM and 100 µM) or with QNP (5 µM, 10 µM, 50 µM and 100 µM) and green fluorescence intensity was quantified on Synergy 2-multimodal lector. Preliminary results showed that both 5µM and 10µM DA and QNP decrease while high DA concentrations increasegreen fluorescence intensity, suggesting opposite effects depending on agonist concentration. Co-transfection experiments with HA-KOR and SepHluorin-D2L-mCherry are currently carrying out.



110) **INFLUENCE OF T_3 ADMINISTRATION ON THE HEPATIC PPAR-A–FGF21 SIGNALING PATHWAY.**

Vargas, R[1]., Cornejo, Pamela[2].,Fernández, Virginia[1].,Tapia, Gladys[1].,Videla, Luis[1].,[1]Farmacologia, Medicina, Universidad De Chile.[2]Escuela de Tecnología Médica, Medicina, Universidad Diego Portales. (Sponsored by Agradecimientos: Proyecto FONDECYT 1150104)

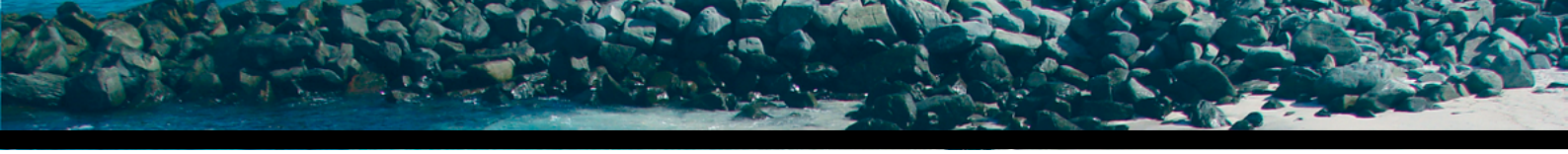
Enhanced mitochondrial activity in the liver, with increased fatty acid oxidation, O_2 consumption and ATP generation, is one of the major effects of T_3. This metabolic pathway may occur through PPAR-α/FGF21. To test this hypothesis a single dose of T_3 (0.1mg/kg) or its vehicle, were given to Sprague-Dawley rats, and parameters of T_3-treatment, as well as PPAR-αand target genes for acetyl-CoA oxidase (ACOX), carnitine-palmitoyltransferase-1α (CPT-1α) and hydroximetilglutaril-CoA synthase-2 (HMGCoAS2), as well as that of FGF21, were evaluated (qPCR). Serum levels of T_3 and the rectal temperature were enhanced ($p<0,05$), in concomitance with liver content of mRNA for PPAR-α and target genes for ACOX, CPT-1α and HMGCoAS2. In concomitance with these effects, FGF21 induction was observed, effect that was significantly correlated with that of PPAR-α ($r=0.98$; $p<0.0002$). It is concluded that T_3 activates PPAR-α signaling pathway, in association with that of FGF21, hepatokine controlling liver energy production needed for preconditioning.

Acknowledgment: Proyecto FONDECYT 1150104

111) **Domains of the human corticotrophin releasing hormone type-2 receptor involved in the interaction with D1 dopamine receptor**

Palominos, Tomás[1]., Fuenzalida, Javier[1].,Slater, Paula[1].,Yarur, Hector[1].,Gysling, Katia[1].,[1]Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by Funded By FONDECYT Grants 1110392 & 1150244, & MSI Grant N° P10/063F)

It has been reported that D1 dopamine receptor (D_1R) and corticotrophin releasing hormone type-2 receptor (CRH_2R) are important in relapse to drug seeking behavior triggered by stressful stimuli. Reports from our group have suggested the presence of both receptors in pre-synaptic inputs to the ventral tegmental area (VTA). Additionally, by heterologous expression in HEK293T cells, it has been assessed the physical and functional interaction of these two receptors. The heteromerization changes the subcellular localization of both receptors. In this work, we have generated several CRH_2R deleterious mutants in order to determine its interacting domains with D_1R. The interaction between D_1R, CRH_2R and CRH_2R mutants was evaluated by checking their capacity to shift their sub-cellular distribution when co-expressed. A loss of this shift indicates a lack of interaction. Our results showed that the intracellular loop 3 and the carboxyl termimal of CRH_2R are not responsible in the heteromer formation. Interestingly, our controls with corticotrophin releasing hormone type-1 receptor (CRH_1R) revealed that CRH_1R and D_1R are capable heteromerize when co-expressed in HEK293 T cells. Thus, D_1R is capable of heteromerizing with both CRH receptors.



112)

N-Acetylcysteine restores prefrontal cortical-accumbens LTD in Swiss CD1 mice fed high-fat diet.

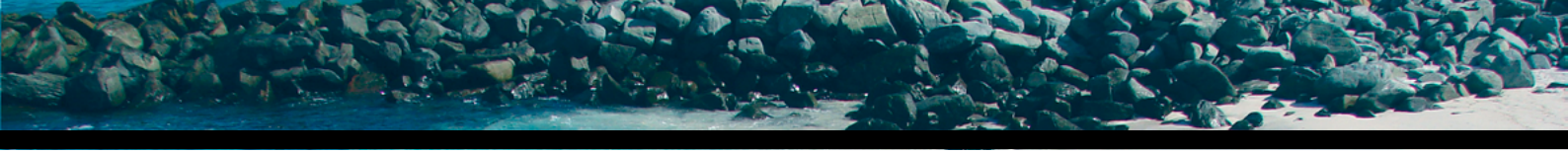
Morgan, Carlos[1]., Hernández, Alejandro[2].,Ramírez, Paulina[1].,Reyes, Andrea[2].,Burgos, Héctor[3].,Constandil, Luis[2].,Sáez-Briones, Patricio[4].,[1]Unidad de Nutrición Humana, INTA, Universidad De Chile.[2]Departamento de Biología, Facultad de Química y Biología, Universidad De Santiago De Chile.[3]Escuela de Psicología, Facultad de Ciencias Sociales, Universidad Central De Chile.[4]Escuela de Medicina, Facultad de Ciencias Médicas, Universidad De Santiago De Chile.

