Name of the Center: CENTRO INTERDISCIPLINARIO DE NEUROCIENCIA DE VALPARAÍSO	
Acronym	CINV
Code	P09-022-F
Reported period	January, 2013 to December 2013
Starting date of the Center	08/08/2011
Address	Pasaje Harrington 287, Playa Ancha, Valparaíso.
Telephone(s)	056-032-2508040
Fax	056-032-2508047
Web Page	www.cinv.cl
Host Institution(s)	Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso, Pontificia Universidad Católica de Chile, Centro de Modelamiento Matemático de la Universidad de Chile, Universidad de Talca
Contact Information	
Scientific Contact	*Ramón Latorre de la Cruz* *CINV Director*
Electronic Address	*Ramón.latorre@uv.cl*

Institute / Nucleus Principal Researcher Name	*Institute / Nucleus Deputy Principal Researcher Name*
Ramón Latorre de la Cruz	*Alan Neely Delgueil*
Principal Researcher's Signature	*Deputy Principal Researcher's Signature*

Index

1.1 Executive Summary:

The main aim of the **Interdisciplinary Center for Neuroscience of Valparaíso (CINV) Millennium Institute** is to transform the CINV into an **outstanding multidisciplinary, interregional science center.** During this second year of activities we have accomplished one of the main goals proposed in the original application namely the Ph.D. Program in Biophysics and Computational Biology. We have also made significant progress in two other important areas for the development of CINV. First, the new building that will host CINV is under the final steps of approval by the Regional Government; and second, a program designed to hire young investigators is underway with the support of the Max Planck Institutes.

The CINV deals with several aspects of a fundamental scientific question: ***How does the Nervous System respond to Stimuli in Health and Disease?*** We are addressing this question along four research lines and one cross-cutting theme, which have continued to show increasing associativity and qualitative and quantitative progress during this second year. Scientific progress made during the second year of the CINV as a Scientific Millennium Institute (ICM) is summarized as follows.

1. Structure and Function of Molecular Sensors. Using electrophysiological, fluorescence techniques (Lanthanide Resonance Energy Transfer (LRET) and voltage clamp fluorometry (VCF)) and molecular simulations (collaborative effort with line 5) we have increased our understanding of the molecular workings of the voltage sensor in voltage-dependent proton (H_v), Ca^{2+}- and voltage-activated K^+ and voltage-dependent Ca^{2+} channels. It is especially noteworthy that we have, for the first time, been able to detect H_v channel gating currents and voltage-dependent movements of extracellular connexin (Cx) domain by VCF (in association with line 2). We also have unveiled the molecular determinants of temperature-sensing in transient receptor potential ankyrin 1 (TRPA1), a cold-receptor channel.

2. **Cellular Signaling.** We studied Neimann Pick disease and occulodentodigital diseases and found an increase in hemichannel (HC) activity while gap junctional communication was reduced. We also demonstrated that HIV viral infection in astroglial cells increases HC activity possibly favoring the neuroinflammation and dementia associated with HIV infection. Furthermore, we discovered that functional Panx1 channelsare required for the infection of T cells by HIV virus, opening novel therapeutical approaches to treat neuroinflammatory diseases. We continued exploring the molecular mechanism of syndromic deafness finding that the disease could be the consequence of aberrant Cxs interactions. We also found that *the novo* expression of Cx hemichannels in denervated fast skeletal muscles leads to atrophy. We demonstrated the role of Panx1 channels in hippocampal synaptic plasticity and showed that they control Ca^{2+} signals induced by activation of cholinergic receptors in chromaffin cells. In the latter, we also found that Src kinase and cortactin promote *de novo* actin polymerization, and that the newly formed actin filaments accelerate the expansion of the initial pore.

3. Genetic and Developmental Neuroscience. We have shown that the odor environment can negatively regulate olfactory receptor expression in early development and are exploring the underlying mechanisms of this epigenetic interaction. Because of our novel work on adult Neural Stem Cells and their capacity to generate neuroendocrine cells essential for puberty, we have initiated a collaboration to analyze new genes underlying human Kallmann syndrome using zebrafish as a model system. In *Drosophila* we have used genetically encoded calcium indicators and mutants to understand how neuropeptides regulate the expression of a sequential behavior. Finally we have also made progress in the understanding how the circadian clock imposes a daily rhythm on behavior.

4. Systems Neuroscience. In system and circuits neuroscience, we discovered that Alzheimer disease biomakers present in the brain of *degus* during aging are also present in their retina, where they affect the normal physiological function of the eye. We are also working to understand the mechanism involved in the alteration of neural plasticity in the pre frontal cortex after induced post natal stress in rodents and we postulate a differential participation of AMPA and NMDA receptors. Sensory coding in the retina is being studied by multielectrode (MEA) and patch clamp assays, complemented by

histological and imaging techniques. A new regulatory pathway of OFF bipolar cells through nitric oxide (NO) was unveiled. In the olfactory system, field potential oscillations and their information contents were studied in the olfactory bulb and telencephalon of teleosts.

5. Cross-cutting: ***Molecular Simulations, and Computational Biology.*** Using non-equilibrium molecular dynamics with the application of external electric fields, we are investigating the ion translocation processes and voltage gating mechanisms that occur in ion channels and gap junction channels. Our simulations also unveiled a novel water pocket located in the cytoplasmic portion of Cx26. We have developed molecular models of ion channels including the pore region from K^+ channels (Shaker and BK), TRP channels, and Ca^{2+} channels, currently under experimental validation. Additionally, we performed large-scale sequence analyses of the TRP channel family based on sequence similarity networks. In the field of neural excitability, a mathematical model for the dynamic response of cold thermoreceptors, allowed us to explain the contribution of the TRPM8 channel in acute cold sensing within a wider context. We are also analyzing the peptidergic neural network that regulates the ecdysis behavior, and a neural circuit that detects motion direction in retina.

Advanced training and new investigators: Through an International search, CINV incorporated two new scientists into its faculty: Dr. Carlos González has made fundamental advances in our understanding of H_v channels and Dr. Andrés Chávez was one of the first to unveil the molecular targets of endocannabinoids and their importance in synaptic function. On the other hand, talks are well underway with the Max Planck Institutes to create at CINV a Max Planck Research Group aiming to hire during 2015 two Max Planck Research Leaders as part of our program to recruit young investigators

In this period many of our Ph.D. students have visited laboratories abroad learning new techniques and/or doing experiments that could not be performed in our center. During this year's recruitment period, our PhD and Master's programs in Neuroscience were very successful. We received 22 and 35 applications for our PhD and Master program, respectively. At the Doctoral level we accepted 7 students while 17 entered the Master program. We believe that our recruitment strategy has become more effective as we now receive enough applications to be able to select the very best.

Networking: The strengthening of our network of international collaborations has also resulted in exciting research projects. A few highlights are listed below: i) new advances in the detailed mechanisms by which the Na^+/K^+ pump translocates K^+ ions; ii) the molecular workings of the voltage sensor of Ca^{2+} channels using voltage clamp fluorometry; and iii) our International Networking with French INRIA researchers organized III LACONEU Summer School in Neuroscience held in Valparaiso and we obtained an ECOS-CONICYT (2014-2016) exchange program.

Outreach: This year, as in the past period, members of CINV delivered lectures in high schools within the Valparaíso Region and in other institutions reaching about 1,500 students. We continued the series "***Tertulias Porteñas***". In the current year, the subject of pain and perception were approached from different perspectives by distinguished panels of scientists, artists and communicators. We created the TV series "***Neuromantes***" set in Valparaíso, which addresses a topic of general interest from 2 or more perspectives but always includes a CINV scientist. We have, through private funding, secured a space (called "Edificio Verde") dedicated to the junior high school science outreach ***Ciencia Al Tiro*** program. We have developed and implemented new laboratory workshops for this program and completed the final layout of our book, "*La Alegria de la Ciencia*", containing 12 workshops of *Ciencia Al Tiro*.***A new house for CINV***. The "Severin" building project, CINV`s new home, is well advanced and is in the last stages of approval by the Regional Government. The University of Valparaíso has reserved 3 million dollars from the 2013 budget for its construction. A recent report of the UNESCO Advisors mentions this project as one that should be supported by the Chilean government

1.2 Resumen Ejecutivo

El principal objetivo del Instituto Milenio **Centro Interdisciplinario de Neurociencia de Valparaíso (CINV)** es transformar al CINV en un **centro científico interregional de excelencia**. Durante este segundo año hemos podido alcanzar una de las principales metas propuestas en el proyecto original: El programa de doctorado en Biofísica y Biología Computacional fue aprobado por la Universidad de Valparaíso. También hemos progresado en dos áreas cruciales para el desarrollo del CINV. Primero, el nuevo edificio que albergará al CINV está en sus etapas finales de aprobación por el Gobierno Regional; y segundo, en colaboración con los Institutos Max Planck diseñamos un convenio cuyo objetivo es atraer investigadores jóvenes.

El principal objetivo del CINV es abordar una pregunta científica fundamental maximizando la colaboración entre nuestras cinco líneas de investigación que incluyen una línea transversal: ***¿Cómo responde el sistema nervioso a estímulos, en condiciones fisiológicas y patológicas?*** Los progresos científicos realizados durante este segundo año como Instituto Científico Milenio se pueden resumir como sigue: 1. **Estructura y función de Sensores Moleculares**. Usando técnicas electrofisiológicas, de fluorescencia [Transferencia de Energía Resonante basada en Lantánidos (LRET) y fluorometría a potencial controlado (VCF)] y de modelación molecular (con la línea de investigación 5) hemos continuado aumentando nuestro entendimiento de cómo funcionan los sensores de voltaje en los canales de protones (H_v), los canales de K^+ activados por Ca^{2+} y voltaje y los canales de Ca^{2+} dependientes de voltaje. Se hace necesario resaltar aquí que hemos sido capaces de develar las corrientes de compuerta en H_v y los movimientos de un dominio extracelular de una conexina (Cx) usando VCF (en asociación con Línea 2). Hemos también descubierto los determinantes moleculares que dan origen a la sensibilidad a la temperatura en el receptor de potencial transitorio ankirina1 (TRPA1) un receptor de frío. **2. Señalización Celular.** Hemos estudiado la enfermedad de Neimann Pick y las enfermedades oculodentodigital, encontrando un aumento en la actividad de los hemicanales (HC) al mismo tiempo que la comunicación mediada por uniones en hendidura disminuye. Hemos demostrado también que la infección por el virus del SIDA (VIH) de las células de la astroglía aumenta la actividad de los HC, posiblemente favoreciendo la neuroinflamación y la demencia asociada a la infección. Descubrimos que se requieren canales funcionales de panexina1 (Panx1) en la infección de las células T por el VIH, lo que abre nuevos acercamientos terapéuticos para el tratamiento de enfermedades neuroinflamatorias. Hemos continuado explorando los mecanismos moleculares de la sordera sindrómica encontrando que la enfermedad puede ser una consecuencia de interacciones aberrantes entre Cxs. Descubrimos que la expresión *de novo* de hemicanales de Cx en músculos esqueléticos rápidos denervados lleva a la atrofia. Revelamos también el papel de la Panx1 en la plasticidad sináptica en el hipocampo y demostramos que Panx1 controla las señales de Ca^{2+}inducidas por la activación de receptores colinérgicos en células cromafines. En estas células encontramos que la quinasa SRc y la contactina promueven la polimerización de la actina y que los nuevos filamentos de actina que se forman aceleran la expansión del poro de fusión. **3. Genética y Neurociencia del Desarrollo.** Hemos demostrado que en el desarrollo temprano los olores ambientales pueden inhibir la expresión de receptores olfatorios y estamos explorando los mecanismos que subyacen esta interacción epigenética. Descubrimos que las células madres adultas tienen la capacidad de generar células endocrinas esenciales en la pubertad. Esta observación nos condujo, usando el pez cebra como sistema modelo, a iniciar un trabajo de colaboración para analizar los genes involucrados en el síndrome de Kallmann. Con el objetivo de entender cómo los neuropéptidos regulan la expresión de un comportamiento secuencial, hemos codificado genéticamente en la *Drosophila* indicadores de Ca^{2+}. También hemos progresado en nuestro entendimiento de cómo el reloj circadiano impone un ritmo diario al comportamiento. **4. Neurociencia de Sistemas.** Descubrimos que los "biomarcadores" de la enfermedad de Alzheimer presentes en el cerebro del *degus* durante el envejecimiento están también presentes en su retina afectando la función fisiológica normal del ojo. Estamos tratando develar los mecanismos involucrados

en la alteración de la plasticidad neural en la corteza pre frontal después de inducir un estrés postnatal en roedores y postulamos la participación diferencial de receptores AMPA y NMDA en el. Usando multielectrodos y "patch clamp" complementados con técnicas histológicas y de imágenes estamos estudiando la codificación sensorial en la retina. Revelamos un nuevo camino regulatorio mediado por óxido nítrico. En el sistema olfatorio (bulbo olfatorio y teléncefalo de teleósteos), estudiamos las oscilaciones en los potenciales de campo y la información que ellas contienen. **5. Transversal: *Simulación Molecular y Biología Computacional.*** Estamos investigando los procesos del movimiento de iones y los mecanismos que median la voltaje dependencia en canales de iones y en los canales de las uniones en hendidura, usando dinámica molecular del no equilibrio y aplicando campos eléctricos externos. Nuestras simulaciones revelaron un bolsillo acuoso localizado en la región citoplasmática de Cx26 que juega un papel importante en la permeabilidad de este canal. Hemos desarrollado modelos moleculares del poro de los canales de K^+ (Shaker y BK), así como de los canales TRP y Ca^{2+}, modelos que se encuentran en estos momentos siendo validados experimentalmente. En el campo de la excitabilidad neural, un modelo matemático para la respuesta dinámica de termoreceptores de frío nos ha permitido explicar la contribución del canal TRPM8 en la sensación de frío agudo. Estamos analizando la red peptidérgica neural que regula el proceso de ecdisis y el circuito neural que detecta dirección del movimiento en la retina.

Entrenamiento avanzado y nuevos investigadores: A través de un llamado internacional hemos incorporado a dos nuevos miembros del CINV: Dr. Carlos González quien ha hecho importantes avances en el campo de los canales H_v y al Dr. Andrés Chávez quien fue uno de los primeros en develar los blancos moleculares de los endocanabinoides y su importancia en la función sináptica. Por otra parte, en conjunto con los institutos Max Planck estamos desarrollando un programa con el objetivo de crear en el CINV un "Max Planck Research Group", que se iniciará con la contratación de dos investigadores jóvenes o "Max Planck Research Leaders".

En este periodo, los estudiantes de doctorado han aprendido nuevas técnicas y/o hecho experimentos en laboratorios en el extranjero, que no se pueden realizar en el CINV. Tanto nuestro programa de doctorado como el de magister han sido exitosos recibiendo 22 y 35 postulaciones, respectivamente. A nivel doctoral se aceptaron 7 y al magister entraron 17 estudiantes. Esto demuestra que nuestras estrategias de reclutamiento han sido exitosas permitiéndonos reclutar a los mejores estudiantes.

Redes: Durante este periodo las redes nacionales e internacionales se reforzaron, y aquí mencionamos solo algunos puntos destactados: i) Nuevos avances en los mecanismos mediante los cuales la bomba de Na/K transporta K^+; ii) Usando VCF hemos podido detectar los cambios de conformación que sufren los sensores de voltaje durante la activación de los canales de Ca^{2+}; and iii) Nuestra red internacional con investigadores Franceses organizó la III Escuela de Verano LACONEU en Neurociencia en Valparaíso.

Proyección al medio: Durante este año, así como en el pasado, los miembros del CINV han dictado clases dentro de la región de Valparaíso a aproximadamente 1500 estudiantes. Hemos continuados con la serie de ***Tertulias Porteñas*** y durante este periodo temas como el dolor y la percepción se trataron desde diferentes perspectivas por paneles de distinguidos artistas, comunicadores y científicos. Creamos la serie de TV ***"Neuromantes"*** ambientada en Valparaíso que trata un tema de interés general desde más de una perspectiva y siempre incluye un científico del CINV. Fondos privados permitieron la construcción de un espacio ("Edificio Verde") dedicado al programa "***Ciencia al Tiro***" para llevar la ciencia a estudiantes de educación media. Nuevos talleres de laboratorio se han implementado en este programa y se completo la primera versión del libro "*La Alegría de la Ciencia*" que contiene la 12 de los talleres de *Ciencia al Tiro**.Una nueva casa para el CINV.*** El proyecto del Edificio "Severín" está en las últimas etapas de aprobación por el Gobierno regional y la Universidad de Valparaíso ha comprometido 1500 millones de pesos de su presupuesto para su construcción. Un reporte reciente de consejeros de la UNESCO menciona que este proyecto debe ser aprobado por el gobierno chileno.

