Annex 3
Publications

Chapter 26
Thermo-TRP Channels: Biophysics of Polymodal Receptors

David Baez-Nieto, Juan Pablo Castillo, Constantino Dragicevic, Osvaldo Alvarez, and Ramon Latorre

Abstract In this chapter we discuss the polymodal activation of thermo-TRP channels using as exemplars two of the best characterized members of this class of channels: TRPM8 and TRPV1. Since channel activation by temperature is the hallmark of thermo-TRP channels, we present a detailed discussion on the thermodynamics involved in the gating processes by temperature, voltage, and agonists. We also review recently published data in an effort to put together all the pieces available of the amazing puzzle of thermo-TRP channel activation. Special emphasis is made in the structural components that allow the channel-forming proteins to integrate such diverse stimuli, and in the coupling between the different sensors and the ion conduction pathway. We conclude that the present data is most economically explained by allosteric models in which temperature, voltage, and agonists act separately to modulate channel activity.

26.1 Introduction

Transient Receptor Potential (TRP) channels play important roles in sensory transduction from insects to mammals. TRP channels conform a superfamily consisting of seven subfamilies with little homology between them. The seven subfamilies include: the classical TRP subfamily (TRPC), the melastatin related subfamily (TRPM), the vanilloid-sensitive TRP subfamily (TRPV), the ankyrin subfamily (TRPA), the polycystin subfamily (TRPP), the mucolipin subfamily (TRPML), and the TRPN subfamily, named after the non mechanoreceptor potential C (nonpC) homologue [1]. Bioinformatic analyses based on primary structure of diverse sequences of TRP and topological predictions, suggest that these channels are

D. Baez-Nieto (✉)
Centro Interdisciplinario de Neurociencias de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile
e-mail: monobolico@gmail.com

M.S. Islam (ed.), *Transient Receptor Potential Channels*, Advances in Experimental Medicine and Biology 704, DOI 10.1007/978-94-007-0265-3_26,

Chapter 28
Voltage Sensing in Thermo-TRP Channels

Sebastian Brauchi and Patricio Orio

Abstract Membrane voltage, ligand binding, mechanical force and temperature can all induce conformational changes that open ion channel pores. A key question in understanding ion channel function is how the protein domains involved in sensing stimuli (sensors) communicate with the pore to gate its opening and closing. TRP channels are considered six-transmembrane cation-permeable channels, distant relatives of voltage-gated potassium channels (Kv), which are known to be activated by membrane depolarization. Understanding the molecular nature of thermo-TRP channel gating offers a fair challenge to biophysicists. This chapter will summarize our present knowledge on the effect of voltage and temperature during thermo-TRP channel activation.

28.1 TRP Channel Family and Thermo-TRPs

Mammalian TRP channel proteins are polymodal cation channels with essential roles in cellular sensing. Other than a loose sequence homology, predicted channel architecture, and a common poor cation selectivity, there are no particular features defining the TRP family. TRP channels are grouped by homology into six subfamilies named C, M, V, A, P, and ML, for canonical, melastatin related, vanilloid binding, ankyrin repeat, polycystin, and mucolipin, respectively [1]. By integrating multiple stimuli they supply signal amplification through calcium permeation and membrane depolarization. Cooperativity intrinsic to TRP channels may result in allosteric coupling of distinct activation stimuli. A good example of the allosteric nature of TRP channels would be the case of temperature-activated TRP channels (*thermo-TRPs*) [2]. Mammalian thermo-TRPs correspond to a subgroup of 9 TRP channels which are expressed in sensory nerve endings and in skin, characterized

S. Brauchi (✉)
Facultad de Medicina, Instituto de Fisiologia, Universidad Austral de Chile,
Valdivia 511-0566, Chile
e-mail: sbrauchi@docentes.uach.cl

M.S. Islam (ed.), *Transient Receptor Potential Channels*, Advances in Experimental Medicine and Biology 704, DOI 10.1007/978-94-007-0265-3_28,

Energy landscape of the reactions governing the Na^+ deeply occluded state of the Na^+/K^+-ATPase in the giant axon of the Humboldt squid

Juan P. Castillo[a,b], Daniela De Giorgis[a,b], Daniel Basilio[a,c], David C. Gadsby[a,d], Joshua J. C. Rosenthal[a,e], Ramon Latorre[a,b,1], Miguel Holmgren[a,f,1], and Francisco Bezanilla[a,g,1]

[a]Laboratorio de Fisiologia Celular, Facultad de Ciencias, Universidad de Chile, Montemar 2366103, Chile; [b]Centro Interdisciplinario de Neurociencia de Valparaiso, Universidad de Valparaiso, Valparaiso 2366103, Chile; [c]Department of Anesthesiology, Weill Cornell Medical College, New York, NY 10065; [d]Laboratory of Cardiac/Membrane Physiology, The Rockefeller University, New York, NY 10065; [e]Department of Biochemistry, University of Puerto Rico–Medical Sciences Campus, San Juan, Puerto Rico 00901; [f]Molecular Neurophysiology Section, Porter Neuroscience Research Center, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892; and [g]Department of Biochemistry and Molecular Biology, University of Chicago, Gordon Center for Integrative Sciences, Chicago, IL 60637

Contributed by Ramón Latorre, October 5, 2011 (sent for review August 9, 2011)

The Na^+/K^+ pump is a nearly ubiquitous membrane protein in animal cells that uses the free energy of ATP hydrolysis to alternatively export $3Na^+$ from the cell and import $2K^+$ per cycle. This exchange of ions produces a steady-state outwardly directed current, which is proportional in magnitude to the turnover rate. Under certain ionic conditions, a sudden voltage jump generates temporally distinct transient currents mediated by the Na^+/K^+ pump that represent the kinetics of extracellular Na^+ binding/release and Na^+ occlusion/deocclusion transitions. For many years, these events have escaped a proper thermodynamic treatment due to the relatively small electrical signal. Here, taking the advantages offered by the large diameter of the axons from the squid *Dosidicus gigas*, we have been able to separate the kinetic components of the transient currents in an extended temperature range and thus characterize the energetic landscape of the pump cycle and those transitions associated with the extracellular release of the first Na^+ from the deeply occluded state. Occlusion/deocclusion transition involves large changes in enthalpy and entropy as the ion is exposed to the external milieu for release. Binding/unbinding is substantially less costly, yet larger than predicted for the energetic cost of an ion diffusing through a permeation pathway, which suggests that ion binding/unbinding must involve amino acid side-chain rearrangements at the site.