Loss of prefrontal cortical (PFC) neuroplastic control on nucleus accumbens activity is involved in triggering drug seeking behavior on several substances of abuse due to impaired glutamate homeostasis in tripartite synapses in the nucleus accumbens core (Nacc) receiving afferents from PFC. We previously showed that PFC-Nacc long-term depression (LTD), normally observed in control mice is suppressed in young mice fed high-fat diet (HFD) *ad libitum* during 2 weeks after weaning, who developed a dietary preference for HFD (primed on HFD). We hypothesized that this LTD suppression is involved in developing and maintaining dietary preference for HFD. Our interest was to restore the PFC-Nacc LTD and assess the effect on dietary consumption of HFD. Then, PFC-Nacc LTD was elicited in young mice fed HFD that were injected either with 100 mg/Kg i.p. N-Acetylcysteine (NAC) or PBS 2 h before. N-Acetylcysteine, a compound able to induce astrocyte release of glutamate, was effective in restoring PFC-Nacc LTD *in vivo*. In addition, daily NAC administration during 5 days to mice primed on HFD progressively diminished its dietary consumption. Our results are consistent with the glutamate homeostasis hypothesis of addiction and extend previous findings in drugs of abuse to consumption of high-fat diet in mice. These neuroplastic changes may be relevant to explain the high prevalence of obesity in western societies.

113) **Putative molecular mechanisms associated to leptin resistance exhibited by Mecp2 null mice.**

Valdivia, Sharin[1]., Hernández-Galaz, Sergio[1].,Guzmán, Luis[1].,Ojeda-Provoste, Patricia[1].,Gutiérrez, Noemí[2].,Kerr, Bredford[2].,[1]Laboratorio de Biología Centro de Estudios Científicos-CECs, Universidad Austral de Chile.[2]Laboratorio de Biología Centro de Estudios Científicos-CECs. (Sponsored by Fondecyt 1140162. PFB 01/2007.)

Leptin is a hormone produced by adipose tissue that mediates long-term regulation of energy balance. Leptin activates a signaling pathway to increase the expression of the anorexigenic gene *Pomc* through the Jak2/Stat3 pathway. After being phophorylated, Stat3 is translocated into the nucleus to bind to the *Pomc* promoter and increase its transcriptional activity, response that require the dissociation of the Pomc-transcriptional repressor FoxO1 and its nuclear exclusion. Several studies have shown that the phosphatidylinositol 3-kinase (PI3K) pathway mediates the effects of leptin on hypothalamic neurons. Leptin-receptor interaction induces the production of phospha- tidylinositol (3,4,5) trisphosphates (PIP3) by PI3K, allowing the phosphorylation of Akt and its upstream activator PDK1, which allows FoxO1 phosphorylation and nuclear exclusion. In addition, the tumor suppressor phosphatase and tensin homolog (Pten) inhibits Akt activity by dephosphorylating PIP3. On the other hand, Methyl CpG binding protein-2 (MECP2) is an epigenetic-associated chromatin-remodeling factor with a dual role on gene expression. Previous evidence from patients carrying *MECP2* mutations and results from our lab demonstrated that this protein has a pivotal role in the control of body weight. Using *Mecp2*-null mice, we found that the absence of Mecp2 alters the basal phosphorylation levels of Akt and its target protein FoxO1 and therefore the activation of *Pomc* expression in response to leptin does not occur. However, the molecular mechanism involved in this disrupted leptin response has not been fully elucidated. Our aim was to determinate the molecular mechanism underlying disrupted leptin signaling observed in the hypothalamus of *Mecp2*-null mice. To accomplish this, we performed a short-term leptin challenge and evaluated the expression of genes and proteins associated to leptin signaling and its post-translational modifications by immunofluorescence and western blot in hypothalamus from *Mecp2*-null mice and their wild type littermates. Our results show that *Mecp2*-null mice are unable to achieve proper leptin signaling. We observed an altered subcellular location of Stat3 and FoxO1, a decreased level of Akt phosphorylation in both nuclear and cytosolic protein fractions and a diminished FoxO1 nuclear exclusion. This alteration in leptin signaling components may be associated with a miss-regulation of Pten, hypothesis that is still being evaluated. Our results allow us to gain insight into the mechanism underlying the leptin resistance observed in the absence of Mecp2, a molecular bridge between epigenetic modifications and the control of body weight.