2. Introduction

a) Description of the Institute

Our Center deals with several aspects of a fundamental scientific question: *How does the* Nervous *System respond to Sensory Stimuli in Health and Disease?* We are addressing this question by investigating not only the channels that transduce the stimuli but also how these stimuli result in a functioning organism. The central themes of our research are: molecular transduction, intercellular communication, neural correlates of perception, the generation of appropriate behavioral responses, and the genetic pathways controlling development and complex behaviors. Our research is organized along four broad lines and one cross-cutting theme. Most investigators are involved in more than one research line and specific research questions are investigated through efforts carried out by different research teams.

b) Research Lines:

Structure and Function of Molecular Sensors: This line of research seeks to understand, at a molecular level, how ion channel proteins are able to sense stimuli such as temperature and voltage, and how the Na^+/K^+ pump transports Na^+ and K^+ across the cell membrane. Using a variety of different experimental techniques that include electrophysiology, molecular biology, fluorescence, and molecular modeling we have made progress in understanding how the voltage sensor of different ion channels work; the molecular nature of cold sensitivity in TRPA1 channels; and how the Na^+/K^+ pump translocate ions (*e.g*., *Neuron* 77:288-98, 2013; *J Gen Physiol*. 141: 275-85, 2013). This line of research is composed by the P.I. (R. Latorre), the Co-PI (A. Neely), Assoc. Invs. D. Naranjo, F. Bezanilla, M. Holmgren and C. González (who was incorporated to CINV last year and recently tenured by the University of Valparaíso). Collaborations between members of this line of research, collaboration with other lines of research (line 2; e.g., *Proc. Natl. Acad. Sci. (USA)* **110:**16229-16234, 2013) and international collaborations (e.g., Martin et al., *Pflügers Archiv* – 2013) have continued to increase.

2. Cellular Signaling: This line of research investigates the physiological mechanisms that regulate cell-to-cell communication pathways and their alterations in neurodegenerative diseases. Cells of the nervous system communicate with each other via extracellular signals released by cytoskeleton- and dynamin-dependent vesicular system. Intercellular communication also occurs via gap junction channels and hemichannels composed of connexins or pannexins, which communicate the cytoplasms of adjacent cells or the intra and extracellular compartments, respectively, to allow electrical and metabolic transfer. The goals are to unveil the functional role of these pathways in physiological function and pathological conditions related to the nervous system, and that could allow us to find therapeutic targets and design molecules that inhibit or activate them. This research line includes the Assoc. Inv. J.C. Sáez and A.M. Cardenas, and the Adjunct Inv. A.D. Martinez. Collaborations within the group (e.g.,*Biochim Biophys Acta.* 1818:1169, 2013) and collaboration with other lines of research (1 & 5) (e.g., *Proc. Natl. Acad. Sci USA **110:** 16229, 2013*) are thriving. The interaction with the Cross-cutting line has been relevant to develop molecular models of hemichannels bearing mutations associated with some forms of deafness as well as to identify specific inhibitors of hemichannels.

3. Genetic and Developmental Neuroscience. This line of research uses modern molecular-genetic tools to dissect the mechanisms underlying development and behavior in intact animals using *Drosophila* and zebrafish as model systems. Using a reporter line generated in the lab to visualize the differentiation of cells *in vivo* we have proposed a new model for vertebrate peripheral nervous system development (Torres-Paz and Whitlock, submitted *Dev. Dyn*). Using *Drosophila* we visualized the patterns of neuronal activity induced by neuropeptides as they express specific behaviors (Mena and Ewer, in preparation). We have built on our strengths in imaging and genetics to visualize neuronal activity in whole animals by expanding our work with genetically encoded calcium and luciferase reporters with the addition of a postdoc (Dr. Isabel Benjumeda, Spain). We secured funding for the

purchase of several specialized imaging systems to monitor *in vitro* activity of clock gene-driven luciferase and olfactory neuron-GCaMP transgenes in flies and fish, respectively. Collaboration between lines has resulted in several publications (e.g., *PLOS One*, 8:e69574, 2013 with line 5 and *Cold Spring Harb Protoc*, 4:312-318, 2013 with line 4). We have continued to work with the line 5 in an effort to identify potential regulatory regions for olfactory receptors. Outside Chile we have initiated collaborations in the area of neural and hormonal control of postecdysial behaviors in insects (White, B. H. and J. Ewer, 2014; Dr. Benjamin White, NIH) and genetic control of Kallmann Syndrome in humans (Dr. William Crowley, Harvard Medical School).This research line is composed of Assoc. Inv. K.Whitlock, J.Ewer, and Adjunct Inv. R. Greenspan.

4. System Neuroscience and Circuits. This line works on Circuitry and Neural Plasticity where we study the neural capacity (mechanism and circuits) for learning and memory under normal and pathological states. Recently, we have suggested that in human and several rodent models, including *Octodon degus*, the retina may be an excellent biomarker for Alzheimer Disease during aging (Chang et al. *Alzheimer & Dement* 2014). In the rodent retina, we are working on the complex regulatory mechanism of glutamate responses in type 3A/B and 4 OFF bipolar cells by NO and on the role of pannexin1, where we found strong electrophysiological evidence that endogenous pannexin 1 form intracellular channels between primary oligodendrocytes in culture (work done with Line 1). We are interested in the mechanism that underlies stress-induced impairment of the recall of conditioned fear extinction, and found that a reduction in the AMPA-, but not the NMDA-mediated response, results in a decrease in the basal synaptic transmission (*Behav Brain Res* 259:342.352, 2014). The research team is composed by the P.I. (A. Palacios) and Assoc. Inv. O. Schmachtenberg, P. Muñoz, and Dr. A. Kirkwood, (J. Hopkins U) and Dr. Andres Chavez is joining the team in 2014.

5. Cross-cutting. *Molecular Simulations, and Computational Biology*. Using non-equilibrium molecular dynamics with the application of external electric fields, we are investigating, at the molecular level, ion translocation processes and voltage gating mechanisms in ion channels and gap junction channels. These simulations revealed that residue Arg143, located within a cytoplasmic water pocket of Cx26 hemichannels, play an important role in the permeability and voltage gating of Gap-Junction channels. We have developed molecular models of the pore region of K^+ (Shaker and BK), TRP (TRPV1 and TRPM8), and Ca^{2+} channels. Additionally, we performed large-scale sequence analyses of the TRP channel family based on sequence similarity networks. Modeling neural excitability, a mathematical model for the dynamic response of cold thermoreceptors, allowed us to explain the contribution of the TRPM8 channel in acute cold sensing at a cellular level. We are also analyzing the peptidergic neural network that regulates the ecdysis behavior, and a neural circuit that detects motion direction in retina.The research team is composed by the P.I. (F. González-Nilo) and Assoc. Inv. T. Pérez-Acle and P Orio. This line of research has built strong ties with lines 1, 2, & 3.

Conclusion. Scientific productivity has kept its pace during this second year and, as the first year, some of our studies entered into high impact journals (e.g., *PNAS, Neuron*). Collaborations between lines have further improved and, in particular, the Cross-cutting line has been fundamental to the interpretation of experimental data of other research lines. CINV has become known by the general public in Valparaíso through its outreach program and Networking is strong.

c) ***Organization of researcher's team:*** As described in the text above

3. Scientific and technological research:

a) Current status of research lines:

1. Structure and Function of Molecular Sensors:

Voltage-dependent K^+ channels are responsible for the repolarizing phase of the action potential. The BK channel is activated by depolarizing voltages and cytoplasmic Ca^{2+}, and is therefore the perfect molecular machine to retard or stop excitatory signals like those produced, for example, by voltage-dependent Ca^{2+} (Cav) channels. Voltage-dependent K^+ channels form a large superfamily characterized by an extremely well conserved voltage sensor and pore domains. Themes of interest to this research lineinclude the mechanisms that allow the transformation of electrical energy into mechanical energy (pore opening), and the one that allows this K^+ channel superfamily to be exquisitely selective to K^+ (Latorre and Contreras, 2013(Commentary); Reviewed in Contreras et al., 2013).

BK channel*: *Role of BK channels in cell viability: pharmacological modulation. We found that BK channel regulates cell viability under hyperkalemia but not hypokalemia conditions. The drugs bendroflumethiazide and acetazolamide were the most potent drugs in activating the BK current and in preventing cell proliferation induced by hyperkalemia. These findings illustrate the relevance of BK channels in disorders associated with abnormal K^+ ion homeostasis that include periodic paralysis and myotonia (Tricarico et al. 2013; work done in collaboration with Dr. Tricarico Laboratory, University of Bari, Italy).

*BK channel external architecture determined with a spectroscopic ruler.*Despite its importance little is known about its detailed structure. We have determined the external architectural intimacies of BK α-subunit using Lanthanide-based Resonance Energy Transfer (LRET) as a molecular ruler. For this we used a genetically encoded Lanthanide Binding Tag (LBT) that binds Tb^{3+} as LRET donor; as acceptor we used the scorpion toxin, iberiotoxin (IbTX) labeled with the fluorophore BODIPY-FL, which binds to the pore domain of the channel near the channel symmetry axis. By analyzing the sensitized emission of BODIPY-FL, we determined a basic extracellular map of the BK channel. We were also able to describe the β1 subunit-induced rearrangements in the α-subunit. The methodology presented gives us the first glimpses to the external surface structure of the BK channel in its different functional states, with and without the β1-subunit *(To be submitted;* work done in collaboration with the laboratory of Dr. Francisco Bezanilla and Line 5.).

The BK Channel S6 Transmembrane Domain is a Stimuli Integration Node. BK channel opening is mediated by a cross-talk between the modular voltage and Ca^{2+} sensors. BK open probability is enhanced by increasing cytosolic Ca^{2+} concentration and/or depolarization. These stimuli activate sensors that are allosterically coupled to channel gating. We found that mutating a phenylalanine (F380A) located in the S6 transmembrane helix profoundly hinders channel opening with minor changes in voltage sensor displacement. Interpretation of these results using an allosteric model suggests that the F380A mutation mainly modifies the closed-open channel equilibrium and uncouples Ca^{2+} binding and voltage sensor activation from channel opening. Based on these functional studies, we propose a structural model that sheds light on the molecular nature of the coupling between Ca^{2+} and voltage sensor activation and pore opening. Moreover, the mutation F380A could be used for detailed studies of the voltage-sensor in the presence of permeant cations (Submitted to *J. Gen. Physiol*. Work done in collaboration with Line 5).

***Shaker K^+ Channel*: *Closing the gap between low and high conductance K-channels*.** The extremely conserved signature sequence in the residues forming the pore selectivity filter allows exquisite K^+ selectivity. However, closely related K-channels display differences of up to ~100-fold in unitary conductance. Can we approach BK-like conductance by increasing channel ion occupancy along the pore? We found that substitution Pro475Asp at the internal entrance, and away from the selectivity filter of the low conductance Shaker K-channel, increases unitary conductance 8-fold, reducing to 3-fold the gap with BK high conductance K-channels. We also made Shaker-variants having additional

negatively charged residues in several positions along the inner cavity and measured their unitary conductance in a wide range of symmetrical [K+]. Because no variant approached BK conductance beyond that of Pro475Asp, we tested for differences in pore architecture. By measuring diffusion-limited outward currents we found that Shaker's internal pore is narrower than BK channels. MD simulations showed that this narrow pore imposes diffusion limit and additional dehydration steps in Shaker-derived channels, likely opposing to this channel's large conductance *(To be submitted in collaboration with line 5).*

Ca_V channel: *Contribution of Cav Channel Domain to Voltage sensing*: In a collaborative effort with Dr. Olcese from UCLA using Voltage-Clamp fluorometry (VFC) we detected voltage-dependent changes in fluorescence (V-ΔF) from probes attached to each individual voltage-sensor of the human calcium channel. Surprisingly, only two of the four voltage sensors contribute significantly to channel opening. These results are well described by a model that assumes that the voltage-sensors are connected to the pore via allosteric interactions. We also made two important discoveries: 1) the absence of an important auxiliary subunit (alpha2delta) results in a channels in which the voltage-sensing structures do not seem to contribute to channel opening. 2) A calcium channel mutation that causes a multisystem disease called Timothy syndrome and thought to impair the channel inactivation gate appears to profoundly modify voltage-sensing activity according to our fluorometric studies.

Proton (H_v) Channels.*H_v voltage sensor revealed.* Hv channels are integral membrane proteins homologous to the Voltage Sensor Domain (VSD) of Kv channels with the capacity to permeate elementary particles in a voltage and pH dependent manner. Our data show that the three S4 charges are the main voltage-sensing residues responsible for the voltage dependence of Hv channels and that the extent of movement of these S4 charges is sufficient to explain the voltage dependence of opening in Hv channels (González et al., 2013). Using voltage clamp fluorometry, we detected two conformational changes reported by a fluorophore attached to the voltage sensor S4 in Hv1 channels. The first conformational change is consistent with the previously reported outward movement of the positively charged S4 upon membrane depolarization. The second one is consistent with a concerted conformational change that opens the two permeation pathways in the two subunits in the dimeric Hv1 channel. (Qiu et al., 2013).

H_v gating currents detected. We have been able to obtain the first Hv1 gating currents recordings from the monomeric Hv form (Hv-ΔNΔC). Interestingly, the voltage sensor activation precedes the H^+ conduction, suggesting that the voltage sensor and the H^+ pore are two different allosterically coupled structures. The Q-V presents a zδ of 1.6 e0, in agreement with previous experiments of voltage-clamp fluorometry. Our data support the hypothesis that the channel opening is associated to a second conformational state of the voltage sensor, associated to an extremely slow component in the gating current of the channel.

H_v channel conduction pathway. Hv1 channels lack a classic pore domain and the mechanisms by means of which protons move through this highly proton selective channel are unclear. Using non-stationary fluctuation analysis we determined that the conductance for the dimer and the monomer are 200 and 100 fS, respectively. Mutations at position S191 (S2) and N264 (S4) modified the unitary conductance of Hv channel. Introducing an asparagine in the equivalent positions in either a nonconductive Shaker K^+ channel or in the non conducting voltage-dependent phosphate Ci-VSP induces the formation of a voltage-gated H^+ channel. Thus, S191 and N264 form the molecular determinants of permeation pathway in Hv

*Structural models of the open H_v channel.*We compared the different structural models of voltage-gated proton channel proposed to date, how they were obtained, their assumptions and predictions, with an emphasis on the experimental data that support them. The highlight of the most significant structural differences, focused in the seven existing models of the open conformation of Hv1 allowed us to

propose a new structural model of Hv1 in the open conformation, which explains better our experimental data (Pupo et al., in Press *Channels*, 2014).

TRP channels.*Gating of Thermally Activated Channels.*A class of ion channels that belongs to the transient receptor potential (TRP) superfamily and is present in specialized neurons that project to the skin has evolved as temperature detectors. Some of these channels are activated by heat (TRPM2/4/5, TRPV1-4), whereas others by cold (TRPA1, TRPC5,TRPM8). We have recently reviewed the general biophysical properties of these temperature receptor channels by considering them as allosteric proteins with polymodal gating. The identification of molecular determinants of temperature sensitivity in TRPV1, TRPA1 and TRPV3 strongly suggest that thermal sensitivity arises from a specific protein domain (Reviewed in Báez et al. In Press *Current Topics in Membranes*).

*Single-point mutations in ankyrin repeat 6 make mouse TRPA1 sensitive to warm temperatures.*Several transient receptor potential (TRP) ion channels are activated with high sensitivity by either cold or hot temperatures. However, the structures and mechanism that determine temperature-directionality (cold vs. heat) are not established. Here we screened 12,000 random mutant clones of the cold-activated mouse TRPA1 ion channel with a heat stimulus. We identified three single-point mutations that are individually sufficient to make mouse TRPA1 warm-activated, while leaving sensitivity to chemicals unaffected. Mutant channels have high temperature-sensitivity of voltage-activation, specifically of channel opening, but not channel closing, which is reminiscent of other heat-activated TRP channels. All mutations are located in ankyrin repeat 6, which identifies this domain as a sensitive modulator of thermal activation. We propose that a change in the coupling of temperature-sensing to channel-gating generates this sensitivity to warm temperatures. Our results demonstrate that minimal changes in protein sequence are sufficient to generate a wide diversity of thermal sensitivities in TRPA1 (Jabba et al. *Neuron*in Press; in collaboration with the laboratory of Dr. Ardem Patapoutian).

Na^+/K^+ Pump. The Na^+/K^+ pump is a membrane protein that plays a fundamental role in maintaining the Na^+ and K^+ electrochemical gradients in animal cells. When Na^+ is absent the pump can only undergo K^+translocation reactions. If K^+ travels a fraction of the membrane electric field in at least in one of these reactions, the K^+ binding-unbinding equilibrium become voltage-dependent and sudden changes in voltage will shift this equilibrium generating a transient current signal. We measured K^+ translocation currents by using H2DTG, a reversible inhibitor of the squid Na^+/K^+ pump. Kinetics of these transient currents shows two main components, which, in contrast to their Na^+ counterpart, appeared to be uncoupled. We also found that only the slow component kinetics and charge distribution are dependent on the external K^+ concentration, revealing that the two K^+ reach their binding site before their occlusion takes place. (*to be submitted;* Work done in collaboration with the groups of Dr. Bezanilla and Holmgren).

*In **all** the studies described above participation of Ph.D. students and Postdoctoral fellows has been fundamental.*

2. Cellular Signaling.

Connexins (Cxs) and Pannexins (Panxs) Gap Junction Channels (GJCs) and Hemichannels (HCs). *The activity of HCs and GJCs is increased and reduced, respectively, by Neuroinflammation.* We have previously shown that acute treatment with pro-inflammatory cytokines or with a neurotoxic amyloid peptides increases the astroglia HC activity and reduces intercellular gap junctional communication. It is unknown whether similar responses occur in other neuro-inflammatory diseases. In astroglial cells of NPC-1 mice, model of Niemann Pick type C disease, we found increased HC activity and reduced GJC activity **(**Sáez et al., 8(8):e71361 2013**)**. We also found that HIV infection increases the astroglia HC activity favoring the release of dikkopf-1 protein and that Panx1 channels are required for HIV virus infection of T- Cells (Orellana et al., 2013a & b,).The possible regulation of HCs and GJCs in parasite infections was discussed in a review article (Vega et al., 2013). Moreover, ATP was found to mediate the cytokine-induced formation of gap junction between microglia (Sáez et al., 216402, 2013) and GJCs

were demonstrated to be essential for cell resistance to oxidative stress (Lee et al., 2014). We also demonstrated that cerebral ischemia-induced injury is enhanced in an animal model of oculodentodigital dysplasia disease, which is associated to an increase in astroglia HC activity (Kozoriz et al., 2013).