P-type ATPases | pump currents | thermodynamics

During each normal transport cycle of the Na^+/K^+-ATPase pump, three Na^+ are exported from the cell in exchange for two K^+ imported, a process driven by hydrolysis of one molecule of ATP. By establishing the Na^+ and K^+ gradients across cell membranes, the Na^+/K^+ pump enables action potentials, synaptic signaling, and most solute transport in and out of cells. Two consequences of the unequal transport stoichiometry of Na^+ and K^+ are that steady pumping produces an outwardly directed current (1), proportional in magnitude to the turnover rate (2), which can be monitored electrically (3–8), and that at least one step in the transport cycle must move charge through the membrane field (9). The latter implies that, under favorable conditions, charge relaxations following voltage jumps can be used to learn details about specific steps during the transport cycle (10–28).

In the absence of internal and external K^+, the nominal absence of internal ADP and presence of an ATP regenerating system, the Na^+/K^+ pump allows exchange of $3Na^+$ between their binding sites in a deeply occluded conformation and the external milieu (21, 29) (Fig. 1*A*; encircled by dotted lines). Under these conditions there is no steady pump current observed at any voltage. Nevertheless, sudden voltage steps produce pre-steady-state currents that can be recorded (12, 13, 16–18, 21, 23, 26). These currents allow direct measurements of the rates of partial reactions of the pump cycle. Using giant axons from the squid *Loligo pealeii*, we have characterized the kinetics of extracellular Na^+ binding/release and Na^+ occlusion/deocclusion and proposed mechanistic and structural hypotheses related to ion movement (18). The time course of the transient pump currents contains a fast, a medium-speed, and a slow component (see *Materials and Methods* and ref. 18). The temporal correlation between these components indicates that they occur in strict sequence (18), likely representing the binding/release and occlusion/deocclusion of individual Na^+ (Fig. 1*B*). The charge distribution of each component can be described by a Boltzmann function with an apparent valence for the charge moved of approximately 1 for the medium (18) and slow (12, 13, 16–18, 21, 23, 26, 30) components but only approximately 0.2 for the fast component, indicating that it does not transport charge across a large portion of the electric field (26). Most importantly, the voltage dependence of the slow relaxation shifts with changes in external Na^+ concentration, $[Na^+]_o$, supporting the interpretation that Na^+ ions must negotiate a high-field access channel as they travel back and forth between the external solution and their binding sites deep within the Na^+/K^+ pump (17, 18, 23).

Giant axons from *Loligo* have provided unique advantages for studying Na^+ translocation events mediated by the pump (18, 29). However, they too have limits. At low temperatures the amplitudes of the signals resulting from the binding/release of extracellular Na^+ are too small to permit study of the thermodynamics of these steps. To overcome these biological limitations, we have performed experiments using the giant axons of the Humboldt squid (*Dosidicus gigas*). In Chile, where these experiments were conducted, these squid commonly reach lengths close to 2 m and weigh up to 40 kg. More importantly for the present purpose, their axons routinely surpass 1.2 mm in diameter (approximately twice the diameter of a large *Loligo* axon), providing a substantially larger membrane area, and hence a greater number of pumps, than comparable lengths of axons from *Loligo*. With the aim of characterizing the energetic landscape of the transitions associated with the extracellular release of the first Na^+ from the deeply occluded state, in this study we have determined the ther-

Author contributions: M.H. and F.B. designed research; J.P.C., D.D.G., D.B., D.C.G., J.J.C.R., M.H., and F.B. performed research; J.P.C., R.L., M.H., and F.B. analyzed data; and R.L., M.H., and F.B. wrote the paper.

The authors declare no conflict of interest.

[1]To whom correspondence may be addressed. E-mail: ramon.latorre@uv.cl, holmgren@ninds.nih.gov, or febesanilla@uchicago.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1116439108/-/DCSupplemental.

RESEARCH REPORTS
Biological

E. Couve[1] * and O. Schmachtenberg[2]

[1]Departamento de Biología, Laboratorio de Microscopía Electrónica, and [2]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Avda. Gran Bretaña 1111, Casilla 5030, Correo 4, 2360102 Valparaíso, Chile; *corresponding author, eduardo.couve@uv.cl

J Dent Res 90(4):523-528, 2011

Autophagic Activity and Aging in Human Odontoblasts

ABSTRACT

Odontoblasts are long-lived post-mitotic cells in the dental pulp, whose function is to form and maintain dentin. The survival mechanisms that preserve the viability of terminally differentiated odontoblasts during the life of a healthy tooth have not been described. In the present study, we characterized the autophagic-lysosomal system of human odontoblasts with transmission electron microscopy and immunocytochemistry, to analyze the mechanisms that maintain the functional viability of these dentinogenic cells. Odontoblasts were found to develop an autophagic-lysosomal system organized mainly by large autophagic vacuoles that are acid-phosphatase-positive to various degrees. These vacuoles expressed the autophagosomal and lysosomal markers LC3 and LAMP2, respectively, in an age-related pattern indicating the organization of a dynamic autophagic machinery. Progressive accumulation of lipofuscin within lysosomes indicates reduced lysosomal activity as a function of odontoblast aging. Our results suggest that autophagic activity in odontoblasts is a fundamental mechanism to ensure turnover and degradation of subcellular components. A reduction in the efficacy of this system might compromise cell viability and dentinogenic secretory capacity. In adult teeth, this condition is described as an 'old odontoblast' stage.