114) **Aldosterone and IL-17 in the genesis of mineralocorticoid arterial hypertension: an *ex vivo* study.**

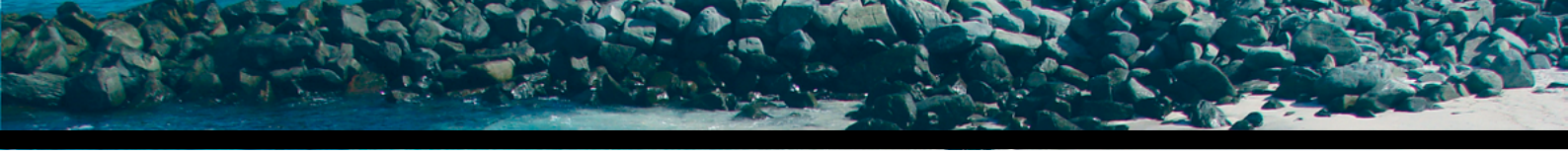
Vecchiola, A[1,2]., Muñoz-Durango, Natalia[2].,Fuentes, Cristobal[1].,Gonzalez-Gomez, Luis Martin[1].,Allende, Fidel[4].,Ortiz-Canales, David[1].,Tapia-Castillo, Alejandra[1].,Valdivia-Pizarro, Carolina[1].,Rojas, Maria[3].,Carrasco, Carmen[1].,Campino, Carmen[1,2].,Solari, Sandra[4].,Baudrand, Rene[1].,Carvajal, Cristian[1,2].,Lagos, Carlos[1,2].,Kalergis, Alexis[2].,Fardella, Carlos[1,2].,[1]Department of Endocrinology, School of Medicine, Pontificia Universidad Catolica de Chile.[2]IMII Millennium Institute on Immunology and Immunotherapy.[3]Department of Family Medicine, School of Medicine, Pontificia Universidad Catolica de Chile.[4]Department of Clinical Laboratories, School of Medicine, Pontificia Universidad Catolica de Chile. (Sponsored by Supported By Proyecto SOCHED 13-6, IMII P09/016-F, CORFO 13CTI-21526-P1, FONDECYT 1150437 & 1130427 Grants. Nothing To Disclose)

Background *In vitro* and *in vivo* studies suggest a broader role for aldosterone, beyond elevating blood pressure. Clinical data support the notion that aldosterone can directly alter the function of the immune system and can participate in systemic low-grade inflammation, which leads to blood pressure elevation and end organ damage. **Objective and Hypothesis**: To assess in humans, whether aldosterone and IL-17 plasma levels modulate immunogenic activity markers in peripheral blood mononuclear cells (PBMCs). **Methods:** We recruited 178 adult subjects (11-67 years, BMI 27.09±4.8 kg/m^2, 61% female) and measured in blood samples: aldosterone, plasma renin activity (PRA), hsCRP, and IL-17. We isolated mRNA from PBMCs and evaluated MR, RAC-1, TLR-4, CD-14, Hsp-90, NGAL and IL-17 expression by q-RT-PCR. **Results**: Aldosterone circulating levels positively correlate with PRA (p< 0,00001) and CD14 expression (p= 0,024). IL-17 circulating levels positively correlates with MR (p= 0,001), Rac1a (p< 0,0001), NGAL (p= 0,005) expression. **Conclusion**: Aldosterone and IL-17 plasma levels are associated to PBMC inflammatory activation markers, which could predispose to autoimmune disorder development.

115) **Changes of BK potassium channel mRNA abundance in gills during the seawater adaptation in *Salmo salar***

Morera, FJ[1]., Saravia, J[1].,Oyarzun, R[2].,Pontigo, JP[2].,Strobel, P[3].,Contreras, C[1].,Loncoman, C[1].,Gutierrez, L[1].,Vargas-Lagos, C[2].,Yañez, A.[4].,Vargas-Chacoff, L[2].,[1]Instituto de Farmacologia, Ciencias Veterinarias, Universidad Austral De Chile.[2]Instituto de Ciencias Marinas y Limnologicas, Ciencias, Universidad Austral De Chile.[3]Instituto de Ciencia Animal, Ciencias Veterinarias, Universidad Austral De Chile.[4]Instituto de Bioquimica y Microbiologia, Ciencias, Universidad Austral De Chile. (Sponsored by Funding By FONDECYT De INICIACION 11130308 (to FJM), INNOVA-Corfo 13IDL2-23565 (to LVC, FJM And AY); Fondap-Incar, No. 15110027 (to AY) And DID-UACH (to FJM))

Historically, research on smoltification has been linked to the aquaculture industry, in particular to the quality of smolts during transfer time from freshwater (FW) to seawater (SW). Biologically, smoltification, also called parr-smolt transformation, is a complex developmental process in salmonids that consists of a number of independent but coordinated changes in the biochemistry, physiology, morphology, and behavior of juvenile salmon in its transition from fresh water to seawater life. Instrumental to smoltification process is the ability of smolt gills to gradually become capable of active salt secretion through specialized cells known as mitochondria-rich (MR) cells, ionocytes or chloride cells. NaCl secretion by teleost gills is therefore accomplished via a secondary active transport of Cl^- and passive transport of Na^+. The driving force for active transport is provided by the Na^+,K^+-ATPase, which maintains a low intracellular Na^+ concentration and high intracellular K^+ concentrations. However, this mechanism of NaCl secretion necessitates a thermodynamic prerequisite to work under conditions imposed by high extracellular salinity in seawater: the recycling of extracellular K^+, most likely through potassium channels. The identities of K^+ channels required for NaCl secretion from MR cells in seawater are still unknown for *Salmo salar* and only recently have begun to be studied in other teleosts. Preliminary data from our laboratory showed the expression of mRNA from a high conductance voltage- and Ca^{2+}-activated K^+ (BK) channel in gills of *Salmo salar.* In this study, we performed a time-course study of the levels of BK potassium channel mRNA in gills of *S. salar* in a seawater challenge experiment and during smoltification in an industrial setting. This was analyzed in conjunction with the enzymatic activity of NKA, the only “molecular marker” of smoltification currently in use by the Chilean salmon industry. We found that the levels of BK potassium channel mRNA increase during the first week in the seawater challenge experiment and in a similar way to NKA activity during smoltification in an industrial setting. These data could be the first step toward considering potassium channels as new molecular marker candidates for smoltification.