In collaboration with Line 4, A. Martínez and A. Palacios wrote a review about *Octodon degus* as a model for biomedicine, in particular its advantage as model for Alzheimer disease **(**Ardiles et al., 2013).

We started to investigate the importance of Panx1 channels in the degranulation response of mast cells promoted by a neurotoxic amyloid peptide and the role of astroglia HCs in perinatal viral infections as well as perinatal stress conditions. These results have been presented in national and international meetings and discussed in a review **(**Glia meeting, JNC meeting and Aguirre et al., 2013**).**

The permeability and regulation of HCs to pro-inflammatory mediators. Essential elements of inflammation responses are Ca^{2+} and nitric oxide (NO). Knowledge of the HC and GJC permeability to these agents is limited or unknown. Purified Cx26 HCs and reconstituted in liposomes were found to be permeable to Ca^{2+} (Fiori et al., 2013) and HCs and GJCs formed by all Cx expressed in microcirculation (Cxs 32, 37, 40, 43 and 45) are permeable to NO, whereas cell membranes devoid of Cx HCs do not allow the transfer of biologically significant amount of NO (Figueroa et al., 2013).

We found that linoleic acid induces opening of Cx HCs through a PI3K/Akt/Ca^{2+}-dependent pathway (Figueroa et al., 2013). In addition, we have demonstrated that perinatal stress induces strong activation of astroglia HCs in the brain of neonates. Moreover, we published two reviews: One on Cx and Panx channels in brain and their regulation by signaling molecules (Orellana et al., 2013) and another on the pharmacological properties of astroglial HCs (Giaume et al., 2013).

*Regulation and function of postsynaptic HCs at the neuromuscular unit.*The development of atrophy in fast skeletal muscles due to denervation has been known for many years but the molecular mechanism that underlies this response remains virtually unknown. We found that denervation induces the expression of Cx HCs in myofibers and the lack of expression (KO animals) drastically prevented the atrophy response and activation of the inflammasome in myofibers **(**Cea et al., *2013*). We also found upregulation of $P2X_7$ receptors and TRPV2 channels. While the former interacted with Cx HCs to increase the membrane permeability, the possible role of TRPV2 channels remains unknown. This work was done in collaboration with Dr. R. Latorre (Line 1). Under control conditions, Cx HCs are not expressed and we found that Panx1 channels are essential for ATP release that is required for the potentiation response of muscle contraction (Riquelme et al., 2013).

Pathogenic mechanism of deafness related Cx26 mutations. We continued studying molecular and cellular mechanisms of mutations in the Cx26 protein that cause syndromic deafness. We characterized the effect of different mutations over channel formation and function.

Characterization of Cx26 N-Terminal (NT) deafness mutations. We combined cellular, biochemical and functional analysis of HCs. Cx43 and Cx26 are co-expressed in human skin, but they do not form heteromeric HCs or GJCs. All syndromic mutations in Cx26 NT domain were found to change their oligomerization compatibility allowing the formation of hyperactive heteromeric HCs with Cx43, but non-functional GJCs (a manuscript is currently under revision).

Molecular mechanism of cytoskeletal regulation of GJCs and HCs trafficking and function. The knowledge on the importance of tubulin and actin cytoskeleton on the functional state of HCs and GJCs is limited. This issue could be relevant in cell responses to numerous physiological and pathological processes, going from cell migration to tumorigenesis. In cells that stably express Cx43, treatment with cytochalasin B decreases the size of GJC plaques. In contrast, the amount and activity of HCs in non-appositional plasma membranes increases significantly. In cells that express Cx26, actin depolimerization reduced the size of gap junction plaques and increased the amount of HCs in non-appositional membranes, but HCs have no gain in function. Thus, Cx26 HCs and Cx43 HCs are differential regulated by the actin cytoskeleton.

Regulation of Panx1 channels in hyppocampal synaptic plasticity. The threshold for bidirectional modification of synaptic plasticity is known to be controlled by several factors but the possible role of Panx1 channels has received little attention. We examined the age-dependent effect of deficiency or blockade of Panx1 channels on excitatory synaptic plasticity in CA1 region of hippocampus. We found that blockade of Panx1 channels precluded the induction of LTD in adult but not in young animals. This study is the postdoctoral work of Dr. A. Ardiles in association with the Master degree student C. Flores and in collaboration with Dr. A. Palacios (Line 4).

Panx1 channels in neuroendocrine chromaffin cells. We found Panx1 channels in the adrenal gland where they regulate the release of catecholamines. Inhibitors of Panx1 channels reduced the secretory activity induced with the nicotinic agonist in whole gland, as well in cultured chromaffin cells. Panx1 channel inhibitors also reduced the Ca^{2+} signals evoked by a nicotine agonist in single chromaffin cells, response that was also observed in cells transfected with Panx1 siRNA. Thus, Panx1 channels are relevant in controlling Ca^{2+} signals that trigger the secretory response of adrenal chromaffin cells. This mechanism could have physiological implications during responses to stress. The work is conducted by Dr. F. Momboisse, post-Doctoral fellow (submitted to *Frontiers in cell. Neurosci*.)

Dynamin-2 a multifunctional GTPase.We demonstrated that in addition to its canonical role in the endocytosis, dynamin-2 also regulates the cortical actin dynamics during exocytosis, and that, in coordination with F-actin, it controls the expansion of the fusion pore, anintermediate structure formed during exocytosis **(**González-Jamett et al., 2013**)**. This work was done in collaboration with Dr. Stéphane Gasman (CNRS, Strasburg) and Dr. Neely (Line 1). Dynamin-2 is also involved in the organization of the cortical actin in myocytes, and as consequence of this action also regulates the trafficking of Glut4-containing vesicles. This dynamin-2 function appears to be altered in myocytes expressing dynamin-2 mutations associated to centronuclear myopathy. In fact the cell transfection with these types of mutants drastically disrupts actin organization. This study is part of the postdoctoral project of Dr. A. González-Jamett, and has generated two reviews (González-Jamett et al., 2013; González-Jamett et al., 2014).

The role of the association of dynamin to the β subunit of the voltage-dependent Ca^{2+} channel was studied in a collaborative project between Drs. Cárdenas and Neely (Line 1). This association occurs via a PRD/SH3 interaction, in which the dimerization of the beta-subunit is required. The injection of the SH3 domain of the beta-subunit in chromaffin cells reduced the Ca^{2+} currents, an effect that was reverted by the co-injection of a peptide that interferes with the association of SH3-containing proteins to the PRD of dynamin. Conversely, the injection of a mutant that is unable to dimerize did not affect the Ca^{2+} currents. This work was done by the Master student M. J. Guerra, and was communicated in an international meeting (17-ISCCB, Rouen, France).

Two articles in collaboration with other national and international collaborators were reported. With Dr. F. Nualart, Univ. of Concepción, we studied the expression of the vitamin C transporter (SVCT2) in progenitor cells of the adult neurogenic niche. Vitamin C was found to play a role in the maintenance of the neurogenic niche and treatment with ascorbic acid increased neuronal differentiation and SVCT2 expression in neuronal progenitor cells **(**Pastor et al., 2013**)**. An opinion article was published (Johnson and Sáez, 2013)

3. Genetic and Developmental Neuroscience.

We continue to use genetic tools to understand the development of the nervous system and the resulting behaviors. During this funding period Line 3 has developed collaborations with labs outside of Chile (Germany, England, USA, France) and these collaborations have resulted in (2) co-authored papers.

Neurogenesis and Behavior in Zebrafish. Notably, we have successfully generated neurospheres (neural stem cells), shown that they give rise to the gonadotropin-releasing hormone neuroendocrine cells, and that hormone treatment can increase the number of GnRH cells. This cell type is essential for puberty where defects in the development of these cells underlie Kallmann Syndrome in humans

(Cortés-Campos, Letelier, Westmiller & Whitlock, submitted *Development*; Letelier, Kim & Whitlock, in preparation). We are currently initiating a collaboration with Dr. Bill Crowley (Harvard Medical School) to analyze Rare Sequence Variants (RSVs) that underlie Kallmann Syndrome in humans using zebrafish as a model system. Using the fluorescent reporter line (*six4b:mCherry*) generated in our lab, (Harden et al., 2012. *Develop. Dynamics* 241:1143-1154) we are following the olfactory sensory system precursors in the nervous system *in vivo*. By combing this *in vivo* analysis with clonal analysis of precursors during early somitogenesis we have shown that the mechanisms of induction are similar for the neuroectoderm giving rise to both the central and peripheral nervous system (Torres & Whitlock, submitted *Develop. Dynamics*). Our characterization of different olfactory behaviors (Stephenson et al., *Zebrafish* 9:68-73, 2012) has allowed us to study the correlation between neuronal differentiation as measured by activity and olfactory behaviors in zebrafish. With the arrival of a new post doc, Dr Isabel Benjumeda, we are taking advantage of recently available lines of zebrafish expressing genetically encoded calcium indicators (GCaMP 7 /GCaMP3) in different cell types to better understand on the onset of activity and plasticity in the peripheral olfactory sensory system. Currently, we have obtained the GFF398A, SAGFF27A, UASGCaMP7a, UASGFP lines from Dr. Yoshihiro Yoshihara from the RIKEN institute in Japan. With these lines we can drive GCaMP expression in specific subsets of olfactory sensory neurons and record their activity (see below) in the olfactory epithelium. In conjunction with activity, we are determining whether early patterns of olfactory receptor expression are plastic. Recently we have finished the first phase of RNAsequence analysis (GeneWIZ Inc, USA) to determine the expression profiles of the olfactory receptors, transcription factors and microRNAs that are associated with olfactory receptor regulation. Our preliminary analysis of these data indicates that specific receptors are differentially regulated by the odor environment and, strikingly there appears to be differential environment effects on cohorts arising from the same parents. This is work of PhD student Cristian Calfún.

Ecdysis Behavior in Drosophila. In order to grow, insects must replace their exoskeleton. The last step in this process is the shedding of the remains of the old exoskeleton, which is accomplished through a complex sequential behavior called ecdysis. Ecdysis is triggered by the release of the neuropeptide ETH (ecdysis triggering hormone), which acts on targets in the CNS that express the A and/or B form of its receptor (ETHR). Targets expressing ETHR-A are peptidergic neurons, which are activated sequentially. We have used mutants null for genes encoding specific neuropeptides that we have isolated and targeted RNAi expression coupled with GCaMP imaging to understand how ecdysis behavior is controlled. We find that the neuropeptides downstream of ETH themselves, in combination with inhibition mediated by GABA, play a critical role in determining the CNS's sequential response to ETH and that of the ensuing ecdysis behavior. These findings have important implications for understanding how neuropeptides modulate the activity of a neuronal network, and how they regulate behavior in all animals (Mena & Ewer, in preparation). Our recent review with Dr. White highlights recent progress in this area (White, and Ewer.2014. Ann. Rev. Entomol. 59:363, 2014).). Dr. White's and our lab are currently carrying out collaborative work. In particular, his lab has created lines that allow us to drive gene expression in ETHR-A and –B neurons, which will be very useful for understanding how ETH acts to control ecdysis behavior.

Circadian Clocks.We continued our analysis of how the circadian clock causes a daily rhythm of emergence of the adult fly, a rhythm that is known to depend on the activity of a circadian clock in the brain and one in the Prothoracic Gland (PG). We investigated how these clocks are coupled by interfering genetically with the brain and PG clock and their interaction (Millán, Ubillo, Palacios& Ewer, in preparation). We were recently awarded a FONDEQUIP grant (CONICYT) for a total of US$212,000 to purchase an extremely high sensitivity camera to be able to monitor the activity of the circadian clock *in vitro*, using flies bearing a clock gene-luciferase transgene. The equipment has arrived and is currently being set up. The award also allowed us to purchase a camera to detect calcium

signals, which will be used in larval zebrafish to correlate olfactory sensory neuron activity with developmental experience in living animals (see above). Thus we have made great strides in developing tools and a knowledge base necessary for unraveling the link between neuronal differentiation and activity underlying the onset and modulation of behaviors essential for survival.

Collaborations.

We are collaborating with Dr. Adrián Palacios (Line 4), assisting with the identification in the Chilean rodent, *Octodon degus*, of homologs of genes that have been implicated in Alzheimer disease. This rodent is a natural model for this important human disease, and we aim to correlate the occurrence of specific variants with the severity of the disease. We co-authored a publication on *O. degus* for Cold Spring Harbor Protocols (Ardiles, et al. 2013). - In collaboration with Dr. Tomas Pérez Acle (Line 5) we identified regulatory elements controlling olfactory receptor expression in zebrafish. The olfactory receptors we previously identified by qt-RTPCR to be down-regulated by odors are consistent with our recent bioinformatic results showing dual activation of homeobox containing transcription factors results in genomic suppression (Calfún, Domínguez, Pérez-Acle & Whitlock, in preparation).

4. System neuroscience.

Circuitry and Neural Plasticity*:** We continued studying the neural capacity (mechanism and circuits) for learning and memory during normal and pathological states.*Octodon degus as a Model of Alzheimer Disease (AD)*. We are interested in the rescue of cognitive capacity loss during aging, due to a neurodegenerative process (Ardiles *et alPNAS* 109(34):13835-13840 2012). One of our strategies was to use an enriched environment with a wheel exercise placed in the *degus* home cage. Our preliminary results show that 4 year old *degus*, which normally have memory defects at this age, are now comparable to 1-year-old degus in object recognition and 8-arm maze tests, [3 master and undergraduate thesis are involved in this work]. We have recently suggested that in human and several rodent models including *degus*, the retina may be an excellent biomarker for AD (Lowe *et al*.,*Alzheimer &Dementia* in press). In a large screen of eyes from degus in our colony, we found that many AD biomarkers, which are present in *degus* brains, are also found in the retina (in preparation). During 2013 we published, using degus model, in sleep (Ocampo-Garces et al., 2013) and in visual anatomy [Vega-Zuniga *et al.,* 2013].Sensory Neuroscience:*** Olfactory transduction and coding in teleost fishes: A summary of our findings from recent years and a comprehensive review of the field was published at the beginning of the year (Bazáes *et al.,* 2013). Work continued on the role of olfactory oscillations and their information contents in the olfactory bulb and telencephalon of teleosts, in collaboration with P. Orio (line 5), helping with wavelet analysis of local field potential oscillations in these brain regions. Research also continued on the role of nitric oxide (NO) in retinal visual coding: A complex regulatory mechanism of glutamate responses in type 3A/B and 4 OFF bipolar cells by NO was dissected and formed part of the PhD thesis of Dr. A. Vielma, defended in September 2013. In parallel, the patterns of glutamate responses in rat OFF bipolar cells were characterized to create an electrophysiological fingerprint for each cell. Two manuscripts are in preparation with these data. Does pannexin 1 form intercellular (gap junction) channels in oligodendrocytes? In a collaboration with J.C. Sáez and A. Martínez (Line 2), we found strong electrophysiological evidence using double patch clamp that endogenous pannexin 1 forms intracellular channels between primary oligodendrocytes in culture, an oligodendrocyte-derived cell line, as well as between HeLa cells transfected with Panx1 (in preparation).

Axonal sprouting induced by caries infection in human teeth: In collaboration Eduardo Couve, we analyzed the innervation patterns of human dental pulp from third molars with caries infections of different stages. Extensive sprouting of nerve endings into the reactionary dentin matrix was observed in caries-infected teeth. At the same time, an elevated number of HLA-DR-positive dendritic cells infiltrate the odontoblast layer and invade reactionary dentin formed underneath the caries-affected

regions, suggesting a coordinated neuro-immune response required to fight caries pathogen invasion (manuscript submitted).

Cortical Neural Plasticity and Stress.Male Sprague-Dawley rats were subjected to seven days of restraint stress on postnatal day forty-two (PND 42, adolescence). One and twenty-one days after the stress period the rats had significantly higher anxiety levels than controls, while the stress-induced impairment of the recall of conditioned fear extinction was recovered twenty-one days after the stress period (Negrón-Oyarzo, et al., 2014). Electrophysiological data showed a reduction in the AMPA-, but not NMDA-mediated response, resulting in a significant decrease in the basal synaptic transmission. At PND70, stressed animals showed a significant recuperation in both synaptic transmission and plasticity (Negrón-Oyarzo, et al 2014; *submitted*) Together, these finding suggest that chronic stress induces transient alterations in the Pre Frontal Cortex function.

Retinal and Computational Neuroscience: During this period we set up a fully operational 256 multi-electrode system able to record from hundreds of retinal ganglion cells per experiment. A. Palacios was a guest editor for a special issue of the Journal of Physiology, Paris (107(5), 2013) in Neural Coding and Natural Images Statistics. Four graduate theses are been carried out in our lab, associating multielectrode recording techniques and theoretical computational analysis. We have contributed to the development of an Open Source platform tools [Event Neural Assembly Simulation [ENAS, http://enas.gforge.inria.fr/v3/] for computational analysis of neuron populations in terms of neural coding used by the retina.