KEY WORDS: dentin, teeth, lysosome, lipofuscin, autophagic vacuole.

DOI: 10.1177/0022034510393347

Received September 17, 2010; Last revision November 12, 2010; Accepted November 15, 2010

INTRODUCTION

In mature human teeth, the dentin-pulp complex works as a sensory and dentin-reparative organ (Mjör *et al.*, 2001). Odontoblasts are dentinogenic cells that differentiate into a terminal state and become long-lived post-mitotic cells within the dental pulp. The life cycle of human odontoblasts has been described only for the developmental period of permanent teeth (Couve, 1986). Once primary dentinogenesis is completed, the odontoblast reduces its secretory machinery by autophagic activity and limits its secretory functionality (Couve, 1986, 1987). This last cellular stage has been characterized as a resting odontoblast condition (Couve, 1985, 1986; Linde and Goldberg, 1993; Ten Cate, 1994). After the active period of primary dentinogenesis, resting odontoblasts maintain their physiological secretory activity, forming secondary dentin, and are capable of responding to moderate injury by the formation of reactionary dentin (*e.g.*, caries lesions of slow progression) (Smith *et al.*, 1995; Murray *et al.*, 2000, 2003; Bjorndal and Mjör, 2001). Under severe injury, such as rapidly progressing dentin lesions, odontoblasts are replaced by odontoblast-like cells from the subodontoblastic region of the dentin-pulp complex, forming irregular atubular fibrodentin, defined as reparative dentin (Baume, 1980; Smith *et al.*, 1995; Bjorndal and Darvann, 1999; Bjorndal and Mjör, 2001; Mitsiadis and Rahiotis, 2004).

While the survival strategies that maintain the viability of terminally differentiated odontoblasts during the whole life of a healthy tooth have not been established, autophagic activity has been proposed as one of the necessary survival mechanisms operating in long-lived post-mitotic cells (Terman *et al.*, 2007). In fact, autophagy is a ubiquitous and hierarchical cellular process that is required for the continuous turnover and removal of excess and damaged organelles by intralysosomal degradation (Klionsky and Emr, 2000; Cuervo, 2004a,b; Klionsky, 2007). Autophagic activity has been described as a fundamental survival mechanism for long-lived post-mitotic cells and is thought to be associated with the aging process (Brunk and Terman, 2002a,b). Long-lived post-mitotic cells, like neurons and cardiac myocytes, are mainly maintained through the ubiquitin-proteasome and autophagic-lysosome systems that guarantee the turnover and degradation of cellular subcomponents (Brunk and Terman, 2002a). However, progressive changes in lysosomal function and a reduction of autophagic activity have been related to the accumulation of lipofuscin, a process that mainly affects long-lived cells (Orenstein and Cuervo, 2010; Terman *et al.*, 2010).

Lysosomal compartments are responsible for the degradation of proteins and cytoplasmic components (mainly mitochondria), yet there are different mechanisms for the delivery of substrates to lysosomes. Three distinct autophagic pathways have been described in mammalian cells: macroautophagy, microautophagy, and chaperone-mediated autophagy (Cuervo, 2004b; Mizushima *et al.*, 2008). Macroautophagy (hereafter referred to as

THE ANATOMICAL RECORD 294:11–15 (2011)

Neurogenesis in the Adult Goldfish Cerebellum

LUZ M. DELGADO AND OLIVER SCHMACHTENBERG*
Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile

ABSTRACT

Neurogenesis was studied in the cerebellum of adult goldfish, to establish the phenomenon in this popular laboratory animal model. BrdU and proliferating cell nuclear antigen labeling revealed a high rate of cell proliferation within the molecular layer of the cerebellar corpus and valve. Most newborn cells expressed the neuronal marker beta-III-tubulin after 24 hr, supporting the goldfish cerebellum as an excellent paradigm to study vertebrate adult neurogenesis. Anat Rec, 294:11–15, 2011. © 2010 Wiley-Liss, Inc.

Key words: brain; teleost; cell proliferation; neuron; regeneration; nervous system

The phenomenon of adult neurogenesis occurs more widespread and at a much higher rate in teleosts than in mammals and terrestrial vertebrates, and has, therefore, aroused considerable interest in the last two decades (Chapouton et al., 2007; Kaslin et al., 2008; Zupanc, 2008). A large amount of information has been gathered principally through studies in zebrafish (Byrd and Brunjes, 2001; Zupanc et al., 2005; Grandel et al., 2006; Ampatzis and Dermon, 2007;), due to its importance as developmental model organism, and in gymnotiformes such as the brown ghost knifefish (Zupanc and Horschke, 1995; Zupanc, 1999), owing to the wealth of information on brain morphology and function available for this electrocommunicating species. However, for most physiological and behavioral studies related to adult neurogenesis, neither species is ideal, because the zebrafish brain and especially its neurons are too small for many types of experiment, and most researchers do not have easy access to gymnotiformes. The goldfish, in turn, is a well-established, omnipresent and hardy laboratory animal perfectly suited for most behavioral and (electro-) physiological trials. It has been the preferred teleost model for the study of visual function, and for learning and memory assays like those concerned with the vestibulo-ocular reflex. Although the putative relationship between learning, memory and adult neurogenesis is intriguing, few studies have addressed this topic in the central nervous system, with the notable exception of the retina (Boucher and Hitchcock, 1998). The goldfish cerebellum was evaluated here as an accessible model system for studies of vertebrate adult neurogenesis, because this part of the brain is central to motor learning and memory, and arguably the site of most widespread neurogenesis in teleosts (Ampatzis and Dermon, 2007). To that end, BrdU, proliferating cell nuclear antigen (PCNA), and beta-III-tubulin immunohistochemistry were combined to compare the cerebellar neuronal proliferation pattern of the goldfish with that of previously scrutinized species like zebrafish and the brown ghost knifefish.