116) **Evaluation of TASK-3 knockdown effect on breast cancer cell proliferation**

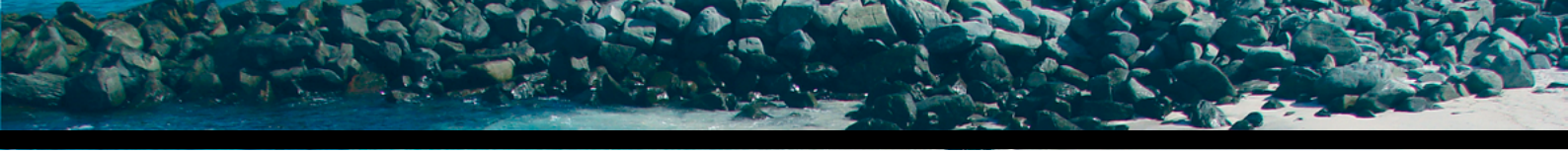
Veneciano, Jocelyn[1]., Zúñiga, Rafael[1].,Valenzuela, Claudio[1].,Brown, Nelson[1].,Zúñiga, Leandro[1].,[1]Escuela de Medicina Universidad De Talca. (Sponsored by This Work Was Supported By FONDEF-IDEA CA13I10223. J.V. Thanks To University Of Talca, Biomedical Science Master Program.)

TASK-3 (also known as K_2P9) is a potassium (K^+) channel that belongs to the family of two-pore domain (K2P), displaying K^+ outwardly rectifying currents independently of membrane voltage. The TASK-3 potassium channel has been identified as a key component in the maintaining of resting potential in excitable cells. This channel also presents an overexpression in several types of human tumors, including those originating from breast tissue and lungs. In these tumors, TASK-3 seems to promote proliferation and survival of cancer cells, most likely by augmenting their resistance to both hypoxia and serum deprivation. Here, we evaluate the overexpression of TASK-3 potassium channels in different breast cancer cell lines (MCF-10F, MCF-10A, MCF-7 y MDA-MB-231), using immunofluorescence and real time PCR (qPCR) analysis to determinate gene expression. Our results corroborate the presence of TASK-3 in different breast cancer cell lines with an expression profile of MCF-10F >> MCF-10A = MCF-7 = MDA-MB-231. Also, we assess the contribution of TASK-3 channel activity over proliferative and tumorigenic properties in MCF-10F and MDA-MB-231, through shRNA approach. Our result shows that the knockdown of TASK-3, in MCF-10F and MDB-MB-231 cancer cells, generated a protein reduction of ~30–50%, evaluated at both protein and mRNA levels. This reduction was also well correlated with a significant reduction in cell proliferation process. Our results suggest that TASK-3 has a critical role in tumor cell proliferation and corroborate the therapeutic potential for designing of new treatment for mammary cancer.

117) **Characterization of a molecular site for the modulation of the glycine receptor α3 subunit by 2,6-di-tert-butylphenol**

Lara, Cesar[2]., Burgos, Carlos[2].,Muñoz, Braulio[2].,Moraga-Cid, Gustavo[1].,Corringer, Pierre-Jean[1].,Yévenes, Gonzalo[2].,[1]Institute Pasteur Institute Pasteur.[2]Department of Physiology Universidad De Concepción. (Sponsored by Supported By FONDECYT 1140515, Fondation De La Recherche Médicale, Institut Pasteur And The Agence Nationale De La Recherche. Sponsored By Dr. Jorge Fuentealba A.)

Glycine receptors (GlyRs) are ligand-gated ion channels that are particularly critical in the spinal dorsal horn processing of nociceptive signals. Recent studies have determined that peripheral inflammation decreases the function of spinal GlyRs composed by the α3 subunit, contributing to the generation of chronic pain. Molecules that enhance α3GlyRs activity thus may exert analgesic actions. However, only few α3GlyR modulators are currently available and the molecular sites involved in these effects are not defined. Using electrophysiology and molecular modeling, here we characterized a molecular site associated with the modulation of α3GlyRs by the non-anesthetic propofol analog 2,6-di-tert-butylphenol (2,6-DTBP). Our results show that 2,6-DTBP (1-300 µM) is a positive allosteric modulator of α3GlyRs. Single channel recordings showed that 2,6-DTBP significantly increases the ion channel open probability without changes in the main conductance. Studying mutant GlyRs, we identified a phenylalanine residue (F388 in α3GlyR) at the beginning of the transmembrane domain 4 (i.e. MA-stretch) which is necessary for the positive allosteric modulation elicited by 2,6-DTBP. Whole-cell recordings revealed that the mutation of this phenylalanine residue to alanine (F388A) significantly impaired the sensitivity to 2,6-DTBP (164 ± 20% in wild-type vs 10 ± 9.0% in F388A mutant). At the single-channel level, 2,6-DTBP did not significantly enhanced the activity of F388A mutant α3GlyRs, confirming the low sensitivity of the mutated receptor to modulation by 2,6-DTBP. We next characterized the potential role of the F388 residue for the binding of 2,6-DTBP in a structural model of α3GlyRs. Three residues (F388, M384 and P381) appeared as critical for hydrophobic interactions of 2,6-DTBP with α3GlyRs. Molecular docking analyses revealed a favorable theoretical energy of interaction with this putative acceptor site ($\Delta G_{binding}$= -42.09 kcal/mol, docking score= -2.133). Interestingly, the introduction of the F388A mutation in the structure caused a significant decrease in these parameters (ΔGbinding= -29.51 kcal/mol, docking score= -0.507), suggesting a direct relationship between the degree of potentiation of α3GlyRs by 2,6-DTBP and the energy of interaction. Our results demonstrate that 2,6-DTBP is a positive modulator the α3GlyR, which may directly interact with an acceptor site within the MA-stretch of the receptor. The detailed characterization of this molecular site could contribute to the screening and development of new α3GlyR allosteric modulators.