5. Cross-cutting: ***Molecular Simulations, and Computational Biology***.

(Structure and Function of Molecular Sensors). To date, Dr. González-Nilo´s team has increased the number of molecular models for different ion channels, such as K^+ channels (Shaker and BK), TRP channels (TRPV1 and TRPM8) and Ca^{+2} channels. These models allowed atomic level interpretation of 25 mutations studied experimentally. We have used different approaches (Free Energy Perturbation calculations, External Electric Field, among others) to study the structural elements that govern the single-channel ion conductance and to understand how mutations perturb the structural properties of the channels. Using these strategies, we have found novel residues and the most relevant structural mechanism involved in the modulation of the conductance of K^+ channels. These mechanisms explain the differences that cause high conductance in BK (collaboration with Dr. Latorre), and low conductance in Shaker (Dr. Naranjo). Our results were presented at the 2013 Biophysical Society meeting in San Francisco, USA, and a manuscript on these findings is currently in preparation.

We built a molecular model of the BK channel using as reference LRET analysis (for detail see Line 1), requiring the implementation of 10 different systems, including a total of 500.000 atoms. We also continue to elucidate the structure of the BK pore (using as reference the MthK structure). We have modeled the structure of a pore hydrophobic ring composed by residue F380 in the sixth transmembrane (S6) domain and leucine 377 in an adjacent subunit and studied the structural consequences of 6 different mutations of F380. This work has been submitted to *J. Gen. Physiol.* In collaboration with Line 1 we have taken advantage of the recently reported TRPV1 channel structure to determine the characteristics of the phosphatidylinositol 4,5-bisphosphate (PIP_2) site. Our recent findings are in agreement with our previous studies regarding the binding site of PIP_2, which identified 4 key positively charged in the PIP_2binding pocket. We found that neutralization of these residues greatly reduces the ability of $PIP_2$to activate TRPV1. A large molecular dynamics simulation of TRPV1 of 200 ns is now giving us new hints to understand PIP_2–dependent TRPV1 activation

Sequence similarity network for the entire TRP superfamily*:* With the aim of using evolutionary information to infer channel function, we implemented the use of sequence similarity networks to study the evolution of the many proteins superfamilies studied by Line 1. Using the sequences of functionally characterized TRP channels we searched in the public repositories of sequences for homologous proteins, and were able to generate a high quality sequence similarity network for the entire TRP

subgroup, consisting of 3028 proteins. We have discovered that there are large groups of uncharacterized sequences within the superfamily, some of which seem to belong to families with novel functional characteristics. Among the latter we have identified a few sequences from algae, one of which from *Chlamydomonas reinhardtii* has been the subject of more extensive primary structure characterization (collaboration with Dr. S. Brauchi (Universidad Austral de Chile, Valdivia; In preparation).

Development of methods for the overexpression and purification of proteins: with the aim of producing our own structural data in the medium term, we have been in contact with groups expert in X-ray crystallography (USA and Brazil) and Cryo-electron microscopy (Germany). We have implemented at CBIB-UNAB a facility for the production of recombinant proteins using microbiology, molecular biology, and biochemistry techniques (PCR, cloning, transformation, electrophoresis and chromatography). According to the plan for the first months of activity, standard protocols and processes based on previously stated methods were performed. The actual focus lies on increasing the purity of the proteins of interest (BK and KcsA) by using different molecular biology approaches (use other expression system pBAD or pET and XL1-Blue cell hosts). After reaching the goals of objective 1, protein crystallization will be implemented using classical protocols of vapor diffusion (hanging drop and sitting drop). Protein crystals will be analyzed by X-Ray diffraction by our collaborators in United States or Brazil.

***Synthetic Toxins*:** In cooperation with the Anillo Project 1107 (T.Pérez-Acle and D. Naranjo), we are creating synthetic toxins based on dendrimers. The aim is to take advantage of the shared properties between dendrimers (nanoparticles) and peptide toxins that are specific for potassium channel (from scorpion, anemonas and sea snails). Using bioinformatics tools we characterized the propensity of some amino acids in the contact zone between channels and toxin, in order to functionalize dendrimers with the identified amino acids. The synthesis of these toxins is in progress. We also tested the concept using commercial dendrimers (PAMAM) ranging in size from 2 to 4 nm in diameter, with surfaces functionalized with amino groups that emulate the conserved lysine residues present in most toxins known. Dr. Naranjo (Line 1), who uses as a case study the Shaker channel, did a preliminary experimental evaluation showing that PAMAM has a high affinity for Shaker when added to the channel intracellular region. These results are in agreement with docking simulations and with conformational explorations of the interaction of the dendrimer and the channel at both intracelullar and extracelullar sides (article to be submitted on 2014). These synthetic toxins will be optimized iteratively so that they bind as specifically as possible to the ion channels used as case studies. Later we will develop specific dendrimer toxins to block other kinds of channels of interest in biomedicine, such as the Na^+, the Ca^{2+} and TRP channels (collaboration with Dr. Ramón Latorre, Line 1). All of these channels are involved in chronic or inflammatory pain and the development of inhibitors may prove to be useful in the treatment of certain type of pain (supported also by Anillo Project ACT1107, Foundation Fraunhofer Chile Research and USA Army (TIP Reference 2050).

***Dissecting structure-function relationships coded into the molecular architecture of human Cx26 hemichannel and Gap Junction channel**:* (Tomas Perez-Acle's team). This project represents a transdisciplinary effort between Line 2 and 5 dedicated to gain insights on the structure-function relationships that are coded into the molecular architecture of the human Cx26 hemichannels and Gap Junction channels. So far, the main results of this project are: i) the discovery of a novel and previously unknown water pocket on the cytoplasmic portion of every Cx26 monomer (the IC-pocket), and ii) a putative functional role for Arg143, an important residue in the IC-pocket, on the human Cx26 hemichannel permeability. Using molecular dynamics (MD), we produced molecular models using as structural reference the crystallographic structure of the human Cx26 hemichannel and Gap Junction channel. Using these models, we conducted large-scale molecular simulations at the BlueGene supercomputing facility of the IBM Watson Research Center, NY, USA. When analyzing the trajectory

data from Cx26WT hemichannel, we discovered a novel water pocket at the cytoplasmic portion of every Cx26 monomer, termed the IC-pocket. Characterization of water behavior within this pocket revealed that residue Arg143 determines the degree of hydration of the pocket. When evaluating water volume within this pocket, a correlation between the volume of water and the position of the Arg143 became apparent. When conducting multiple sequence alignments among human connexin families we realized that Arg143 is 100% conserved among beta connexins, being replaced by Lys, another polar and positively charged residue, in other Cx families. We hypothesized that the functional role of Arg143 may play an important role on the regulation of the IC-pocket water volume, controlling the position of the N-terminal helix (NTH) by a sort of hydraulic mechanism able to modulate channel gating. To test this hypothesis, we performed a hemichannel functional assessment through time-lapse imaging of ethidium bromide uptake. Our experiments demonstrated that changing the nature of Arg143 to an opposite polarity (E) or by a hydrophobic residue (A), dramatically diminish the hemichannel activity in cells expressing these mutants, as compared to the WT control and the HeLa parental cells with MOCK transfection. As a whole, our current results demonstrate that Arg143 may play a key functional role for the permeability of the Cx26 hemichannel. These results were submitted for publication to the *Biophysical Journal* and are at present under revision.

Modeling neural excitability (Patricio Orio team). We study conductance-based models of neural excitability to understand the role of some ion channels in the generation of behavior. During 2013 we worked on a mathematical model for the dynamic response of cold thermoreceptors. This is the first model that reproduces the response of cold thermoreceptors to rapid changes in temperature, explaining the contribution of the TRPM8 in acute cold sensing within a neural context. We have started a collaboration with Line 3 (Dr. J. Ewer) modeling a peptidergic neural network that regulates ecdysis. This project will dig into how biological systems generate robust behaviors from variable inputs. In collaboration with Line 4 (Dr. A. Palacios), we are developing a biophysical model of a neural circuit in retina selective to direction of motion. These projects have been boosted by the installation of a new HPC cluster (256 cores) at CINV facilities in Valparaíso. This facility was possible as a joint effort with other projects in which Dr. Orio participates: Fondecyt 1130862, Anillo ACT-1104 (Carlos González, PI; Line 1), and Anillo ACT-1113 (with researchers from the Universidad de Santiago de Chile).

b) Publications:

Summary table

Category of Publication	**MSICenter Members**	**Number of Publications coauthored by students**	**Total Number of Publications**
ISI Publications or Similar to ISI Standard	Associate Researchers	20	29
	Other Researchers	2	9
SCIELO Publications or Similar to SCIELO Standard	Associate Researchers	0	1
	Other Researchers	0	0
Scientific Books and chapters	Associate Researchers	3	6
	Other Researchers	0	0
Other Scientific Publications	Associate Researchers	0	0
	Other Researchers	0	0
Total of Publications		**25**	45

c) Other achievements:

Patents: NONE
Intellectual property:NONE
Congress Presentations:
Summary Table

Type of presentation	**National Events [Number]**	**International Events [Number]**
A. Associate Researchers		
Conferences, oral communications, poster communications, others (specify)	**2**	**5**
Invited presentations (not included in above row)	**5**	**13**
B. Other researchers (Adjunct Researchers, Senior Researchers, Young Researchers, Postdoctoral Researchers and Students)		
Conferences, oral communications, poster communications, others (specify)	**9**	**2**
Invited presentations (not included in above row)	**4**	**4**

Organization of Scientific Events:
*III Latin-American school on ion channel biophysics:*Organized by Dr C. González .Students were exposed to both theoretical and practical courses of confocal spectroscopy, voltage-clamp fluorometry (VCF), cut-open voltage-clamp fluorometry, LRET, FRET and TIRF. Further details in the network section.
VIII Ibero-American Congress of Biophysics together with the IX Annual Meeting of the Chilean Neurosciences Meeting. Chaired Dr. A. Neely (also president of the Chilean Neuroscience Soc.) and Dr. M. Holmgren (also president of SOBLA). Several of the meeting symposia were also organized by CINV investigators. More detailed description in the network section.
Symposium "Physical Biology of the Cell": Held in Valparaíso, January 8. at the Universidad de Valparaíso. Talksby Rob Phillips, Hernán García and Jane Kondev, editors of the second edition of the Book"Physical Biology of the Cell".
Scientific Editorial Boards:
R. Latorre: Biological Research (ISI) since 1992, Proceedings of the National Academy of Sciences(ISI) since 2004, Journal of General Physiology(ISI) since 2004, Channels (ISI) since 2007, Journal of Biological Chemistry (ISI) (2008-2013), Frontiers in Pharmacology (since 2103) and Frontier in Temperature (since 2013).
J. Ewer: Journal of Insect Science(ISI) since 2006.
A. Palacios: Biological Research (ISI) since 2008, J.Pol.Complex Sys since 2013.
T. Pérez-Acle:_ PeerJ since 2012.
J.C. Sáez. *Frontiers in Neurosciences* (ISI) since 2010.Guest editor, special issue Connexin and pannexin channels of Neuropharmacology.
C.González Journal of Biological Chemistry (ISI) since 2013.
D. F. González Editors-in-Chief of Current Opinion in Structural Biology since 2013

Awards:

New grants awarded: During this period 3 FONDECYT were awarded to CINV investigators, 2 as P.I and one as Co.P.I. Each investigator can be a P.I. in only one FONDECYT and most CINV investigators is directing one. There are also 2 Scientific Ring project from PIA CONICYT directed by CINV investigator and one Co-directed by CINV member. Our Ph.D. graduate G. Contreras and A. Vielma were granted postdoctoral fellowships from FONDECYT.

Special awards and honors:

Dr Kathleen Whitlock was selected to organize the **Third Latin American Zebrafish Network (LAZEN) Meeting in 2014**. Through an international competition she was awarded 10,000 Euros from The International Centre for Genetic Engineering and Biotechnology (ICGB) to help cover costs of the meeting.

Dr. Juan Carlos Saez elected to organize the **International Gap Junction Conference 2015 to be held in Valparaiso** due to the increasing international importance of Chilean laboratories working in the field of Gap Junction Channels and Hemichannels.

Dr. Ramón Latorre was honored with the **SOBLA award lecture** during VIII Ibero-American Congress of Biophysics.

Dr. Carlos González was invited to join the **editorial board** of the prestigious **Journal of Biological Chemistry**.

Several of our students and postdocs received special fellowships to participate at international scientific meetings.

4. Education and Capacity Building

a) Education and Capacity Building:

ThePhD Programin Neuroscience was created in 2002 by CINV members and is accredited through 2017. Its Director is currently Dr. John Ewer (CINV, Research line 3). It is designed to train researchers interested in the development, the structure, and the function of the nervous system. The strengths of our Program are in the areas of molecular physiology and biophysics, computational neuroscience, sensory neuroscience, neuronal plasticity and neuropathology, and development and neurogenetics.
During this period, the CINV-ICM granted 8 graduate fellowships. Guaranteed funding ensures that students can devote full time to their Ph.D. work. Agreements with other graduate programs allow students to take elective courses at other universities in Chile and abroad, as well to carry out research internships. Our Program has a double Ph.D. agreement with the Scuola Internazionale Superiore di Studi Avanzati (SISSA) of Trieste, Italy (www.sissa.it), which has expanded the range of thesis topics. We are also in the process of making a similar agreement with the University of Sao Paulo, Brazil.
Requirementsandapplication process for entering the program were explained in the first CINV-Report.
Web pages: www.uv.cland www.dnuv.cl.
TheMasters Programin Neurosciencewas foundedin 1999 and is currently directed by Dr. AgustínMartinez (CINV Research line 2). It was recently reaccredited for 8 years (through 2018), becoming the longest accredited program at the University of Valparaíso. To date it has graduated43students. The program distinguishes itself byits high content ofbasic Neuroscience as well as for its multidisciplinarity.Its studentscomefrom various disciplines: biologists and biochemists as well ashealth professionals, engineersormathematicians, eager tounderstandthe biological basis of the functioningof the nervous system.
Requirementsandapplication process for entering the program were explained in the first CINV-Report.
Web pages:www.uv.cl and www.magisterneurociencia.cl

b) Achievements and results:

In the **PhD Program**, 24 students have graduated since 2002 (8 female; 16 male), of which 8 graduated during this period (see list below). The Program currently has 32 students (8 female, 24 male). During this year's recruitment period we received 22 applications of which 12 were considered admissible and were interviewed. Of these, 8 students were selected, of which 7 accepted, which is close to the maximum number of students we are able accept. We believe that our recruitment strategy has become more effective as we now receive enough applications to be able to select the very best. All current students typically attend one national or international conference in their area of study per year; funding is provided through their fellowship or by their advisor. (See Annexes 5.1 and 5.2).
In the **Master Program**, 42 students have graduate since 1999 (22 females; 20 male), of which 5 graduated during this period; three of them did thesis in our center (see list below). During this year's recruitment period, the Masters program in Neuroscience was very successful receiving 35 applications of which 17 were accepted, being close to the maximum number of students we are able accept. In addition, this is the second year in a row in which a high number of fellowships from CONICYT were awarded to our students (7). This recruitment success has been coincidental with the positioning of CINV as a center of excellence at the national and international level.

Main achievements of our Ph.D. and Master Students during the period:

Ph.D. Thesis Project Approvals and Qualifying exams CINV students (Jan - Dec. 2013):

1. Cristian Alfonso Calfún Medina, "*Genomic Plasticity in the Olfactory Epithelium mediated by Odorant Exposure in Zebrafish"*. Advisor: Kathleen Whitlock (Line 3).

2. Miguel Andrés Piñeiro Feick, "*Study of the properties of the circuit associated with the AN1-AN4 CCAP neurons and motoneurons during pupal ecdysis in Drosophila*". Advisor: Patricio Orio (Line 5).

Master Thesis Project Approvals and Qualifying exams of students (Jan - Dec. 2013):

1. Alfredo Cordero, "*Comorbidity Psychiatric and School Performance in adolescents with Epilepsy*". Advisor: Ana María Cárdenas.
2. Ester Otarola, "*Mechanism of permeation in the Hv channel*. Advisor: Carlos González
3. Gaspar Herrera, "Modular nature of potassium channels". Advisor: David Naranjo
4. Mauricio Segura, "*Circadian Rhythms and anticipative locomotor activity in Octodon degus*". Advisor: John Ewer.

Graduations of Ph.D. students (Jan-Dec 2013):

1. Tatiana Cevo Espinoza, "*Fotobiostimulation with near-infrared light for the treatment of glaucomatous optic neuropathy"*. Advisor: Adrían Palacios (Line 4).
2. Alex Vielma Zamora, "*Modulation by nitric oxide of the activation of the rat internal nuclear layer of the retina"*. Advisor: Oliver Schmachtenberg (Line 4).
3. Gustavo Contreras Cáceres, "*Modulation by the beta subunit of calcium activated potassium channels*" Advisor: Alan Neely (Line 1).
4. Natalia Alejandra Raddatz Cárdenas, *"Detection and characterization of the temperature sensor of TRPM8 channels"*. Advisor: Ramón Latorre (Line 1).
5. Arlek Marion González Jamett, "*Dynamin-2 regulates the late steps of exocytosis in adrenal chromaffin cells through a mechanism that involves actin polymerization*". Advisor: Ana María Cárdenas (Line 2).
6. David Enriquez Báez Nieto, "*Study of TRPV1 channel activation by voltage. In the quest of the voltage sensor module*". Advisor: Ramón Latorre (Line 1).
7. Sergio Ignacio Negron Oyarzo, "*Long- and short-term effects of chronic stress on anxiety, fear, and synaptic plasticity in the prefrontal cortex*". Advisor: Pablo Muñoz (Line 4) and Alexies Dagnino (University of Valparaíso).