For this study, eight male spawning-stage comet goldfish (*Carassius auratus*) were obtained from a local breeder during the spring of 2008, maintained in the laboratory for up to 1 month under a natural light/dark cycle at 20 ± 2°C, and fed twice daily. Females were not analyzed, because possible gender differences were not the aim of this study. The fish were injected intraperitoneally with 50 μL/g of body weight of a 3 mg/mL solution of 5-bromo-2-deoxyuridine (BrdU, Sigma-Aldrich). This dosage of 150 mg/kg body weight has been shown previously to effectively label proliferating cells in zebrafish (Zupanc et al., 2005). After 2 or 24 hr, the animals were sacrificed and their brains were fixed in 4%

Grant sponsor: FONDECYT; Grant number: 1090343.

*Correspondence to: Oliver Schmachtenberg, CINV, Facultad de Ciencias, Universidad de Valparaíso, Avda. Gran Bretaña 1111, Casilla 5030, Correo 4, 2360102 Valparaíso, Chile. Fax: +56-32-2508027. E-mail: schmachtenberg.uv@gmail.com or oliver.schmachtenberg@uv.cl

Received 6 January 2010; Accepted 21 September 2010
DOI 10.1002/ar.21291
Published online 2 December 2010 in Wiley Online Library (wileyonlinelibrary.com).

OPEN ACCESS Freely available online

PLoS one

Molecular Basis of Ligand Dissociation in β-Adrenergic Receptors

Angel González[1,2], Tomas Perez-Acle[3,4,5], Leonardo Pardo[1], Xavier Deupi[6]*

1 Laboratori de Medicina Computacional, Unitat de Bioestadística, Facultat de Medicina, Universitat Autònoma de Barcelona, Bellaterra, Catalunya, Spain, **2** Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile, **3** Computational Biology Lab, Center for Mathematical Modeling, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Santiago, Chile, **4** Centro Interdisciplinario de Neurociencias de Valparaíso, Playa Ancha, Valparaíso, Chile, **5** Fundación Ciencia para la Vida, Ñuñoa, Santiago, Chile, **6** Condensed Matter Theory Group and Laboratory of Biomolecular Research, Paul Scherrer Institut, Villigen PSI, Switzerland

Abstract

The important and diverse biological functions of β-adrenergic receptors (βARs) have promoted the search for compounds to stimulate or inhibit their activity. In this regard, unraveling the molecular basis of ligand binding/unbinding events is essential to understand the pharmacological properties of these G protein-coupled receptors. In this study, we use the steered molecular dynamics simulation method to describe, in atomic detail, the unbinding process of two inverse agonists, which have been recently co-crystallized with β_1 and β_2ARs subtypes, along four different channels. Our results indicate that this type of compounds likely accesses the orthosteric binding site of βARs from the extracellular water environment. Importantly, reconstruction of forces and energies from the simulations of the dissociation process suggests, for the first time, the presence of secondary binding sites located in the extracellular loops 2 and 3 and transmembrane helix 7, where ligands are transiently retained by electrostatic and Van der Waals interactions. Comparison of the residues that form these new transient allosteric binding sites in both βARs subtypes reveals the importance of non-conserved electrostatic interactions as well as conserved aromatic contacts in the early steps of the binding process.

Citation: González A, Perez-Acle T, Pardo L, Deupi X (2011) Molecular Basis of Ligand Dissociation in β-Adrenergic Receptors. PLoS ONE 6(9): e23815. doi:10.1371/journal.pone.0023815

Editor: Hendrik W. van Veen, University of Cambridge, United Kingdom

Received June 3, 2011; **Accepted** July 25, 2011; **Published** September 7, 2011

Funding: This work was supported by the Swiss National Science Foundation (SNF) grant 31003A_132815 and the ETH Zürich within the framework of the National Center for Competence in Research Structural Biology Program (to XD); by grants from Ministerio de Ciencia e Innovación (MICINN) SAF2010-22198-C02-02 and Instituto de Salud Carlos III (ISCIII) RD07/0067/0008 (to LP); and by the Proyectos de Financiamiento Basal PFB03 and PFB16 grants and the Millenium Institute Centro Interdisciplinario de Neurociencias de Valparaíso (CINV) (to TPA). AG is recipient of a Comisión Nacional de Investigación Científica y Tecnológica de Chile (CONICYT) doctoral fellowship. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: xavier.deupi@psi.ch

Introduction

G protein-coupled receptors (GPCRs) represent one of the largest protein families in mammals [1] and constitute 2% 3% of the human proteome [2]. GPCRs transduce sensory signals of external origin, such as photons, odors or pheromones, and endogenous signals including biogenic amines, (neuro)peptides, proteases, glycoprotein hormones and ions, into the cell. Thus, these receptors are essential in cell physiology, and their malfunction is commonly translated into pathological outcomes [3]. As a result, GPCRs constitute one of the most important pharmaceutical targets, as around 40% of prescribed drugs act through this family of proteins [4]. These receptors feature a common fold of seven transmembrane helices (TMs 1 to 7) connected by three extracellular (ECLs 1 to 3) and three intracellular (ICLs 1 to 3) loops [5], with an extracellular N-terminus and an intracellular C-terminus. Extracellular regions are very diverse in structure and amino acid composition, and in many GPCRs, as glycoprotein hormone and peptide receptors in family A or most receptors in families B and C, they are directly involved in ligand binding [6]. While smaller ligands bind in a pocket relatively buried within the TM bundle, they must also interact with the extracellular regions in order to reach the binding site. Understanding the molecular basis of ligand-receptor interactions in the extracellular domains is of great importance, as they are implicated in many aspects of receptor function, as ligand binding [7] and specificity [8], allosterism [9] or receptor activation [10,11]. Importantly, recent NMR data show ligand-specific conformational changes in the extracellular surface of the β_2-adrenergic receptor (β_2AR) [12].