118) **Exploring the molecular mechanisms underlying the functional inhibition of the glycine receptor α3 subunit by PKA-mediated phosphorylation.**

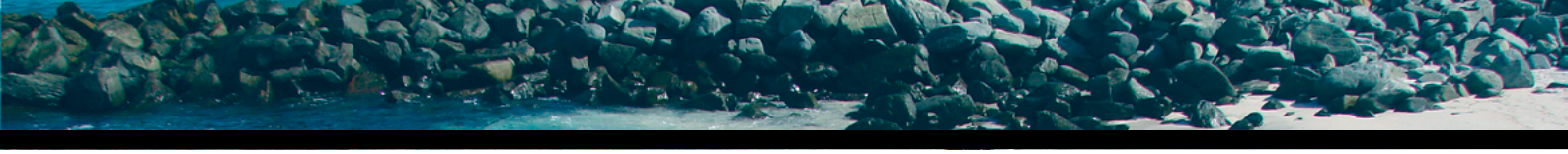
San Martin, Victoria[1]., Lara, Cesar[1].,Yévenes, Gonzalo[1].,[1]Department of Physiology Universidad De Concepción. (Sponsored by Supported By FONDECYT 1140515 Sponsored By Dr. Jorge Fuentealba A.)

Diminished inhibitory glycinergic neurotransmission at the level of the spinal dorsal horn makes a relevant contribution to the generation and maintenance of chronic pain. Inflammation decreases synaptic glycinergic currents primarily through the PGE_2-mediated activation of spinal EP2 receptors, which leads to a PKA-dependent phosphorylation of glycine receptors (GlyRs) composed of α3 subunits. Despite the importance of these events on chronic pain, the molecular and cellular mechanisms involved in the functional inhibition of α3GlyRs by PKA-induced phosphorylation has been not yet established. In this work, we explore the nature of these mechanisms using immunocytochemistry and electrophysiology of α3GlyRs expressed in HEK293 cells. We first analyzed whether the α3GlyR phosphorylation by PKA can modify the receptor stability at the plasma membrane through immunocytochemistry. The results showed that PKA activation elicited by EP2 receptor activation, by incubation with forskolin or by the expression of constitutively active versions of Gαs (Gαs Q-L) or PKA (PKA-C_{Q-R}) did not altered the plasma membrane localization of α3GlyRs. We next studied the effects of PKA phosphorylation at the functional level using whole-cell electrophysiology of HEK 293 cells expressing α3GlyRs and EP2 receptors. Application of PGE_2 inhibited the glycine-activated currents through α3GlyRs in a time-dependent manner. The maximal inhibition (-27±9%) was reached after 6 min of sustained application of PGE_2. To determine whether this current inhibition is related with changes on the apparent affinity or on the efficacy of α3GlyRs, we next analyzed concentration-response curves to glycine before and after EP2 receptor activation. Our data showed that the apparent affinity for glycine (i.e. the EC_{50} for the agonist glycine) was not significantly modified by phosphorylation (Control EC_{50}=136±19 μM vs PGE_2 EC_{50}=123±16 μM). On the other hand, the maximal efficacy (i.e. maximal current) of α3GlyRs was significantly diminished by PKA activation (control I_{max}=2119±405 pA vs PGE2 I_{max}=1560±319 pA). Altogether, these data suggest that the mechanisms involved in the functional inhibition of α3GlyRs by PKA-induced phosphorylation are more likely related with biophysical changes on the ion channel rather than modifications in the number of receptors at the plasma membrane. Ongoing studies using α3GlyRs carrying mutations mimicking either the phosphorylated or the non-phosphorylated state (S346E or S346A) combined with rapid optical stimulation of the PKA signaling cascade and single channel recordings will help to define the nature of these biophysical changes.

119) **Influence of lipid rafts in the regulation of two-pore domain potassium channels in rat cerebellar granule neurons**

Zúñiga, Rafael[1]., Valenzuela, Claudio[1].,Brown, Nelson[1].,Zúñiga, Leandro[1].,[1]Escuela de Medicina, Ciencias de la Salud, Universidad De Talca. (Sponsored by Acknowledgments: This Work Was Supported By Fondecyt Grant 11110217. R.Z. Thanks The University Of Talca For A PhD Fellowship.)

Two-pore domain potassium (K2P) channels are highly expressed in central nervous system (CNS), where they have been proposed to play important roles in modulating neuronal excitability. These channels give rise to leak K^+ currents, voltage-independent and primarily important in the maintenances of resting membrane potential. In neurons, this current, is known as IK_{SO} (standing-outward potassium current). Several lines of evidence have been shown that the cholesterol is critical for the activity of different plasma membrane proteins, such as ion channels. Suggesting that these proteins are intimately associated with cholesterol rich membrane microdomains (lipid rafts). And whereas the importance of lipid rafts integrity is essential for neuronal activity. We evaluate the effect cholesterol depletion by methyl-β-cyclodextrin (MβCD) on background K^+ currents in cerebellar granule neurons (CGNs) by patch clamp experiments. Cholesterol depletion showed a reduction of IK_{SO} currents by ⍰40%, when the CGN were exposed to 5 mM of MβCD. In addition, the presence of different K2P subunits were confirmed by immunocytochemistry and their association with lipid rafts was identified with immunocolocalization with Flotillin-2 (a lipid rafts marker in neuron). Finally, those results were confirmed by co-immunoprecipitation with Flotillin-2. These results suggest strongly that background K^+ channels are associated to lipids raft environments and that the modification of this behavior, by cholesterol depletion, affect the ion channel activity.