Graduations of CINV Master students(Jan-Dec 2013):

1. Alfredo Cordero, "*Comorbidity Psychiatric and School Performance in adolescents with Epilepsy*". Advisor: Ana María Cárdenas (Line 2).
2. Catherine Estay, "*Expression, localization and role of Panx1 in the secretory activity in primary culture of chromaffin cells and adrenal glands sliced*". Advisor: Ana Maria Cárdenas(Line 2).
3. Natalia Barraza, "*Hyperphosphorylation of Tau protein in cell lines of mouse brain with trisomy 16, a cellular model of down syndrome*". Advisor: Ana María Cárdenas (Line 2).

Participation in graduate and undergraduate programs from other institutions:

Graduations of Ph.D. students (Jan-Dec 2013):

1. Calixto Dominguez Portilla, PhD in Biotechnology, UNAB (Santiago). Advisor: Tomas Perez-Acle (Line 5).
2. Raúl Araya Secchi, PhD Biotechnology, UNAB (Santiago). Advisor: Tomas Perez-Acle (Line 5).
3. Raul C. Lagos, PhD in Physiological Science, PUC. Advisor: J.C. Saez. (Line 2)
4. Horacio Poblete, PhD in Applied Sciences, University of Talca. Advisor: Danilo González (Co-Advisor: Ramón Latorre). (Line 1)

Ph.D. Thesis Project Approvals and Qualifying exams (Jan - Dec. 2013):

1. Carlos Lagos, PhD Biotechnology, UNAB (Santiago). Advisor: Tomás Perez-Acle (Line 5).
2. Sebastián Gutiérrez, PhD Biotechnology, UNAB (Santiago). Advisor: Tomás Perez-Acle. (Line 5)
3. Juan Pablo Castillo, Dr. en Ciencias con Mención en Biología Molecular, Celular y Neurociencias, Faculty of Science, University of Chile. Advisor: Ramón Latorre (Líne 1).
4. Paloma Harcha, Ciencias Fisiologicas, PUC. Advisor: J.C. Sáez. (Line 2)
5. Anibal Vargas, Ciencias Fisiologicas, PUC. Advisor: J.C. Sáez (Line 2)
6. Gonzalo Gomez, Ciencias Fisiologicas, PUC. Advisor: J.C. Sáez. (Victoria Velarde, co-advisor) (Line 2)
7. Romina Hernadez, Ciencias Fisiológicas, PUC. Advisor: J.C. Sáez. (Victoria Velarde, co-advisor) (Line 2)
8. Dusan Racordon, Ciencias Fisiológicas, PUC. Advisor: J.C. Sáez. (Gareth Owen, co-advisor) (Line 2)
9. Daniela Salas, Bioquímica, U. de Chile. Advisor: J.C. Sáez. (Sergio Lavandero, co-advisor) (Line2)
10. Carola Maturana, Physiology, PUC. Advisor: J.C.Sáez. (Line 2)

Master Thesis Project Approvals and Qualifying exams of CINV students (Jan - Dec. 2013):

1. Yerko Escalona, Faculty of Science, University of Chile (Santiago). Advisor: Tomás Perez-Acle. (Line 5)
2. Carolina Urrutia, Magister en Radicales Libres, Universidad de Valparaiso. Advisor: J.C. Saez. (Line 2)

Undergraduate Thesis Project Approvals and Qualifying exams of CINV students (Jan - Dec. 2013):

1. Angelica Benbenuto, Biochemistry, PUC. Advisor: J.C. Sáez (Line 2)
2. Fijiko Saavedra, Biochemistry, PUCV. Advisor: J.C. Sáez (Line 2)
3. Paula Mujica, Biochemistry, PUCV. Advisor: J.C.Sáez (Line 2)
4. Patricio Cáceres, Pharmacy, PUC. Advisor: J.C. Sáez (Victoria Velarde, co-advisor) (Line 2)

Students visiting laboratories abroad. We continued supporting the travel and stay of many of our students in the laboratories of members of our international network to do research that cannot be done in Chile because lack of equipments or experience.The following students did research stays during this period:

1. Isaac Garcia (Ph.D. Student), Rutgers University (Laboratory of Dr. Jorge Contreras) Thesis work2.
2. María José Guerra Fernández (Master Student), Universidad de La Laguna Tenerife (Laboratory of Dr Borges).
3. Raul Araya (Ph.D. Student), IBM Thomas J. Watson Research Center (Laboratory of Dr. Ruhong Zhou).

4 Paloma Harcha (Ph.D. Student), Institut National de la Santé et de la Recherche Médicale, Collège de France (Laboratory of Dr. Giaume).

5. Carola Maturana (Ph.D. Student), Department of Surgery. School of Medicine, University of California, San Diego (Laboratory of Dr. Antonio Maio)

The new Ph.D. program in Biophysics and Computational Biology (Program Director: Dr. Alan Neely). This Program, which was proposed in the original grant, recently received final approval by the University Board. The Program will start September 2014 by accepting no more than five students following a worldwide call for application in the first semester 2014. We have already received inquiries from 5 candidates.

c) Destination of Students:

PhD Program: Twenty-four students have graduated since 2002, starting with Dr. A. Chavez in 2007. The majority of graduates of our Program are currently carrying out postdoctoral work (with the exception of a few graduates from 2013 who are either looking for postdoctoral positions or are completing experiments and preparing their thesis work for publication).

Master program: Forty-two students have graduated since 1999, starting with Dr. Chavez in 2000. About half of our graduates have gone back to their professional practice, and the other half have followed the scientific academic pathway, entering to diverse Ph.D. programs in Chile and abroad, including our PhD in Neuroscience. Some of them are professors in Chile or abroad.

Summary Table:

Obtained Degree	Academy	Industry and Services	Studies	Research	Other (Specify the other type of activity
Doctoral		1		8	
Master		1			
Undergraduate		5	2	2	
TOTAL		7	2	10	

5. Networking and other collaborative work

a) Networking:

The creation, maintenance and strengthening of scientific networks is at the core of CINV mission. These initiatives are grouped, depending on CINV scientific areas, into four main networks of scientific collaboration: the Biophysics and Computational Neurosciences Network, the Genetic and Development Network, and the Intercellular Communication at the Nervous System Network. During 2013, these networks organized workshops, international schools, and congresses, which are briefly presented below.

Biophysics and Computational Neurosciences Network

1. *VIII Ibero-American Congress of Biophysics together with the IX Annual Meeting of the Chilean Neurosciences Meeting:*

Held in Valparaiso from October 1st to 4th, at the Parque Cultural de Valparaíso and organized by CINV, these meetings joined scientist and students from Ibero-America and renowned international researchers from US and Europe with the Chilean Neurosciences and Biophysics community. During the congress the following symposia were organized: Membranes, New insights on calcium signaling, Diversity of chemical senses, Exocytosis, neurotransmission and synapse structure, Synaptic plasticity, Protein structure, Role & regulation of channels and hemichannels formed by connexins or panexins in the nervous system, Calcium channels, and Lightning the structure and function of ion channels.

2. *III Latino-American School on Computational Neurosciences (LACONEU):*

Held in Valparaiso from January 13th to the 31st, the aim of this school is to promote the field of Computational Neuroscience through cutting edge mathematical and computational tools and their applications in biomedical and clinical research. With the support of the Max Planck Institutes, INRIA France, ISCV, Universidad Federico Santa María, ANR-CONICYT and CINV, the school gathered 23 students from all over the world. They joined researchers from France, Germany, USA, and Chile, to participate in both theoretical and practical courses on advanced topics in computational neurosciences.

Several grants were also awarded to CINV researchers to support the strengthening of national and international networks of scientific collaboration.

Cellular signaling Network.

During this period we organized several seminars and symposia in the context of National and International Meetings with our national and international collaborators.

1.-IX Annual meeting of the Chilean Society of Neuroscience & VIII Ibero American Congress of Biophysics

Symposium: *Role and regulation of channels and hemichannels formed by connexins or pannexins in the nervous system.* Chairs: Juan C. Sáez, Agustín Martínez .

Symposium:*Exocytosis, neurotransmission and synapse structure.* Chair: *Ana M. Cárdenas.*

2.- Chilean Society for Cell Biology xxvii annual meeting October, 23 – 27th, 2013, Puerto Varas. CINV sponsored the Symposium: *"Cell Membrane Channels Made by Connexins or Pannexins are Key Players in Genetic and Acquired Diseases".* Chairs: Agustín Martinez and Juan Carlos Saez.

International Gap Junction Conference 2015. During this period, Chile was selected to host the next International Gap Junction Conference that will be held in Valparaíso and organized by Line 2 in the year 2015 (IGJC-2015). This is the first time in the history of this scientific meeting that a country outside of US or Europe will organize this conference. The majority voted in favor of Chile in the past IGJC-2013 in Charleston USA was mainly due to the increasing international importance of Chilean laboratories working in the field of Gap Junction Channels and Hemichannels

b) *Other collaborative activities:*

Different projects awarded to CINV researchers supported the strengthening of national and international networks of scientific collaboration.

1. A CONICYT MEC grant will allow Dr. Ximena Nelson (Canterbury U., New Zealand) to spend 2 months in John Ewer Lab (Line 3) in 2014. This is the second MEC award we have obtained to

fund joint work with Dr. Nelson, an expert on jumping spiders, to investigate the circadian clock of spiders, about which little is known despite being the second most abundant group of animals on Earth.

2. J. Ewer (Line 3) was awarded with an ECOS/CONICYT grant with Dr. Jean-François Ferveur (Université de Bourgogne) to investigate the process of maturation of the insect exoskeleton. This structure is essential for insect survival and must be hardened, waterproofed and pigmented very rapidly after ecdysis.
3. A. Palacios (Line 4) was awarded with an ECOS/CONICYT grant with Dr. Frederique Alexandre (INRIA, Université de Bordeaux) to work on neural coding from small retina networks and natural images.
4. C.arlos González (Line 1) was awarded with a CONICYT/Redes project that supports the Creation of International Networks between Research Centers 2013 - REDES130006. Other researchers involved are: Gonzalo Ferreira (Department of Biophysics, Faculty of Medicine, Universidad de la República, Uruguay), Ramón Latorre (Line 1), Alan Neely (Line 1), Gustavo Brum (Department of Biophysics, Faculty of Medicine, Universidad de la República, Uruguay). The overall objective of this proposal is research, human resource development, regional cooperation, and technological innovation, in order to study the impact of environmental contaminants on ion channels, in expression systems and animal models,
5. A project (Development of a lipidic biosensor platform for the *in situ* detection of Red Tide -; II Contest for Applied Science 2013 - Program IDeA # CA13I10274) involving Carlos González (Line 1) and Pablo Conejeros (Facultad de Ciencias, Universidad de Valparaíso)was awarded to Dr. Patricio Villalobos (Universidad Técnica Federico Santa María). The objective of this proposal is the development of a portable colorimetric kit based on channels reconstituted in liposomes, in order to detect the Red Tide more easily and with a higher sensitivity and specificity than can be achieved with existing kits.
6. A joint collaboration agreement was signed between the Soft Matter Group of the Center for Computational Biology at the IBM Watson Research Center, Yorktown Heights, NY, USA and the Computational Biology Lab at Fundación Ciencia para la Vida headed by Dr. Tomas Perez-Acle (Linea 5) in order to conduct joint research on the topic of Connexins Hemmichannel and Gap Junction channel structure and function. This agreement includes access to IBM's Supercomputing capacities.
7. A collaboration with the laboratory of Dr. A. Patapoutian (The Scripps Research Institute, California Campus) that started in 2010 has continued and now includes a collaboration with the laboratory of Dr. Jorg Grandl (Duke University). Through this collaboration one of Dr. Latorre (Line 1) PhD student (Hans Moldenhauer) learned the high throughput mutagenesis technique applied to the cold receptor TRPA1. This technique was crucial for the discovery of some of the molecular determinants of temperature sensitivity in this channel (Jabba et al. *Neuron* in Press).
8. During 2012 a collaboration with Dr. R. Olcese laboratory at UCLA to perfect voltage-clamp fluorometry technique was strengthened by a visit by A. Neely and G. Contreras. This collaboration, which is partially supported by an international collaboration grant from FONDCYT, resulted first in an abstract describing the first fluorometric detection of voltage-sensor movement in Ca channels submitted to the 57^{th} meeting of the Biophysical Society and later a full paper submitted to *Neuron*. We are preparing a re-submission after receiving encouraging comments. This joint research effort contributed to a full grant application to NIH with Dr. Olcese as the P.I. and Dr. Neely as Co. PI. We were scored within the top 20% and re-submitted a new application this year.
9. A collaboration between Dr. Stéphane Gasman (CNRS, Strasburg, France) and Ana M. Cárdenas (Line 2) to investigate the contribution of dynamin in the endo/exocytosis was initiated in 2008. It was supported by the grant ECOS-CONICYT C08-B01. In 2013 we published a paper (González-

Jamett et al., *PLoS One* 8:e70638) describing our findings.

10. A collaboration between Dr. Narcisa Martínez-Quiles (U. Complutense de Madrid, Spain) and Ana M. Cárdenas (Line 2) to study the role of actin-binding proteins in exocytosis was initiated in 2012. We have a paper in revision in *PLoS One* (PONE-S-13-65632).
11. During Jul/2012 –Jul/2013 D. Naranjo (Line 1) visited Dr. Paul Brehm and Gail Mandel´s labs in the Vollum Institute at OHSU, Portland, USA to study how P/Q-type and N-type calcium channel kinetics could produce calcium signals at the zebrafish neuromuscular synapses. We propose that P/Q -type channels can elicit larger and sharper Ca-signals during an action potential than N-type calcium channels, thereby supporting higher and more synchronous neurotransmitter release probability. Using a primary motor neuron expressing channelrhodopsin, we investigated the ability of inactivated of P/Q-type Ca-channels to sustain spontaneous neurotransmitter release in zebrafish. This work has continued in Valparaiso with the support of Dr. Whitlock (Line 3) to maintain optogenetic fish variants. These collaborative efforts will include Drs. Neely (Line 1) and Cardenas (Line 2).
12. Since November 2012, Dr. Patricio Orio is principal investigator in a collaborative Grant with researchers from Universidad de Santiago de Chile, USACH (Proyecto Anillo ACT-1113, Director Dr. Rodolfo Madrid, USACH). This three-year project studies the role of TRP channels in peripheral sensory transduction and in synaptic plasticity, in health and disease.
13. Dr. AM Cárdenas has been collaborating with Dr. P. Caviedes (ICBM, Facultad de Medicina, U. Chile) since 1995. They investigate the contribution of different proteins on the cellular alteration observed in a cellular model of human trisomy 21 (Dow Syndrome) and are making headway in the identification of therapeutical target among over expressed proteins coded in chormosom 21.
14. Dr. AM Cárdenas with P. Caviedes and J. Bevilacqua (I.C.B.M., Facultad de Medicina, U. Chile) are studying the molecular and cellular mechanisms of myopathies caused by mutations in dysferlin or dynamin-2 that lead the a collaboration grant Anillo ACT112. Several papers have come out of this collaboration; González-Jamett et al "Dynamin-2 in nervous system disorders" J Neurochem. 128:210-23; González-Jamett et al. "Dynamin-2 function and dysfunction along the secretory pathway" Front. Endocrinol. 4:126

6.Outreach and connections with other sectors:

a) *Outreach*:

The CINV's mission is to become a reference for scientific research in the field of Neuroscience and a bridge that brings science closer to the community, by actively collaborating in the development of Valparaíso. Activities that have been implemented with this purpose have resulted in the CINV being recognized by the region's diverse community leaders, and raising awareness of the importance of science for the country's development. This process has also strengthened our relationship with a wide range of local institutions along with a progressive positioning in the local media, which shows a greater interest in covering CINV's activities.

General Objective: CINV's goal is to bring science closer to diverse audiences, from elementary school students to university students, professionals and the community in general. Specifically, the activities have focused on: i) bringing science closer to other disciplines (Tertulias Porteñas); ii) promoting awareness of the scope of the field of Neuroscience and CINV's research (Neuromantes; DVD enclosed); iii) bringing science to local schools in order to integrate it into the curriculum (¿Qué tienes en mente? and Ciencia al Tiro); and iv) the transmission of the science developed at the CINV to the community in each of our meetings and symposia, through conferences designed to be reachable by the general public.

Criteria of the Center for Promoting Outreach Activities:

-To promote increased knowledge about science using simple language to draw comparisons with day to day experiences and using common technological tools;

-To promote practical, "learning by doing".

-To include professionals from other disciplines in order to enrich the teaching-learning processes, making an effort to ensure that the knowledge contributed is comprehensive and lasting;

-To promote discussion of issues that are of interest to our center through social networks and active participation in activities organized by CINV.

Achievements: These activities have increased CINV recognition by various community leaders. In addition, we have progressively positioned our center in the local electronic and print media, which have begun to show greater interest in and familiarity with our activities. This has allowed us to develop strong relationships with political leaders in the city and the region resulting in widespread support for our scientific endeavors. CINV has also obtained country-wide recognition as a center of excellence in Neuroscience residing outside Santiago, helping to value science as a motor for regional development.

Brief description of the key activities implemented during this period. (The remaining activities are described in the corresponding appendix.)

Opening of the VIII Iberoamerican Congress of Biophysics - IX Annual Meeting Sociedad Chilena de Neurociencia. In an alliance with the Parque Cultural de Valparaíso, the CINV hosted this Congress. The Congress Opening was a general conference describing the marvels of animal electricity given by the President of the Society of Latin American Biophysicists and member of CINV, Dr. Miguel Holmgren. The City Mayor and about 300 high-school students were present.