While there is a vast amount of pharmacological, functional and pathophysiological information about GPCRs deposited in specialized databases (e.g. IUPHAR-DB, at http://www.iuphar-db.org), structural data of GPCRs is still scarce. Presently, only the structures of eight Class A GPCRs (bovine and squid rhodopsins, human β_2-adrenergic, turkey β_1-adrenergic, human A_{2A} adenosine (reviewed in [13,14,15]), human CXCR4 chemokine [16], human dopamine D_3 [17] and human histamine H_1 [18] receptors) are known. The availability of the structure of the β_1AR [19] and β_2AR [20] represents a unique opportunity to investigate the similarities and/or differences in the ligand entry process between these closely related subtypes. While these receptors have slightly different pharmacological properties [21], they present a strong similarity in sequence and structure, particularly in the TM bundle and orthosteric binding pockets [19]. Thus, it is plausible to argue that extracellular regions can

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 22, pp. 19830–19839, June 3, 2011

Functional Apical Large Conductance, Ca^{2+}-activated, and Voltage-dependent K^+ Channels Are Required for Maintenance of Airway Surface Liquid Volume*

Received for publication, September 14, 2010, and in revised form, March 23, 2011 Published, JBC Papers in Press, March 31, 2011, DOI 10.1074/jbc.M110.185074

Dahis Manzanares[‡1], **Carlos Gonzalez**[§¶1], **Pedro Ivonnet**[‡], **Ren-Shiang Chen**[§], **Monica Valencia-Gattas**[‖], **Gregory E. Conner**[‡‖], **H. Peter Larsson**[§], **and Matthias Salathe**[‡2]

From the [‡]*Division of Pulmonary and Critical Care,* [§]*Department of Physiology and Biophysics, and* [‖]*Department of Cell Biology and Anatomy, University of Miami, Miami, Florida 33136 and* [¶]*Centro Interdisciplinario de Neurociencia, Universidad de Valparaiso, Valparaiso, Chile*

Large conductance, Ca^{2+}-activated, and voltage-dependent K^+ (BK) channels control a variety of physiological processes in nervous, muscular, and renal epithelial tissues. In bronchial airway epithelia, extracellular ATP-mediated, apical increases in intracellular Ca^{2+} are important signals for ion movement through the apical membrane and regulation of water secretion. Although other, mainly basolaterally expressed K^+ channels are recognized as modulators of ion transport in airway epithelial cells, the role of BK in this process, especially as a regulator of airway surface liquid volume, has not been examined. Using patch clamp and Ussing chamber approaches, this study reveals that BK channels are present and functional at the apical membrane of airway epithelial cells. BK channels open in response to ATP stimulation at the apical membrane and allow K^+ flux to the airway surface liquid, whereas no functional BK channels were found basolaterally. Ion transport modeling supports the notion that apically expressed BK channels are part of an apical loop current, favoring apical Cl^- efflux. Importantly, apical BK channels were found to be critical for the maintenance of adequate airway surface liquid volume because continuous inhibition of BK channels or knockdown of *KCNMA1*, the gene coding for the BK α subunit (KCNMA1), lead to airway surface dehydration and thus periciliary fluid height collapse revealed by low ciliary beat frequency that could be fully rescued by addition of apical fluid. Thus, apical BK channels play an important, previously unrecognized role in maintaining adequate airway surface hydration.

Large conductance, voltage- and Ca^{2+}-activated K^+ (BK)[3] channels control a variety of physiological processes in different tissues. The BK pore-forming structure is a homotetramer of the α subunit (KCNMA1, Slo1, KCa1.1), which is encoded by a single gene (Slo1 gene, named *KCNMA1*). Four β regulatory subunits (KCNMβ1–4), encoded by the genes *KCNMB1–B4*, modulate the kinetics and the calcium and voltage dependence of BK channels, thereby contributing to the functional versatility of BK in different tissues (1–6).

Ion transport plays an important role in maintaining adequate water supply for the apical airway surface, which is critical for mucus hydration and ciliary beating because the periciliary fluid level has to be maintained for cilia to be effective. Both mucus hydration and ciliary beating are critical components of mucociliary function. The importance of ion transport for adequate airway surface liquid (ASL) volume is illustrated by multiple airway diseases where decreased Cl^- secretion and increased Na^+ absorption cause airway surface dehydration and thereby mucociliary dysfunction. A prime example is cystic fibrosis, a disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (7, 8). However, other channels contribute directly to or maintain the electrochemical gradient necessary for apical Cl^- secretion. For example, calcium-activated chloride channels, recently identified as TMEM16 (9–11), secrete Cl^- and are important for airway hydration (12). In addition, basolateral K^+ channels contribute to apical Cl^- transport by maintaining the electrochemical gradient required for Cl^- movement (13–17). Mall *et al.* (17) found that UTP-induced Cl^- currents in human nasal tissue were dependent on both clotrimazole-sensitive, calcium-activated K^+ channels (SK4, KCa3.1, gene KCNN4) and clofilium-sensitive voltage-activated K^+ channels (hKvLQT1, gene KCNQ1). Bernard *et al.* (18) also found that SK4 channels contribute to calcium-dependent chloride secretion in the human bronchial cell line 16HBE14o-.