120) **Properties of the neural circuit associated to the CCAP AN1-AN4 and motoneurons during the ecdysis into pupa of the Drosophila melanogaster**

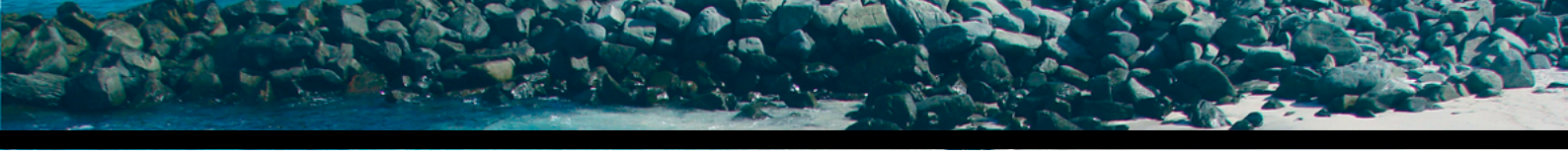
Pineiro, Miguel[1]., Mena, Wilson[1].,Orio, Patricio[1].,John, Ewer[1].,[1]Centro Interdisciplinario de Neurociencia Valparaiso, Facultad de Ciencias, Universidad De Valparaíso.

Ecdysis is the moulting of the cuticle in many invertebrates, which allows them to continue growing. The ETH hormone synthesized in the Inka endocrine cells is released into the hemolymph and initiates the ecdysis sequence in the Drosophila melanogaster. This sequence can be divided in three subphases: pre-ecdysis, ecdysis and post-ecdysis. Of the different neurons that express the ETH receptor, the CCAP neurons from the segment AN1 to AN4 have been identified as the generators of the motor pattern during the ecdysis. The way these CCAP neurons modulate the motor pattern is not evident, mostly because the circuits, neurons and neuropeptide dynamics are not completely understood. We are using calcium imaging to capture the neuronal activity patterns of CCAP and motoneurons during the ecdysis. We developed a clustering algorithm to perform video neural segmentation using pixel intensity variation as cue for pixels belonging to neurons, and coordinated pixel intensity variation as cue for pixels belonging to a single neuron. Pixel intensity variation is measured by the dynamic range of pixel intensity, while coordinated pixel variation is measured by its Pearson correlation. We are measuring different properties of the neural activity pattern during the ecdysis to better characterize its dynamics. We have found that the slow CCAP calcium oscillations apparently increase the probability of motoneurons to switch from a non-oscillatory to a faster oscillatory state . In order to infer the functional connectivity between CCAP and motoneurons, we are fitting a continuous time Markov chain model of two states (oscillatory and non-oscillatory) with transition rates function of the CCAP time series to the experimental data. This model is compatible with the apparent stochasticity of the of the CCAP – motoneuron communication.

121) **EFFECT OF ANGIOTENSIN CONVERTING ENZYME INHIBITION ON THE DEVELOPMENT OF MESONEPHROS IN CHICKEN EMBRYOS.**

Moya, Víctor[1]., Olivares, Natalia[1].,Ansaldi, Valentina[1].,Alexis, Gonzalez[2].,[1]Instituto de Biología, Facultad de Ciencias, Pontificia Universidad Católica De Valparaíso.[2]Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica De Valparaíso.

While the role of the renin angiotensin aldosterone system (RAAS) in the regulation of blood pressure and volume is known, recent evidence suggest the participation of RAAS in the development of mammalian kidney and particularly in developing of metanephros. In order to evaluate the effects of captopril (angiotensinc converting enzyme inhibitor) on the development of mesonephros and expression of some components RAAS, chicken embryos were treated daily with 5 mg captopril per kg from 48 hrs up to 14 days during the development. By histochemical and immunohistochemical techniques mesonephros morphology was analyzed and the presence of components of the RAAS was evidenced. Protein expression of RAAS components was analyzed by western blot. Embryos treated with captopril showed increased weight (43.3%), wide (25.4%) and length (23.8%) of the mesonephros as well as the numbers of mesonephric tubules (86.7%) compared to the control. Renin, AT1 receptor and aquaporin 2 protein levels were slightly decreased by captopril treatment. The effects of captopril on chicken mesonephros suggest that RAAS plays a role in the regulation of renal development in birds, but remains to be determined whether the mechanisms are equivalent to those described in mammals. Aknowledegments: FONDECYT 11121217



122) **Role of the PON1$_{Q192R}$ polymorphism in the cognitive performance of agricultural workers exposed to organophosphate pesticides in the north of Chile (Coquimbo Region).**

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Organophosphate pesticides (OPs) are widely used worldwide with both domestic and industrial purposes. Studies on situations of chronic or acute exposure have revealed numerous health effects attributed mainly to the inhibition of the enzyme acetylcholinesterase (AChE). Besides the peripheral physiological function of this enzyme, AChE is also involved in cognitive processes within the brain. On the other side, it has been described that the paraoxonase 1 (PON1) enzyme involved in the detoxification route of OPs, displays polymorphisms that account for human susceptibility to OP exposure. Although the physiological and toxicological role of PON1 has been well described, its role in the cognitive impairment observed in people chronically exposed to OPs has not been well established yet. In this study we wanted to evaluate the effect of the chronic exposure to OPs on cognitive performance and on the activities of the enzymes used to evaluate acute poisoning (erythrocyte AChE and plasma cholinesterase, ChE) of 93 agricultural workers and 85 non-exposed people and correlate these data with the PON1$_{Q192R}$ polymorphism. The neuropsychological battery consisted of 31 tests that evaluated general mental state, memory, attention, praxis, executive functions, motor coordination, language and mood.