- Date: August 1-4, 2013

Tertulias Porteñas. The CINV organized the second season of this activity with scientists, artists and intellectuals who talk about subjects close to neuroscience and other disciplines, creating spaces for an interdisciplinary discussion. The target audiences included university students, professionals, and community groups. During 2013, this event took place at the cultural and convention center "Parque Cultural de Valparaíso". At present, this activity organized by CINV scientists is recognized by the community as an important cultural activity for the city.

-Dates: July 29 (What do we know about pain?) and December 19 (What do we know about perception?), 2013
-Attendees: 300 people each
-Sponsoring institutions: Universidad de Valparaíso, Parque Cultural de Valparaíso, Radio Valentín Letelier.
-Sponsors: local newspaper Diario El Mercurio de Valparaíso, Hotel Gervasoni.
Neuromantes. A television series created by CINV and set in Valparaiso. The series addresses topics of universal interest like beauty, perception, emotions, etc., approached by different characters of the city, and integrates the perspectives of neuroscience, innovation and culture. The opening and presentation of the series was in Valparaiso and Santiago. This production has been broadcasted by local TV channels as well as nation-wide cable channels to the whole country. Neuromantes was also presented at the International Festival of Science on TVG "VerCiencia" Brazil. (www.vercencia.com.br).
-Associated institutions: Universidad de Valparaíso, EXPLORA CONICYT Program, NOVASUR (a program of the National Council of Television).
- Chapters: Beauty, Emotions, Memory, Perception, Networks, Biological clocks.
¿Qué tienes en mente? After the success of this activity in 2012, CINV continued for a second year the scientific talks in high schools of the Valparaíso Region with the collaboration of the EXPLORA CONICYT program. Fifteen hundred students of different high schools participated in seven lectures offered by CINV researchers in different provinces of the region.
-Events: Seven lectures in schools from seven different cities of the region
-Total number of participating students: 1,500
-Associated Institutions: EXPLORA CONICYT, Universidad de Valparaíso.
3D exhibition of Biological Systems, Arts and Science. Dr. Fernando González using 3-D technology showed to students the essential molecules of life: proteins and water this as part of a 3D exposition of Biological Systems, Arts and Science supported by the "Fundación más Ciencia" that took place in the Museum of Fine Arts in Santiago, October 3-November 16, 2013.http://www.fundacionmasciencia.cl/2013/10/22/arte-y-ciencia-en-el-museo-de-bellas-artes/
-Total number of participants in 3 days of the exposition: 500
Ciencia al Tiro. This project, led by Dr. Kathleen Whitlock, was initiated with funding from MSI Millennium Nucleus Center for Genomics of the Cell. In the year 2013, we conducted three activities: 1) Production of the book "The Joy of Science" in order to disseminate information about our workshops to students and adults in Region V and throughout Chile. The book is in final stages of development. 2) Installation of an "Aquaponics " System and incorporation of workshops, with the aim to teach science through "hands -on " activities for students and adults in Region V. We have a new building, made possible through private funding, dedicated to the "Ciencia Al Tiro" Program where the Aquaponics equipment is located. We translated Aquaponics material to Spanish and developed new workshops. 3) We repaired and improved the Ciencia Al Tiro website (www.cienciaaltiro.cl) with the goal of disseminating information about our program in Region V and throughout Chile. Dates: Ongoing since 2009.
Participants: 25 students / week during the year 2013; Institutions participating: Middle School Basica Pacifico, Doctorate and Masters in Neuroscience at the University of Valparaiso.
-Participating Institutions: Básica Pacífico Middle School, Neuroscience PhD. and Masters Programs from the Universidad de Valparaíso.

***b) Connections with other sectors*:**

During its second year as a Millennium Institute, the CINV set as a goal to consolidate itself at the local level as a key institution in the region's development. In this context, we focused our efforts on

developing connections with public sector institutions. We have implemented the following actions related to this goal in collaboration with those entities.

Agreement with City of Valparaiso for transmission of the TV series Neuromantes: With the aim of showing neuroscience as a subject close to people's experience, the CINV proposed to the city of Valparaíso an agreement to transmit the TV series "Neuromantes" in the waiting room of the City Hall and other public buildings. This is the first time that a scientific TV series is shown in an institutional building in Valparaíso.

Communication strategy of CINV to the media: With the goal of having a permanent visibility in different media (newspapers, TV and internet), the CINV has hired a Communication agency since September 2013. The agency is in charge of disseminating through newspapers and TV the research and outreach activities done by CINV, emphasizing the importance of the science for the development of the country and, in specific, for Valparaíso.

The presence in the press of CINV`s name and the research done in the Center has increased the visibility of the Institute in Valparaíso and across the country.

Meetings with political and business authorities of the region of Valparaíso: In this second year as a Millenium Institute, the CINV organized lunch meetings with different political authorities of the Valparaíso region, including the Mayor, regional counselors, congressmen, senators, and a presidential candidate. The purpose of these meetings was to present the research of the CINV, its outreach activities, and the project of the new building in the historic neighborhood of Valparaiso. The CINV also started to invite different business personalities of the region, with the purpose of creating a non-scientific advisory board composed by politicians and business people.

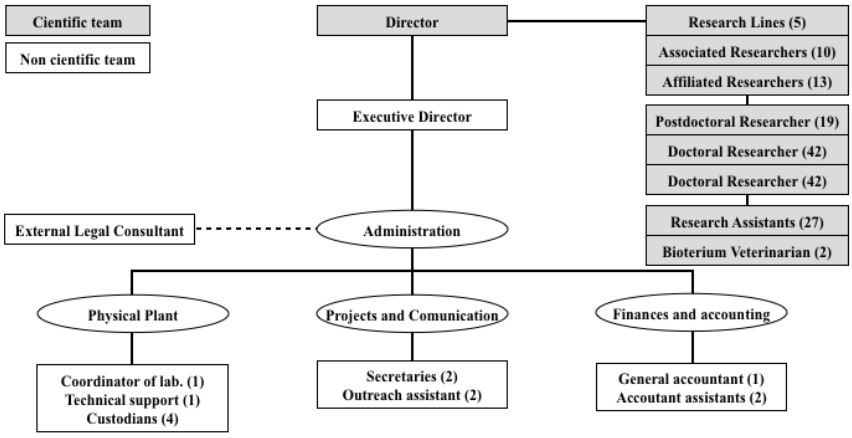
Invitation to present the CINV building project to the UNESCO advisors: In November 2013 the UNESCO advisors visited Valparaiso to evaluate the progress of Valparaíso as a world heritage city. CINV was invited by the UNESCO advisors to present the Severin building project, located in the historical neighborhood, as one of the most important projects for the development and recovery of an heritage site. The report has an explicit mention of this project with the recommendation to the Chilean State to support its construction.

Other partnerships. Through the projects FONDEF-VIU Applied Research & Development (110059) and Foundation COPEC–UC Projects for the development of natural resources (8C055), both completed during the 2013, we designed a chitosan nanoparticle wrapper of compounds that act as antioxidants and iron chelator. An important link with the private sector, particularly with the consortium Biorend S.A. and Foundation COPEC–UC, has been generated. With the support of JARRY IP Services (www.jarryip.com), specialist in evaluation and feasibility of intellectual property, and University of Valparaiso, Metropolitan University of Science of the Education (UMCE), Universidad de Santiago (USACH), we are working on the analysis of patentability. The filing of the patent "Nanofibers of chitosan wrapper antioxidant compounds for use in neuroprotection" is expected for the first semester of 2014.

The partnership between the consortium Biorend S.A. was strengthened with the award of the following project: 2013-2016 INNOVA-CORFO I+D (13IDL2-18271).

7. Administration and Financial Status

a) *Organization and administration*:



An Executive Director supervises and coordinates all the administrative duties according to the need of the Director and other Investigators. He also coordinates outreach and networking together with all efforts aimed at securing funds for the construction of the new building to house the CINV. Beside all the book keeping and accounting, there is a team to fulfill researchers' needs for purchasing and computer maintenance and another team supporting development and submission of grants, and outreach activities.Each host institution provides office and laboratory space to individual investigators holding faculty positions as well as their base salaries. The "Universidad de Valparaíso" set up an institutional grant for operational expenses.

Category	Female	Male	TOTAL
Assistant & Technicians	17	15	32
Administrative Staff	9	6	15
TOTAL	26	21	47

b) ***Financial Status:*** CINV total income for 2013 was USD$4.741.322, 41 % of which came from MSI fund and 37.5% from other national funding agency. Other sources included institutional, international and private sources. Less that 8 % of the total was used to cover administrative human resource. Of this amount, MSI contributed 46% while CONICYT and Universidad de Valparaíso with 54% each.

MSI outcome concentrates mainly in equipments (26%) to increase scientific productivity. In human resources, we have increased the item "students and technicians" and Postdoctoral Fellowship Program (25.6%) over incentives to investigators (18%). 8% of MSI budget was spend on tickets and travel expenses. This amount was complement with funds from other grants andNetworksMSI funds.

Annex 1.- Institute/Nucleus Researchers

1.1 AssociateResearchers

Name	Research Line	Nationality	Gender	Date of birth dd/mm/yy	Profession	Academic Degree	Affiliation	Current Position	Relation with Center
Adrián Palacios	4	Chilean	M	18-3-1958	Psychologist	D	Universidad de Valparaíso	Professor	2
Alan Neely	1	Chilean	M	15-4-1956	Biologist	D	Universidad de Valparaíso	Professor	2
Ana María Cardenas	2	Chilean	F	1-4-1960	Pharmacist	D	Universidad de Valparaíso	Professor	2
David Naranjo	1	Chilean	M	17-10-1957		D	Universidad de Valparaíso	Professor	2
Fernando González	5	Chilean	M	9-12-1968	Chemist	D	Universidad Andres Bello.	Professor	2
John Ewer	3	Chilean	M	23-2-1961	Biologist	D	Universidad de Valparaíso	Professor	2
Juan Carlos Saez	2	Chilean	M	2-2-1956	Biochemist	D	Universidad Católica de Chile	Professor	2
Kathleeen Whitlock	3	North American	F	27-8-1963		D	Universidad de Valparaíso	Professor	2
Ramón Latorre	1	Chilean	M	29-10-1941	Biochemist	D	Universidad de Valparaíso	Professor	1
Tomás Pérez Acle	5	Chilean	M	9-9-1970		D	Fundación Ciencia para la Vida	Assoc. Inv.	2

1.2 Young Researchers

Name	Research Line	Nationality	Gender	Date of birth dd/mm/yy	Profession	Academic Degree	Affiliation	Current Position	Relation with Center
Daniel Almonacid	5	Chilean	M	28-03-81		D	Universidad Andrés Bello	Researcher	2
José Pérez	5	Chilean	M	23-11-78		D	Universidad Andrés Bello	Researcher	2
Paola Soto	2	Chilean	F			D	Universidad Católica de Chile	Researcher	2

1.3 Senior Researchers

NONE

1.4 Others

Name	Research Line	Nationality	Gender	Date of birth dd/mm/yy	Profession	Academic Degree	Affiliation	Current Position	Relation with Center
Agustín Martínez	2	Chilean	M	14-08-68		D	Universidad de Valparaíso	Professor	2
Alfredo Kirkwood	4	Chilean	M		Biologist	D	John Hopkins University	Professor	2
Carlos González	1	Cuban	M	13-12-65		D	Universidad de Valparaíso	Assoc. Professor	2
Francisco Bezanilla	1	Chilean	M	17-05-44	Biochemist	D	Chicago University	Professor	2
José Hurtado	4	Chilean	M	19-02-69		D			2
Miguel Holmgren	1	Chilean	M			D	NIH-NINDS.	Senior Investigator	2
Oliver Schmachtenberg	4	Chilean	M	12-12-70		D	Universidad de Valparaíso	Professor	2
Osvaldo Alvarez	1	Chilean	M	14-10-42	Biochemist	D	Universidad de Chile	Professor	2
Pablo Muñoz	4	Chilean	M	19-01-73		D	Universidad de Valparaíso	Assoc. Profesor	2
Patricio Orio	5	Chilean	M	03-12-73	Biochemist	D	Universidad de Valparaíso	Professor	2
Ralph Greenspan	3	American	M			D	Kavli Institute for Mind and Brain	Professor	2
Verónica Milesi	1	Argentinian	F	02-12-62		D	Universidad Nacional de La Plata	Professor	2
Gonzalo Ferreira	1	Uruguayan	M	20-01-64			Universidad de la República de Uruguay	Professor	
Daniel Aguayo	5	Chilean	M	08-08-78		D	Universidad Andrés Bello	Professor	2

Annex 2.- Research Lines

Nº	Research Line	Research Line Objectives	Description of Research Line	Researcher	Research Discipline	Starting Date [dd/mm/yy]	Ending Date [dd/mm/yy]
1	STRUCTURE AND FUNCTION OF MOLECULAR SENSORS	We try to understand how ion channels and pumps can respond to a variety of stimuli.	It is a combination of molecular biology, electrophysiology, modern fluorescence techniques, simulations and molecular modeling.	D. Naranjo, R. Latorre,A. Neely, O. Alvarez, F. Bezanilla, M. Holmgren V. Milesis & C. González.	73	8/8/2011	
2	CELL SIGNALING	Investigate how protein-protein interactions and covalent modifications of dynamin control neurosecretion and trafficking of ion channels.	Using patch clamp amperometry and total internal reflection fluorescence microscopy the handling by the cell of vesicles containing neurotransmitters is characterized.	JC Saez, AM Cardenas, A. Martinez	61	8/8/2011	
3	DEVELOPMENTAL GENETICS AND BEHAVIOR	Understanding how the nervous system develops and produces complex behaviors.	Using zebrafish and Drosophila as biological models, the development of the olfactory system and the genetic pathways controlling behavior are studied.	K Whitlock J. Ewer, R. Greenspan-	63 y 74	8/8/2011	
4	SENSORY AND SYSTEMS	To investigate the mechanisms of neuronal encoding the visual, olfactory and cerebral physiological and pathological conditions.	Using different animal models, including Degu, a natural model for studying AD. The molecules identified by Group 2 as regulators of neurosecretion will be tested in the context of neuronal plasticity.	A.Palacios, A. Kirkwood, P. Muñoz, O. Schmachtenberg..	61 y 73	8/8/2011	
5	AND BIOLOGY COMPUTATIONAL MOLECULAR SIMULATION	Using high performance computing for molecular modeling of membrane proteins, drug design assisted by computer, and inference and dynamics of biological networks.	Interaction between theoretical and experimental biologist to create new methods, models and hypothesis suitable to be tested by the experimental groups	F.D. González. T. Perez-Acle. P. Orio. D. Aguayo, J.P. Almonacid	6, 59 y 73	8/8/2011	

Annex 3.- Publications (Total or partially financed by ICM)

Students co-authoring a paper are underlined and CINV investigator shown in bold face.

3.1.- ISI Publications or Similar to ISI Standard

3.1.1 Associate Researchers:

1. Aguirre A, Maturana CJ,Harcha PA, **Sáez JC**. (2013) Possible Involvement of TLRs and Hemichannels in Stress-Induced CNS Dysfunction via Mastocytes, and Glia Activation. *Mediators Inflamm. 2013:893521*
2. Aguirre A, Shoji KF, **Sáez JC**, Henriquez M, Quest AFG. (2013) FasL-Triggered death of jurkat cellsrequires caspase 8-induced, ATP-dependentcross-talkbetween fas and the purinergic receptor P2X7. *Journal of Cellular Physiology 228 (2): 485–493.*
3. Ardiles A, Ewer J, Acosta ML, **KirkwoodA, Martinez A**, Ebensperger LA, Bozinovic F, Lee TM, **Palacios AG**. (2013). *Octodon degus* (Molina 1782): A model in comparative biology and biomedicine. *Cold Spring Harbor Protocols. 2013(4): 312-18.*
4. Boric, K, **Orio, P**, Viéville, T., **Whitlock, KE** (2013). Quantitative analysis of cell migration using opticalflow. *PLOS One, 8(7): e69574.*
5. Cea LA, Cisterna BA, Puebla C, Frank M, Figueroa XF, Cardoso C, Willecke K, **Latorre R**, **Sáez JC**. (2013) De novo expression of connexin hemichannels in denervated fast skeletal muscles leads to atrophy. *Proc Natl Acad Sci USA. 110(40): 16229-34.*
6. Contreras GF, Castillo K, Enrique N, Carrasquel-Ursulaez W, Castillo JP, **Milesi V**, **Neely A**, **Álvarez O**, Ferrerira G, **González C**, **Latorre R**. (2013). A BK (Slo l) cannel journeyfrommolecule to physiology. *Channels (Austin). 7(6): 441-457*
7. Escobar MJ, **Palacios AG**. (2013). Beyond the retina neural coding: OnModels and Neural Rehabilitation. *Journal of Physiology Paris. 107(5):335-7*
8. Figueroa V, Sáez PJ, Salas JD, Salas D, Jara O, **Martínez AD,Sáez JC,** Retamal MA. (2013) Linoleicacid induces opening of connexin26 hemichannels through a PI3K/Akt/Ca(2+)-dependent pathway. *Biochim Biophys Acta. 1828(3):1169-79.*
9. Figueroa XF, Lillo MA, Gaete PS, Riquelme M, **Sáez JC**. (2013) Diffusion of nitric oxide across cell membranes of the vascular wall requires specific connexin-based channels. *Neuropharmacology. 75: 471–478.*
10. Giaume C, Leybaert L, C Naus C, **Sáez JC**. (2013) Connexin and pannexin hemichannels in brain glial cells: properties, pharmacology, and roles. *Front Pharmacol. (2013) 4:88.*
11. González-Jamett AM, Momboisse F, Guerra MJ, Ory S, Báez-Matus X, Barraza N, Calco V, Houy S, Couve E, **Neely A**, **Martínez A**, Gasman S, **Cárdenas AM** (2013) Dynamin-2 regulates fusion pore expansion and quantal release through a mechanism that involves actin dynamics in neuroendocrine chromaffincells. *PLOS ONE, 8(8):70638.*
12. González-Jamett AM, Momboisse F, Haro-Acuña V, Bevilacqua JA, Caviedes P, **Cárdenas AM.** (2013) Dynamin-2 Function and Dysfunction Along the Secretory Pathway, *Front Endocrinol (Lausanne). 4:126.*
13. Hernández-Salinas R, Vielma AZ, Arismendi MN, Boric MP, **Sáez JC,**Velarde V. (2013) Boldine prevents renal alterations in diabetic rats. *J Diabetes Res. 2013:593672.*
14. Johnson RG, **Sáez JC**. (2013) "We´ve Had Important Advancez in the Connexin/Pannexin Field, Yet There Is Still Much to Do" *Neuropharmacology. 75: 467–470*
15. Kozoriz MG, Lai S, Vega JL, **Sáez JC**, Sin WC, Bechberger JF, Naus CC. (2013) Cerebral ischemic injury is enhanced in a model of oculodentodigital dysplasia. *Neuropharmacology. 75: 549-556*