Physiological ATP release onto apical surfaces of airway epithelial cells plays an important role in regulating water balance (19–21) and thereby mucociliary transport (22). Apical ATP is well known to increase $[Ca^{2+}]_i$ via P_2Y_2 receptors. Because BK channels are sensitive to intracellular Ca^{2+}, we hypothesized that these channels may be involved in ion transport responses to apically released ATP in human bronchial epithelia, which would make these channels important for the regulation of ASL volume in normal human bronchial epithelial (NHBE) cells.

Using electrophysiological techniques (patch clamp and Ussing chamber) and RNA level modifications, we identified functional BK channels at the apical but not basolateral membrane of fully differentiated NHBE cells. These apical BK channels play a key role in ion transport in response to apically

* This work was supported, in whole or in part, by NHLBI, National Institutes of Health Grants HL-089399 and HL-060644 (to M. S.), HL-095920 (to H. P. L.), AR32805 (to R.-S. C.), and HL-066125 (to G. E. C.). This work was also supported by American Heart Association Grant 10GRNT4150069 (to H. P. L.).

[1] Both authors contributed equally to this work.

[2] To whom correspondence should be addressed: 1600 NW 10th Ave., RMSB 7063, Miami, FL 33136. Tel.: 305-243-6388; Fax: 305-243-6992; E-mail: msalathe@med.miami.edu.

[3] The abbreviations used are: BK, large conductance, Ca^{2+}-activated, and voltage-dependent K^+; NHBE, normal human bronchial epithelial; ALI, air-liquid interface; qPCR, quantitative PCR; Ω, ohm; TES, *N*-tris-(hydroxymethyl) methyl-2-amino-ethane-sulfonic acid; CBF, ciliary beat frequency.

nature COMMUNICATIONS

OPEN

ARTICLE

Received 30 Mar 2011 | Accepted 20 Jul 2011 | Published 16 Aug 2011

DOI: 10.1038/ncomms1446

Editing of human $K_V1.1$ channel mRNAs disrupts binding of the N-terminus tip at the intracellular cavity

Carlos Gonzalez[1], Angelica Lopez-Rodriguez[2], Deepa Srikumar[2], Joshua J.C. Rosenthal[3,4] & Miguel Holmgren[2]

In the nervous system, A→I RNA editing has an important role in regulating neuronal excitability. Ligand-gated membrane receptors, synaptic proteins, as well as ion channels, are targets for recoding by RNA editing. Although scores of editing sites have been identified in the mammalian brain, little is known about the functional alterations that they cause, and even less about the mechanistic underpinnings of how they change protein function. We have previously shown that an RNA editing event (I400 V) alters the inner permeation pathway of human $K_V1.1$, modifying the kinetics of fast inactivation. Here we show that the channel's inactivation gate enters deep into the ion permeation pathway and the very tip establishes a direct hydrophobic interaction with the edited position. By converting I to V, the intimacy of the interaction is reduced, allowing the inactivation gate to unbind with much faster kinetics.

[1] Centro Interdisciplinario de Neurociencia de Valparaiso, Universidad de Valparaiso, Valparaiso V 2340000, Chile. [2] Molecular Neurophysiology Section, Porter Neuroscience Research Center, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892, USA. [3] Institute of Neurobiology, University of Puerto Rico-Medical Sciences Campus, San Juan, Puerto Rico 00901, USA. [4] Department of Biochemistry, University of Puerto Rico-Medical Sciences Campus, San Juan, Puerto Rico 00936, USA. Correspondence and requests for materials should be addressed to J.J.C.R. (email: Joshua.rosenthal@upr.edu) or to M.H. (email: holmgren@ninds.nih.gov).

ARTICLE

doi:10.1038/nature10640

The genome of *Tetranychus urticae* reveals herbivorous pest adaptations

Miodrag Grbić[1,2]*, Thomas Van Leeuwen[3]*, Richard M. Clark[4]*, Stephane Rombauts[5,6], Pierre Rouzé[5,6], Vojislava Grbić[1,2], Edward J. Osborne[4], Wannes Dermauw[3], Phuong Cao Thi Ngoc[5,6], Félix Ortego[7], Pedro Hernández-Crespo[7], Isabel Diaz[8], Manuel Martinez[8], Maria Navajas[9], Élio Sucena[10,11], Sara Magalhães[12], Lisa Nagy[13], Ryan M. Pace[13], Sergej Djuranović[14], Guy Smagghe[3], Masatoshi Iga[3], Olivier Christiaens[3], Jan A. Veenstra[15], John Ewer[16], Rodrigo Mancilla Villalobos[16], Jeffrey L. Hutter[17], Stephen D. Hudson[17], Marisela Velez[18], Soojin V. Yi[19], Jia Zeng[19], Andre Pires-daSilva[20], Fernando Roch[21], Marc Cazaux[1], Marie Navarro[1], Vladimir Zhurov[1], Gustavo Acevedo[1], Anica Bjelica[1], Jeffrey A. Fawcett[5,6]†, Eric Bonnet[5,6]†, Cindy Martens[5,6], Guy Baele[5,6], Lothar Wissler[22], Aminael Sanchez-Rodriguez[23], Luc Tirry[3], Catherine Blais[24], Kristof Demeestere[25], Stefan R. Henz[26], T. Ryan Gregory[27], Johannes Mathieu[28], Lou Verdon[29], Laurent Farinelli[30], Jeremy Schmutz[31,32], Erika Lindquist[32], René Feyereisen[33] & Yves Van de Peer[5,6]

The spider mite *Tetranychus urticae* is a cosmopolitan agricultural pest with an extensive host plant range and an extreme record of pesticide resistance. Here we present the completely sequenced and annotated spider mite genome, representing the first complete chelicerate genome. At 90 megabases *T. urticae* has the smallest sequenced arthropod genome. Compared with other arthropods, the spider mite genome shows unique changes in the hormonal environment and organization of the Hox complex, and also reveals evolutionary innovation of silk production. We find strong signatures of polyphagy and detoxification in gene families associated with feeding on different hosts and in new gene families acquired by lateral gene transfer. Deep transcriptome analysis of mites feeding on different plants shows how this pest responds to a changing host environment. The *T. urticae* genome thus offers new insights into arthropod evolution and plant–herbivore interactions, and provides unique opportunities for developing novel plant protection strategies.