The results indicate that the AChE and ChE activities from the exposed group did not differ from those measured in the unexposed group. In the exposed group the mean scores of 21 tests were significant lower compared to the scores of the unexposed group. Furthermore, the individuals who showed a performance under the normal scores in the 90% of the tests belong to the exposed group. The analysis of the sample after stratifying the population according to 4 levels of impairment (0=normal; 1=borderline; 2=mild impairment and 3=severe impairment) reveals that only the activity of AChE displays a significant inhibition showing less catalytic activity in the individuals with higher deterioration. Finally, our results do not show a role of PON1$_{Q192R}$ polymorphism in global cognitive impairment; however, lead us to put special interest in individuals with QQ genotype (or Q allele carriers) due to they showed a higher percent of impairment in areas such as attention, executive function and motor coordination. This work provides information of great importance in terms of occupational health and environmental toxicology, as it has been described that the Q allele (present in about 60% of the studied population) is less efficient than the R allele for metabolizing chlorpiryfos, the main OP pesticide used in the study area.

This research was supported by FONDEF (Fondo de Fomento al Desarrollo Científico y Tecnológico, CONICYT Chile) Grant number D09I1057 and FONDECYT (Fondo Nacional de Desarrollo Científico y Tecnológico, CONICYT Chile) Grant number 3120231.

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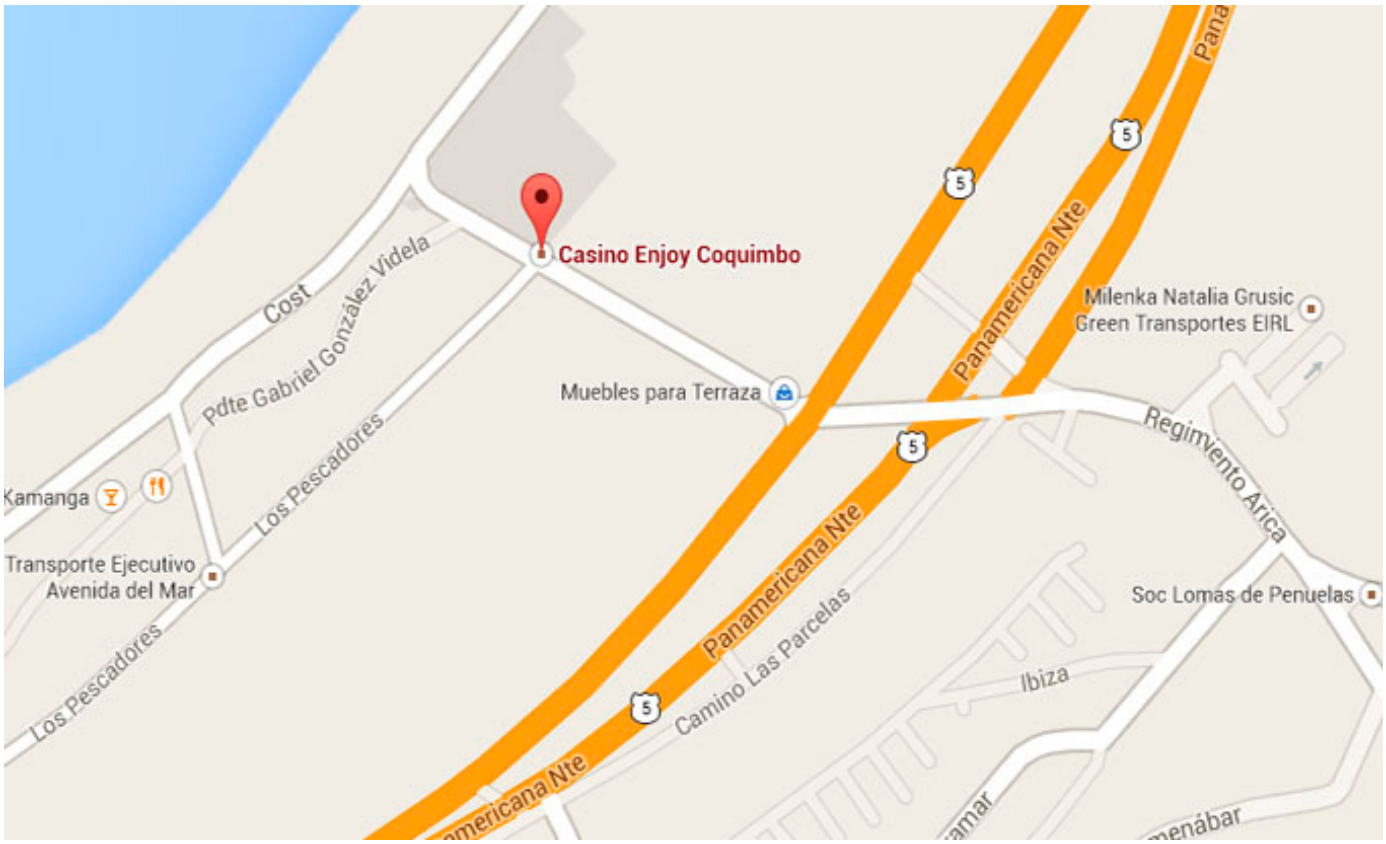