16. **Latorre R**, Contreras G (2013) Keeping you healthy: BK channel activation by omega-3 fatty acids. *Journal of General Physiology. 142:347-350*
17. Ocampo-Garcés A, Hernandez F, **Palacios AG**. (2013) REM sleep preference in the crepuscular *Octodon degus* assessed by selective REM sleep deprivation. *Sleep. 36(8):1247-56*
18. Orellana JA, Velasquez S, Williams DW, **Sáez JC**, Berman JW, Eugenin EA. (2013) Pannexin1 hemichannels are critical for HIV infection of human primary CD4+ T lymphocytes. *J Leukoc Biol. 94(3):399-407*
19. **Palacios AG**, Lee TM. (2013). Husbandry and Breeding in the*Octodon degus* (Molina 1782). *Cold Spring Harbor Protocols. 2013(4): 350-53.*
20. Ravanal MC, Alegrá-Arcos M, **González-Nilo FD**, Eyzaguirre J. (2013) *Penicillium purpurogenum* produces two GH family 43 enzymes with β-xylosidase activity, one mono functional and the other bifunctional: biochemical and structural analyses explain the difference. *Arch Biochem Biophys. 540(1-2):117-24*
21. Riquelme MA, Cea LA, Vega JL, Boric MP, Monyer H, Bennett MV, Frank M, Willecke K, **Sáez JC.** (2013) The ATP Required for Potentiation of Skeletal Muscle Contraction is released via Pannexin Hemichannels. *Neuropharmacology. 75:594–603*
22. Sáez PJ, Orellana JA, Vega-Riveros N, Figueroa VA, Hernández DE, Castro JF, Klein AD, Jiang JX, Zanlungo S, **Sáez JC**. (2013) Disruption in connexin-based communication in associated with intracellular Ca(2+) signal alterations in astrocytes from Niemann-Pick type C mice. *PLoSOne. 8(8):e71361.*
23. Sáez PJ, Shoji KF, Retamal MA, Harcha PA, Ramírez G, Jiang JX, von Bernhardi R, **Sáez JC.**(2013) ATP is required and advances cytokine-induced gap junction formation in microglia in vitro. *Mediators Inflamm. 2013:216402.*
24. Soto-Liebe K, López-Cortés XA, Fuentes-Valdes JJ, Stucken K, **González-Nilo F**, Vásquez M. (2013). In silico analysis of putative paralytic shell fish poisoning toxins export proteins in cyano bacteria. *PLoSOne. 8(2):e55664*
25. Tricarico D, Mele A, Calzolaro S, Cannone G, Camerino MG, Dinardo MM, **Latorre R**, Conte Camerino D. (2013) Emerging Role of Calcium- Activated Potassium Channel in the Regulation. *PLosOne. 8 (7). E69551.*
26. Vega-Zúñiga T, Medina FS, Fredes F, Zúñiga C, Severín D, **Palacios AG**, Karten HJ, Mpodozis J. (2013). Does nocturnality drive binocular vision? Octodontine rodents as a case study. *PlosOne.8(12):e84199*
27. Vega JL, Subiabre M, Figueroa F, Schalper KA, Osorio L, González J, **Sáez JC**. (2013) Role of Gap Junctions and Hemichannels in Parasitic Infections. *Biomed* Res Int. 2013:589130Review.
28. Vergara-Jaque AP, Comer JR, Monsalve LF, **González-Nilo FD**, Sandoval C. (2013) ComputationallyEfficientMethodologyforAtomic-LevelCharacterization of Dendrimer-Drug Complexes: A Comparison of Amine- and Acetyl-Terminated PAMAM. *Journal of PhysicalChemistry B. 117(22):6801-13*
29. Vilos C, Morales FA, Solar PA, Herrera NS, **González-Nilo FD**, Aguayo DA, Mendoza HL, Comer J, Bravo ML, González PA, Kato S, Cuello MA, Alonso C, Bravo EJ, Bustamante EI, Owen GI, Velasquez LA. (2013) Paclitaxel-PHBV nanoparticles and their toxicity to endometrial and primary ovarian cancer cells. *Biomaterials. May. 34(16):4098-108.*

3.1.2 Other researchers:

1. Bazáes A, Olivares J, **Schmachtenberg O**. (2013) Properties, projections and tuning of teleostolfactory receptor neurons. *Journal of Chemical Ecology. 39(4):451-6*4.
2. Castillo K, Valenzuela V, Matus S, Nassif M, Oñate M, Fuentealba Y, Encina G, Irrazabal T, Parsons G, Court FA, Schneider BL, Armentano D, Hetz C. (2013) Measurement of autophagy flux in the nervous system in vivo. *Cell Death Dis. 14;4:e917*.
3. Couve, E., Osorio, R. and **Schmachtenberg, O**. (2013) The Amazing Odontoblast: Activity, Autophagy and Aging. *J Dental Res. 92(9):765-72*
4. Escobar MJ, Pezo D, **Orio P**. (2013) Mathematic analysis and modeling of motion direction selectivity in the retina. *Journal of Physiology Paris. 107(5):349-59*
5. **González, C,** Rebolledo S, Perez M and Larsson HP. (2013) Molecular mechanism of voltage sensing in voltage-gated proton channels. *Journal of General Physiology. 141(3):275-85.*
6. Orellana JA, **Martinez AD**, Retamal MA. (2013) Gap junctionchannels and hemichannels in the CNS: Regulation by signaling molecules. *Neuropharmacology. 75: 567–*582
7. Osorio R, **Schmachtenberg O** (2013) Calcium-activated chloride channels do not contribute to the odorant transduction current in the marine teleost Isacia Conceptionis. *Journal of Fish Biology. 83(5): 1468-73*.
8. Pastor P, Cisternas P, Salazar K, Silva-Álvarez C, Oyarce K, Jara N, Espinoza F, **Martínez AD** and Nualart F (2013) SVCT2 vitamin C transporter expression in progenitor cells of the postnatal neurogenic niche. *Frontiers in Cellular Neuroscience. 7:119.*
9. Qiu F, Rebolledo S, **González C,** Larsson HP. (2013) Subunit Interactions during Cooperative Opening of Voltage-Gated Proton Channels. *Neuron. 77(2), 288–98*.

3.2.- SCIELO Publications or Similar to SCIELO

3.2.1 Associate Researchers:

1. Almeyda Campos MV, Costa C, **González C, Latorre R**, Milesi V, Ferreira G. (2013) New biomedical and physiological knowledge of the sport (with emphasis on ion channels in skeletal muscle). *In "New Technologies and Innovation in Human Movement". (Book VI REMH). Ed. Universidad de Chihuahua.*

3.2.2 Other researchers:

3.3.- Scientific Books and Chapters

3.3.1 Associate Researchers:

1. Cessac B and **Palacios AG.** (2013) Spike train statistics from empirical facts to theory: the case of the retina. In Mathematical Problems in Computational Biology and Biomedicine. Cazals F, Kornprobst P, pag. 261-302. Springer-Verlag, Berlin Heidelberg, Germany.
2. Giaume C, Froger N, Orellana JA, Retamal M, **Sáez JC.**(2013) Impact of Microglial Activation on Astroglial Connexin Expression and Function in Brain Inflammation. Chapter 11 in: E Oviedo-Orta, BR Kwak, WH Evans, eds: "Connexin Cell Communication Channels. Roles in the Immune System and Immunopathology", pp 219-232. CRC Press: Taylor and Francis group. Florida, USA.
3. **Latorre R** and Báez-Nieto, D. (2013) Ca2+ activation of K+ channels: RCK domains. In: Encyclopedia of Biophysics. Pag. 201-205. SpringerVerlag. Heidelberg, Germany.

4. **Latorre R**, **González C**, Rojas P. (2013) Signal-Transduction-Dependent Channels, Neuroscience in the 21st century, Eugene Martin and Donald Pfaff, eds. pag. 81-107.Springer. New York, USA
5. **Latorre R**, Morera FJ, Zaelzer C. (2013). Voltage-dependent K+ channels. In: Encyclopedia of BiologicalChemistry. 2nd Edition. Lane, M.D and W.J. Lennarz, editors. Vol 1, pp. 399-404. Elsevier. London, UK
6. Sáez PJ, Shoji KF, **Sáez JC**. (2013) Gap Junctions in Antigen-Presenting Cells. In "Connexin cell communication channels: roles on the immune system and immunopathology" (ed. Oviedo-Orta, Kwak and Evans), pp 61-88. CRC Press: Taylor and Francis group. Florida, USA.

3.3.2 Other researchers:

3.4.- Other Publications

3.4.1 Associate Researchers:

3.4.2 Other researchers:

3.5.- Collaborative publications:

Category of Publication	1 researcher		2 researchers		3 researchers		4 or more researchers	
	Nº	**%**	**Nº**	**%**	**Nº**	**%**	**Nº**	**%**
ISI Publications or Similar to ISI Standard	32	72%	3	7%	2	4%	1	2%
SCIELO Publications or Similar to SCIELO Standard					1	2%		
Books and chapters	5	11%	1	2%				
Other Publications								
Total of publications	37	83%	4	9%	3	6%	1	2%

Annex 4.- Organization of Scientific Events

Scope	Title	Type of Event	City	Country	Responsible Researcher
National	2° Meeting Anual Instituto Milenio CINV	Symposia	Valparaiso	Chile	Alan Neely
International	Latin american School on Ion Channel Biophysics	Workshop	Valparaiso	Chile	Carlos González
National	Physical Biology of the Cell	Symposia	Valparaiso	Chile	Ramón Latorre
International	VIII Iberoamerican Congress of Biophysics	Congress	Valparaiso	Chile	Alan Neely

Annex 5.- Education and capacity building

Annex 5.1 CapacityBuilding inside MSI Centers

MSI RESEARCHER	NUMBER												TOTAL NUMBER PER MSI RESEARCHER		
	Undergraduate students			Graduate students						Postdoctoral researchers					
				Masters			Doctoral								
	F	M	**T**	F	M	**T**	F	M	**T**	F	M	**T**	F	M	**T**
Adrián Palacios	1	1	**2**	1	1	**2**	1	1	**2**	1		**1**	4	3	**7**
Agustín Martínez	1		**1**	1	3	**4**			**0**		1	**1**	2	4	**6**
Alan Neely			**0**	1		**1**		1	**1**			**0**	1	1	**2**
Ana María Cárdenas			**0**	1	1	**2**		1	**1**	2		**2**	3	2	**5**
Carlos González			**0**	1	1	**2**	1		**1**	1	1	**2**	0	2	**5**
David Naranjo			**0**		0	**0**	2		**2**	0	0	**0**	0	2	**2**
Fernando González			**0**			**0**		2	**2**			**0**	0	2	**2**
John Ewer			**0**		1	**1**	1	1	**2**		1	**1**	1	3	**4**
Juan Carlos Sáez	2		**2**	1		**1**	4	4	**8**		4	**4**	7	8	**15**
KathleenWhitlock			**0**			**0**	1	3	**4**	1	1	**2**	2	4	**6**
Oliver Schmachtenberg		1	**1**			**0**		2	**2**			**0**	0	3	**3**
Pablo Muñoz	1		**1**			**0**	1	1	**2**			**0**	2	1	**3**
Patricio Orio	1	1	**2**		1	**1**		2	**2**			**0**	1	4	**5**
Ramón Latorre			**0**	2		**2**	1	3	**4**			**0**	3	3	**6**
Tomás Pérez-Acle	1	1	**2**		1	**1**		2	**2**		3	**3**	1	7	**8**
A. Cárdenas-A. Neely			**0**	1		**1**		1	**0**			**0**	1	0	**1**
A. Martínez-C. González			**0**			**0**		2	**2**			**0**	0	2	**2**
A. Neely - C. González			**0**			**0**			**0**		1	**1**	0	1	**1**
C. González - R. Latorre			**0**			**0**		1	**1**			**0**	0	1	**1**
O. Schmachtenberg - A. Palacios			**0**		1	**1**			**0**			**0**	0	1	**1**
TOTAL	7	4	**11**	9	10	**19**	12	26	**38**	6	14	**20**	31	54	**85**

Annex 5.2.- Short-term Traineeships of MSI students

Student name	Institution	Country	Advisor	Project Description	Starting Date [dd/mm/yy]	Ending Date [dd/mm/yy]
Carola Maturana	University of California, San Diego	USA	Juan Carlos Sáez	Doctorate thesis research	01-04-2013	28-02-2014
Isaac García	RutgersUniversity	USA	Agustín Martínez	Doctorate thesis research	17-12-13	15-01-14
María José Guerra	U. de la Laguna Tenerife	España	Ana María Cárdenas	Master´s thesis research	09-06-13	10-07-13
Paloma Harcha	Collège de France	Francia	Juan Carlos Sáez	Doctorate thesis research	01-05-13	06-08-13
Raul Araya	IBM Thomas J. Watson Research Center	USA	Tomás Pérez-Acle	Doctorate thesis research	18-11-13	15-01-14
Sara Ganados	Pontificia U. Javeriana	Colombia	Ramón Latorre	Doctorate thesis research	18-06-13	06-10-13

Annex 6.- Networking and other collaborative work

Annex 6.1 Networking

NOMENCLATURE:
[Network Scope]
[N] National [I] International [LA] Latin American

Network Name	Network Scope	Network Participants [Number]				Institutions
		From the Center		External		
		Researchers	Postdocs/Students	Researchers	Postdocs/Students	
Biophysics and Computational Neurosciences Network	**I**	7	10	32	46	Max Planck Inst. Germany INRIA, France UTFSM, Chile CMM, Chile
Cellular signaling Network	**I**	6	8	7	63	U of Aucklan, New Zeland U. W Ontario, Canada NIH-NINDS,USA U. de Sevilla, Spain UBA Argentina UNAB , Chile U de Chile, Chile PUC, Chile College de France, France

Annex 6.2.- Other collaborative activities

Activity Name	Co-Participant Institution(s)	Participants [Number]				Products [Type &Number]
		MSI center		External		
		Researchers	Postdocs/Students	Researchers	Postdocs/Students	
CONICYT MEC grant an expert on to investigate the circadian clock of jumping spiders.	Dr. Ximena Nelson (Canterbury U., New Zealand)	1		1		1 Grant 1 Sci. Exch
ECOS/CONICYT grant to investigate the process of maturation of the insect exoskeleton	Dr. Jean-François Ferveur (Université de Bourgogne	1	1	1		1 Grant 1 Sci. Exch
ECOS/CONICYTto work on neural coding from small retina networks and natural images	Dr. Frederique Alexandre(INRIA, Université de Bordeaux)	2	3	3	3	1 Grant
CONICYT/Redes project that supports the Creation of International Networks between Research Centers 2013	Department of Biophysics, Faculty of Medicine, Universidad de la República, Uruguay	2	2	2	1	1Grant 1Paper 1 Sci. Exchg
Development of a lipidic biosensor platform for the *in situ* detection of Red Tide - ; II Contest for Applied Science 2013	Universidad Técnica Federico Santa María	1	1	2	1	1 Grant

Activity Name	Co-Participant Institution(s)	Participants [Number]				Products [Type &Number]
		MSI center		External		
		Researchers	Postdocs/Students	Researchers	Postdocs/Students	
Connexins Hemmichannel and Gap Junction channel structure and function	Center for Computational Biology at the IBM Watson Research Center, Yorktown Heights, NY, USA	2	1	1	2	1 Manusc. 1 Abstract
High throughput mutagenesis technique applied to the cold receptor TRPA1	Scripps Research Institute, California Campus Duke University	1	1	21	3	1 Paper 1 Sci. Xchg 1 Abstract
Voltage-Clamp fluorometry applied to voltage-dependent Calcium channels	Department of Anesthesiology David Geffen School of Medicine University of California Los Angeles	1	1	1	2	3 Sci. Xchg. 1 Manusc. 1 Grant appl 2 Abstract 1 Conference
ECOS-CONICYT C08-B01. to investigate the contribution of dynamin in the endo/ exocytosis	Stéphane Gasman (CNRS, Strasburg, France)	3	3	1	2	1 Paper 1 Absract
The role of actin-binding proteins in exocytosis	Dr. Narcisa Martínez-Quiles (U. Complutense de Madrid, Spain)	1	2	1		2 Sci. Xchg. 1 Manusc
The role of P/Q-type and N-type calcium channel on calcium signals at the zebrafish neuromuscular synapses	Dr. Paul Brehm and Gail Mandel´s labs atthe Vollum Institute at OHSU, Portland, USA	1	1	2	3	1 Sci. Xchg. 2 Manusc. 1 Conference