Mites belong to the Chelicerata, the second largest group of terrestrial animals. Chelicerates represent a basal branch of arthropods. Subsequent to their origin in the Cambrian period, arthropods radiated into two lineages: the Chelicerata and the Mandibulata (comprising the Myriapoda and the Pancrustacea (which includes both crustaceans and insects))[1,2]. Extant lineages of chelicerates include Pycnogonida, Xiphosura (horseshoe crabs) and Arachnida (a large group comprising scorpions, spiders and the Acari (ticks and mites))[3,4] (Supplementary Fig. 1.1). Within the Acari, *T. urticae* belongs to the Acariformes with the earliest fossils dating from the Lower Devonian period (410 million years ago). The Acari represent the most diverse chelicerate clade, with over 40,000 described species that exhibit tremendous variations in lifestyle, ranging from parasitic to predatory to plant-feeding. Some mites are of major concern to human health and include allergy-causing dust mites, scabies mites and mite vectors of scrub typhus[5].

The two-spotted spider mite, *Tetranychus urticae*, is a cosmopolitan agricultural pest[6] belonging to an assemblage of web-spinning mites. The name 'spider' highlights their ability to produce silk-like webbing used to establish a colonial micro-habitat, protect against abiotic agents, shelter from predators, communicate via pheromones and provide a vehicle for dispersion[7].

Tetranychus urticae represents one of the most polyphagous arthropod herbivores, feeding on more than 1,100 plant species belonging to more than 140 different plant families including species known to produce toxic compounds. It is a major pest in greenhouse production and field crops, destroying annual and perennial crops such as tomatoes, peppers, cucumbers, strawberries, maize, soy,

[1]Department of Biology, The University of Western Ontario, London N6A 5B7, Canada. [2]Instituto de Ciencias de la Vid y el Vino (CSIC, UR, Gobiernode La Rioja), 26006 Logroño, Spain. [3]Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, B-9000 Ghent, Belgium. [4]Department of Biology, University of Utah, Salt Lake City, Utah 84112, USA. [5]Department of Plant Systems Biology, VIB, Technologiepark 927, B-9052 Ghent, Belgium. [6]Department of Plant Biotechnology and Bioinformatics, Ghent University, Technologiepark 927, B-9052 Ghent, Belgium. [7]Department of Environmental Biology, Centro de Investigaciones Biológicas, CSIC, 28040 Madrid, Spain. [8]Centro de Biotecnología y Genómica de Plantas, UPM-INIA, 28223 Madrid, Spain. [9]INRA, UMR CBGP (INRA/IRD/Cirad/Montpellier SupAgro), Campus international de Baillarguet, 34988 Montferrier-sur-Lez, France. [10]Instituto Gulbenkian de Ciência, 2781-901 Oeiras, Portugal. [11]Universidade de Lisboa, Faculdade de Ciências, Departamento de Biologia Animal, 1749-016 Lisbon, Portugal. [12]Universidade de Lisboa, Faculdade de Ciências, Centro de Biologia Ambiental, 1749-016 Lisbon, Portugal. [13]Department of Molecular and Cellular Biology, University of Arizona, Tucson, Arizona 85721, USA. [14]Johns Hopkins University School of Medicine, Department of Molecular Biology & Genetics, Baltimore, Maryland 21205, USA. [15]Institut de Neurosciences Cognitives et Intégratives d'Aquitaine Université de Bordeaux 1, 33405 Talence, France. [16]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso 2360102, Chile. [17]Department of Physics and Astronomy, The University of Western Ontario, N6A 5B7 London, Canada. [18]Instituto de Catálisis y Petroleoquímica CSIC, Madrid, Spain; IMDEA Nanociencias, Facultad de Ciencias, Universidad Autonoma de Madrid, 28050 Madrid, Spain. [19]School of Biology, Georgia Institute of Technology, Atlanta, Georgia 30332, USA. [20]Department of Biology, University of Texas at Arlington, Arlington, Texas 76019, USA. [21]Universite de Toulouse, UPS, Centre de Biologie du Developpement, Universite Paul Sabatier, 31062 Toulouse, France; Centre National de la Recherche Scientifique, UMR 5547, Centre de Biologie du Developpement, 31062 Toulouse, France. [22]Westfälische Wilhelms University, Institute for Evolution and Biodiversity, Evolutionary Bioinformatics Group, Hüfferstrasse 1, D-48149 Münster, Germany. [23]CMPG, Department of Microbial and Molecular Systems, K.U. Leuven, B-3001 Leuven, Belgium. [24]UPMC Univ Paris 06, UMR CNRS 7622, Equipe Biogenèse des signaux hormonaux, Case 29, 75005 Paris, France. [25]Research Group EnVOC, Department of Sustainable Organic Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, B-9000 Ghent, Belgium. [26]Max Planck Institute for Developmental Biology, D-72076 Tübingen, Germany. [27]Department of Integrative Biology, University of Guelph, N1G 2W1 Guelph, Canada. [28]Boyce Thompson Institute for Plant Research, Ithaca, New York 14853, USA. [29]Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, N5V 4T3 London, Canada. [30]Fasteris SA, CH-1228 Plan-les-Ouates, Switzerland. [31]HudsonAlpha Institute for Biotechnology Huntsville, Alabama 35806, USA. [32]DOE Joint Genome Institute, Walnut Creek, California 94598, USA. [33]UMR 1301, INRA, CNRS and Université de Nice Sophia Antipolis, 06903 Sophia Antipolis, France. †Present addresses: Institut Curie, 26 rue d'Ulm, Paris 75248, France; INSERM, U900, Paris 75248, France; Mines ParisTech, Fontainebleau 77300, France (E.B.); Graduate University for Advanced Studies, Hayama, Kanagawa 240-0193, Japan (J.A.F.). *These authors contributed equally to this work.