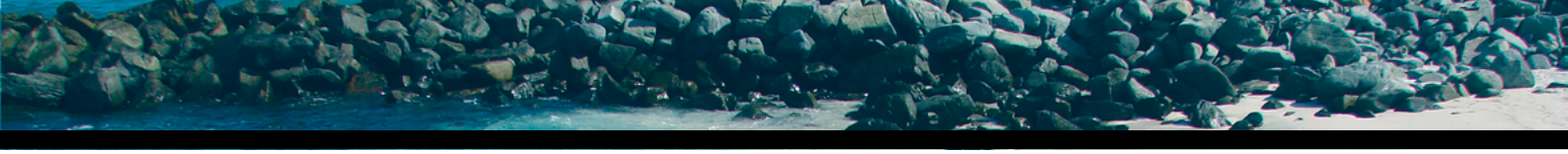

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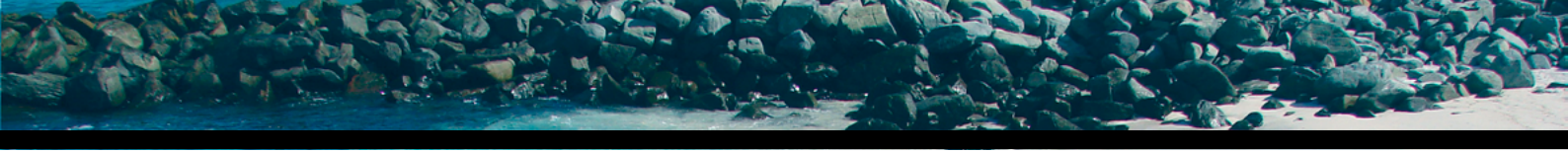

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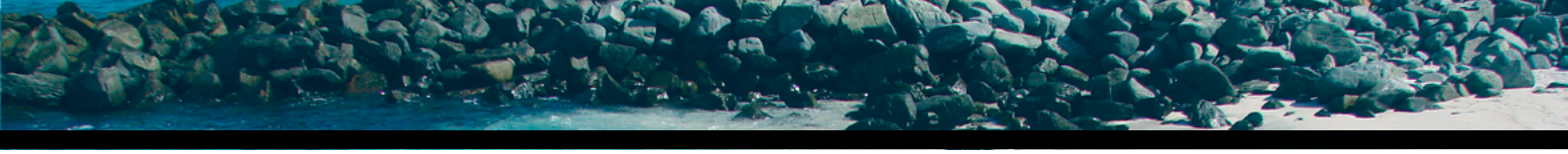

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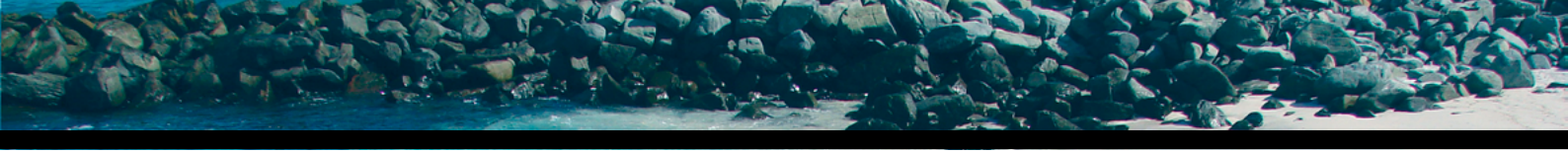

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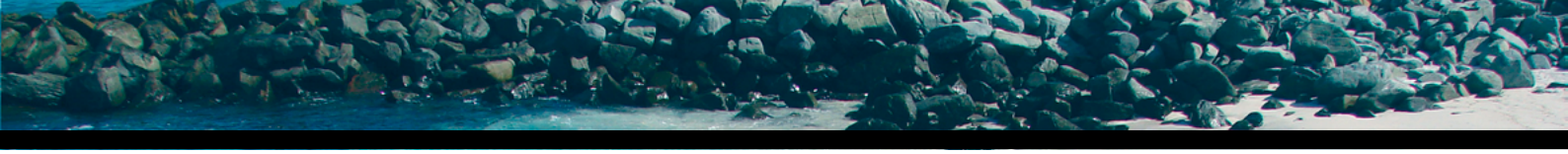

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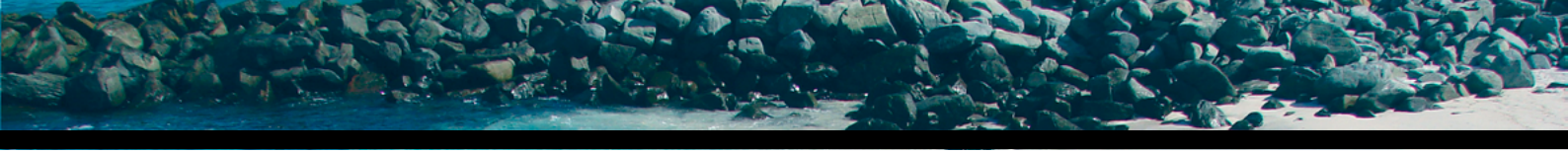

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