Activity Name	Co-Participant Institution(s)	Participants [Number]				Products [Type &Number]
		MSI center		External		
		Researchers	Postdocs/Students	Researchers	Postdocs/Students	
The role of TRP channels in peripheral sensory transduction and in synaptic plasticity, in health and disease	Universidad de Santiago de Chile, USACH (Proyecto Anillo ACT-1113, Director Dr. Rodolfo Madrid, USACH	1	1	1	1	3Abstracts: 2Papers
The involvement of NO in cell death mechanisms in hereditary retinal degeneration	University of Tuebingen Centre for Ophthalmology Cell Death Mechanisms Group Dr. Francois Paquet-Durand,	1	1	1	2	1 Sci. Xchg
FONDECYT Role of overexpressed DSCAM and APP in PAK kinase derregulation, and the consequent neuronal dysfunction in in vitro models of Down syndrome: A quest for cellular therapeutical targets	Dr. Pablo Caviedes, from ICBQ, Facultad de Medicina, Universidad de Chile.	1	1	2	2	3 abstract 2 papers
ACT1121: Molecular and cellular mechanisms of muscular dystrophy related to mutations of dysferlin.	Dr. Pablo Caviedes, from ICBQ, Fac. Medicina, U. Chile is IP. AM Cárdenas is an associated investigator.	1	2	3	1	2 absctracts

Annex 7.- Outreach

Annex 7.1.- Outreach activities throughout the period

a) International events

Description of Activity		Date	Location Region	Target Audience
"The wonders of animal electricity" by the President of the Society of Latin American Biophysicists and member of CINV, Dr. Miguel Holmgren	Conference	1-09-2013	Valparaíso, Parque Cultural de Valparaíso	General Community and primary school students
Closingconference at Nexos 2013: Fourth Reunion of Chilean Scientists in the United States. " Haciendo Ciencia en el fin del mundo" by A. Neely	Conference	11-10-2013	Montgomery County Campus, Johns Hopkins University Washington DC	Chilean Scientist and authorities abroad

b) National events

Description of Activity		Date	Location Region	Target Audience
"Role of synaptogyrin in synaptic function at Drosophila NMJ". Ramón Jorquera (Universidad Central del Caribe)	Seminar	10-01-2013	Universidad de Valparaíso	University students
"A network of integrate and fire neurons. Existence of solution, blow up effects". Etienne Tanre (INRIA Sophia-Antipolis)	Seminar	11-01-2013	Universidad de Valparaíso	University students
"Role of synaptogyrin in synaptic function at Drosophila NMJ". Ramón Jorquera (Universidad Central del Caribe)	Seminar	10-01-2013	Universidad de Valparaíso	University students
Drosophila as a model for the study of human diseases. Florencia Tevy (IRCCS, Italy)	Seminar	15-03-2013	Universidad de Valparaíso	University students
Student seminar: Jacqueline Vásquez and Ingris Oyarzún	Seminar	22-03-2013	Universidad de Valparaíso	University students
Student seminar: Isaac García and Fanny Momboisse	Seminar	05-04-2013	Universidad de Valparaíso	University students
"Función de las espinas dendríticas en el procesamiento, almacenaje e integración sináptica". Roberto Araya (University of Montreal)	Seminar	08-04-2013	Universidad de Valparaíso	University students
"Aumentando la validez ecológica para lidiar con la complejidad cognitive: Posibles soluciones desde la electroencefalografía". Carlos Hamamé (CNRS, Aix-Marseille Université)	Seminar	12-04-2013	Universidad de Valparaíso	University students

Description of Activity		Date	Location Region	Target Audience
Student seminar: Roxana Contreras and Ignacio Díaz	Seminar	19-04-2013	Universidad de Valparaíso	University students
Programa Científico Educacional: Ciencia Al Tiro (CAT). Kathleen Withlock (CINV-UV	Workshop	22-04-2013	Santiago	Companies
Student and Postdoc seminar: Karen Castillo and Cristian Calfún	Seminar	03-05-2013	Universidad de Valparaíso	University students
Cervantino School of Putaendo visited the laboratories of CINV	Other (Lab visit)	03-05-2013	Universidad de Valparaíso	Secondary students
Student and Postdoc seminar: Willy Carrasquel and Anna Kedzierska.	Seminar	10-05-2013	Universidad de Valparaíso	University students
¿Qué tienes en mente?: "La neurociencia de los sentidos". Patricio Orio (CINV-UV)	Conference	15-05-2013	Los Andes, V Región	Primary - Secondary Students
"Conexinas y Panexinas, de la sordera al movimiento". (Agustín Martínez, CINV-UV)	Seminar	17-05-2013	Universidad de Valparaíso	University students
¿Qué tienes en mente?: "Pasas para la memoria". Pablo Muñoz (CINV-UV)	Conference	15-05-2013	Putaendo, V Región	Primary - Secondary Students
Postdoc seminar: Álvaro Ardiles	Seminar	24-05-2013	Universidad de Valparaíso	University students
"Epigenética del desarrollo cerebral: Mecanismos moleculares de la regulación génica a través del remodelamiento de la cromatina". Matías Alvarez-Saavedra (OHRI, Canada)	Seminar	30-05-2013	Universidad de Valparaíso	University students
¿Qué tienes en mente?: "Exprimir mi cerebro puede ser muy divertido". Carlos González (CINV-UV)	Conference	03-06-2013	Limache, V Región	Primary - Secondary Students
"La familia Slo: De las aventuras y desventuras de los canales Slo2 (sodium-activated potassium chanels)". Gonzalo Ferreira (Universidad de la República, Uruguay)	Seminar	07-06-2013	Universidad de Valparaíso	University students
"Detection and characterization of the structural determ inants of temperature sensitivy in TRPM8". Natalia Raddatz, Tesis (Universidad de Valparaíso)	Seminar	13-06-2013	Universidad de Valparaíso	University students
"Señalización y plasticidad neuronal en condiciones normales y ELA". Brigitte van Zundert (Universidad Andrés Bello, Chile)	Seminar	14-06-2013	Universidad de Valparaíso	University students
Student and Postdoc seminar: Óscar Jara and Isabel Benjumeda.	Seminar	21-06-2013	Universidad de Valparaíso	University students
Launching of TV Serie Neuromantes (Chapter: La percepción)	Exhibition	27-06-2013	Severin Library, valparaíso	General public
¿Qué tienes en mente?: "El cerebro funciona a baterías". Alan Neely (CINV-UV)	Conference	03-06-2013	La Ligua, V Región	Primary - Secondary Students

Description of Activity		Date	Location Region	Target Audience
Student and Postdoc seminar: Mauricio Aspe and Wilson Mena	Seminar	05-07-2013	Universidad de Valparaíso	University students
Tertulias Porteñas, ¿Qué sabemos del dolor?	Conference	29-07-2013	Parque Cultural de Valparaíso	General Community
"El estado redox celular condiciona la señalización por calcio en neuronas". Cecilia Hidalgo (Universidad de Chile)	Seminar	05-08-2013	Universidad de Valparaíso	University students
¿Qué tienes en mente?: "Exprimir mi cerebro puede ser muy divertido". Carlos González (CINV-UV)	Conference	07-0-2013	Quillota, V Región	Primary - Secondary Students
"Neuromodulación de la plasticidad neuronal". Alfredo Kirkwood (Johns Hopkins University)	Seminar	09-08-2013	Universidad de Valparaíso	University students
"Ciencia Al Tiro: Acuaponia, Educación y Esperanza". Kathleen Withlock (CINV-UV)	Seminar	23-08-2013	Universidad de Valparaíso	University students
¿Qué tienes en mente?: "Los sentidos, nuestra ventana al mundo". Oliver Schmachtenberg (CINV-UV)	Conference	28-08-2013	Quintero, V Región	Primary - Secondary Students
¿Qué tienes en mente?: "La qu{imica del cerebro". Ana María Cárdenas (CINV-UV)	Conference	28-08-2013	Valparaiso, V Región	Primary - Secondary Students
"The neuroethology of the C. elegans escape response". Mark Alkema (University of Massachusetts)	Seminar	30-08-2013	Universidad de Valparaíso	University students
¿Qué tienes en mente?: "Del azar molecular al orden neuronal" David Naranjo (CINV-UV)	Conference	04-09-2013	San Antonio, V Región	Primary - Secondary Students
"Development and organization of taste and smell". Tom Finger (University of Colorado)	Seminar	30-09-2013	CINV	University students
"Temporal characteristics and mechanisms of photoreceptor degeneration". Francois Paquet-Durand (University of Tuebingen)	Seminar	11-10-2013	Universidad de Valparaíso	University students
"El problema de la percepción consciente en Ciencias Cognitivas". Diego Cosmelli (Universidad Católica de Chile)	Seminar	18-10-2013	Universidad de Valparaíso	University students
"Lessons learned from disease-causing connexin mutations". W. Laird (University of Western Ontario)	Seminar	21-10-2013	Universidad de Valparaíso	University students
"De máquinas moleculares y sueños". Ramón Latorre (CINV-UV)	Seminar	23-10-2013	Universidad de Chile, Santiago.	University students
"Neuromantes: Las emociones". 19th International tv shows exhibition – Ver Ciencia	Exhibition	23-10-2013 03-11-2013	Rio de Janeiro, Sao Paulo, Brasilia.	General public

Description of Activity		Date	Location Region	Target Audience
"New insights in the modulation of synaptic strength" Andrés Chávez (Albert Einstein College of Medicine)	Seminar	29-10-2013	(Video conference) Universidad de Valparaíso	University students
"Sistemas sensoriales en contexto". Daniel Rojas (Biomedical Neuroscience Institute)	Seminar	30-10-2013	Universidad de Valparaíso	University students
"Gap junctions hemichannels mediated vacular leak in the inflammatory response". Colin R. Green (University of Auckland)	Seminar	30-10-2013	Universidad de Valparaíso	University students
"Mitochondrial structure-function relationship: organelle dynamics and Ca2+ homeostasis in the skeletal muscle". Verónica Eisner (Thomas Jefferson University)	Seminar	04-11-2013	(Video conference) Universidad de Valparaíso	University students
"El canal de protons, ¿un sensor de potencial o un poro de conducción?". Carlos González (CINV-UV)	Seminar	05-11-2013	(Video conference) Universidad de Valparaíso	University students
"Experiencias y Plasticidad Neuronal". Pablo Muñoz (CINV-UV)	Seminar	08-11-2013	Universidad de Valparaíso	University students
"Un, dos, tres Neurociencia es". Adrián Palacios (CINV-UV)	Conference	14-11-2013	Universidad Católica de Valparaíso	Primary, Secondary, University students.
"Effects of prenatal stress on memory consolidation and functional connectivity between the hippocampus and the prefrontal cortex". Ignacio Negrón (Universidad Católica de Chile)	Seminar	15-11-2013	Universidad de Valparaíso	University students
"Large Scale Biomolecular Modeling with IBM Blue Green". Ruhong Zhou (IBM Thomas J. Watson Research Center)	Seminar	20-11-2013	Universidad de Valparaíso	University students
"Papel de los transportadores de serotonina y glutamato en el Transtorno Obsesivo-Compulsivo". Pablo Moya (Universidad de Valparaíso)	Seminar	22-11-2013	Universidad de Valparaíso	University students
Visit to Valparaíso and CINV labs of students of PENTA Programme of Santiago	Exhibition	24-11-2013	Valparíso	Secondary students
Launching of TV Serie Neuromantes (Chapter: La belleza)	Exhibition	05-12-2013	Library of Providencia, Santiago	General public
Tertulias Porteñas, ¿Qué sabemos de la conciencia?	Conference	19-12-2013	Parque Cultural de Valparaíso	General Community
"Ojo, cerebro y envejecimiento en Octodon Degus". Adrián Palacios (CINV-UV)	Seminar	19-12-2013	Universidad de Chile. Santiago.	University students

Description of Activity		Date	Location Region	Target Audience
"Mechanisms for membrane transport homeostasis at the interface between the endoplasmic reticulum and the Golgi complex". Jorge Cancino (Consiglio Naxionale delle Ricerche, Italy)	Seminar	20-12-2013	Universidad de Valparaíso	University students
"Neuromodulación en el bulbo olfatorio y el proceso de olores". Ricardo Araneda (University of Maryland)	Seminar	27-12-2013	Universidad de Valparaíso	University students

Annex7.2.- Products of outreach

Type of Product	Target Public	Scope	Total
TV series	1	Community in general	International
Audiovisual recordings	5	Community in general	National
2013 Calendar with co focal microscopy images	1	Secondary Students	National
Color printed map of historical Valparaiso	1	Community in general	National
Documentary for public TV	1	Community in general	International
Documentary for public TV.	1	Community in general	National
Documentary for public TV	1	Community in general	National

Annex 7.3.- Articles and Interviews

Type of media and scope	Local/Regional		National		International		
	N° Interviews	N° Articles	N° Interviews	N° Articles	N° Interviews	N° Articles	TOTAL
Written	1	2	1	5			**9**
Internet	1	35	1	13			**50**
Audiovisual		2		1			**3**
TOTAL	**2**	**39**	**2**	**19**			**62**

Annex 8.- Connections with other sectors:

Activity and Objective	Expected Impact	Obtained Results	Type of Connection [Number]	Type of Activity [Number]	Institution Name	Institution City, Region & Country	Agent Type [Number]	Economic Sector
Meetings with National and regional authorities: 1 Senator : Ricardo Lagos Weber, 2. Congressman: Joaquín Godoy 3. Presidential candidate: Andrés Velasco 4.City Major: Jorge Castro To promote CINV in the city and region and fund-raising for the new building to house CINV	Lobbing for the development of basic sciencesat the national and regional level. Increase visibility of CINV research and outreach, activities. To secure funding for the new building of the CINV	Agreement with Central and Local Authorities to support funding the new building of CINV.	2	4	1. Senate 2. Congress 4, City	Valparaiso, Valparaiso, Chile	2	2
Visit by a fact finding mission from the US embassy. To identify potential research that will be of interest to the US	To develop new research with US funding	2 proposal were submitted	2	2	US embassy	USA	2	2
Meeting with the CEO of TPS the main port company of the city: Francesco Schiaffino, To include the private sector in an advisory board for CINV	To develop a strategic partnership with the private sector for the promotion of science at the local level	TPS willingness to join the advisory board	2	4	TPS Puerto Valparaíso	Chile	1	1

Annex 9.1 Total incomes*:*

Exchange rate: US$ 1 = $ 490

Funds	Accumulated incomes to last year [$]	2013 Incomes		Total incomes to 2013 [$]
		Amount [$]	Percentage of resources used by the Center [%]	
ICM (CINV, Redes y PME)	3.049.128	863.830.000	100%	863.830.000
CONICYT (FONDECYT, PIA)	2.633	156.803.000	100%	785.096.000
UV (Department Direc. Invest.)	1.516.980	965.006.521	100%	379.588.521
FIRCA	20.505	17.170.380	5%	17.170.380
Corporación CINV	4.082	13.182.580	100%	13.182.580
OTHER AGENCIES (CNTV, FNDR)	90.816	6.000.000	100%	6.000.000
Others (N62909-13-N251)	57.179	28.017.600		28.017.600
TOTAL	4.741.321,87	2.050.010.081		2.092.885.081

Annex 9.2 Outcome structure

ITEM	Accumulated expenses to last year [$]	2013 Expenses [$]				Total expenses to 2013 [$]	%
		Operative	Networking	Outreach	**Total**		
Honoraria Researchers	195,549,930	104,600,000	0	0	104,600,000	300,149,930	**18%**
Honoraria students and other personnel	229,642,278	205,446,072	0	0	205,446,072	435,088,350	**26%**
Tickets and travel expenses	46,296,451	71,014,170	22,675,000	4,076,287	97,765,457	144,061,908	**8%**
Materials/supplies	56,877,829	24,425,187	421,298	7,240,909	32,087,394	88,965,223	**5%**
Goods and equipment	324,548,723	108,054,389	0	6,806,944	114,861,333	439,410,056	**26%**
Infrastructure	11,870,682	3,948,600	0	0	3,948,600	15,819,282	**1%**
Administrative expenses	67,327,239	56,666,785	0	0	56,666,785	123,994,024	**7%**
Publications and subscriptions	4,524,899	3,428,454	0	0	3,428,454	7,953,353	**0%**
Consultancies	29,380,533	64,974,066	0	12,875,134	77,849,200	107,229,733	**6%**
Overhead	21,600,000	11,582,580	0	0	11,582,580	33,182,580	**2%**
Insurance costs	882,213	2,367,343	0	0	2,367,343	3,249,556	**0%**
Legal personality expenses	0	0	0	0	0	0	**0%**
Others	986,208	1,637,234	0	0	1,637,234	2,623,442	**0%**
Total Expenses ($)	**989,486,985**	**658,144,880**	**23,096,298**	**30,999,274**	**712,240,452**	**1,701,727,437**	**100%**

Annex 9.3 Financial accounting

ITEM	2013 [$]				TOTAL TO 2013
	Operative	Networking	Outreach	**Total [$]**	
Income	$802,620,000	$28,800,000	$32,410,000	$863,830,000	**$802,620,000**
Outcome	$658,144,880	$23,096,298	$30,999,274	$712,240,452	**$658,144,880**
Annual balance	$144,475,120	$5,703,702	$1,410,726	$151,589,548	**$144,475,120**