REVIEWS

PHYSIOLOGY 26: 273–285, 2011; doi:10.1152/physiol.00004.2011

A Cool Channel in Cold Transduction

Ramón Latorre,[1] Sebastián Brauchi,[2] Rodolfo Madrid,[3] and Patricio Orio[1]

[1]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile; [2]Instituto de Fisiología, Universidad Austral de Chile, Valdivia, Chile; and [3]Laboratorio de Neurociencia, Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile

Transient receptor potential melastatin 8 (TRPM8), a calcium-permeable cation channel activated by cold, cooling compounds and voltage, is the main molecular entity responsible for detection of cold temperatures in the somatosensory system. Here, we review the biophysical properties, physiological role, and near-membrane trafficking of this exciting polymodal ion channel.

From insects to mammals, the transient receptor potential (TRP) channels play important roles in sensory transduction. TRP channels give origin to a super family consisting of seven subfamilies with scarce homology between them. The seven subfamilies are the classical TRP subfamily TRPC, the melastatin-related subfamily TRPM, the vanilloid-sensitive TRP subfamily TRPV, the ankyrin subfamily TRPA, the polycystin subfamily TRPP, the mucolipin subfamily TRPML, and the TRPN subfamily, after the non-mechanoreceptor potential C (nonpC) homologue (78, 97, 123). This review discusses the case of receptor potential melastatin 8 (TRPM8) channel, an ion channel that is the predominant thermoreceptor for cellular and behavioral responses to cold temperatures.

Cold is detected by specific cutaneous thermoreceptor neurons of the somatosensory system, which include unmyelinated primary afferent C-fibers and thinly myelinated Aδ-fibers (12, 44, 47, 49, 53). The transduction of cold stimuli into propagated electrical impulses takes place in the free endings of the thermoreceptor fibers, which correspond to axonal endings of cold-sensitive neurons from trigeminal (TG) and dorsal root ganglion (DRG). At resting temperature of the skin (~34°C), receptors detecting and encoding innocuous cold exhibit spontaneous electrical activity that increases in response to temperature reductions as small as 1°C or less (18). This response is inhibited by heating and sensitized by menthol (20, 46). Cold-thermoreceptor neurons express a wide variety of ion channels, including transduction channels as well as voltage-dependent channels, which give shape to their net excitability. A widely accepted model today maintains that the nonselective Ca^{2+}-permeable cationic channel TRPM8 is the main molecular transducer entity responsible for the sensitivity to innocuous cold in the somatosensory system.

TRPM8 is a Polymodal Receptor

Identified in 2001 as a messenger RNA upregulated in prostate cancer (120), TRPM8 was cloned and characterized in 2002 by two groups independently (75, 90). TRPM8 is a tetramer, with each subunit consisting of six transmembrane domains (S1–S6) and intracellular COOH and NH_2 terminals (FIGURE 1). Coiled coil domains located in the distal portion of COOH terminal of the channel appear to be important in the assembly process of TRPM8 (39, 121). Phelps and Gaudet (92) showed that functional channels need the presence of the COOH terminal as well as a region comprised by amino acids 40–86 of the intracellular NH_2 terminal. The same authors also showed, however, that deletion of the COOH-terminal region prevents function but not tetramerization.

TRPM8 behaves as a polymodal receptor activated by membrane depolarization, cold, and chemical compounds such as menthol, icilin, and several inflammatory agents (16, 66, 75, 90, 125). Also, the activity of TRPM8 appears to require the presence of phosphatidylinositol-4,5-biphosphate [$PI(4,5)P_2$] (31, 102) (see below).

Voltage Dependence and Ion Selectivity

TRPM8 is a channel with a weak voltage dependence, and in the absence of agonists activation requires strongly depolarized membrane voltages (16, 125). Although the voltage sensor domain remains elusive (see Ref. 65), neutralization of positively charged residues in the S4 of TRPM8 causes a decrease in its voltage dependence (FIGURE 1, A AND B)(126). Voets et al. (126) neutralized all the charged residues contained in the S4 segment and in the S4-S5 linker of the TRPM8 channel. They found that the total apparent number of gating charges per channel is 0.85*e* on average and that neutralization of R842 in S4 and K856 located in the S4-S5 linker (FIGURE 1B) decreased this number to 0.7 and 0.5*e*, respectively. These findings suggest that the contribution of these two charges to the total amount of gating charges/channel is not enough to explain the global voltage dependency of the channel (0.85*e*). Therefore, it is possible that at least part of the total gating charge is actually located in another position within the channel structure. It is noteworthy that TRPM2 has a S4 with the same pattern of positively charged residues as the S4 of TRPM8 but is utterly voltage insensitive. However, a chimera containing the putative voltage sensor (S1–S4) of TRPM2 and the

BJ www.biochemj.org

Biochem. J. (2011) **436**, 35–43 (Printed in Great Britain) doi:10.1042/BJ20110008

online data

Different domains are critical for oligomerization compatibility of different connexins

Agustín D. MARTÍNEZ*[1], Jaime MARIPILLÁN*, Rodrigo ACUÑA*, Peter J. MINOGUE†, Viviana M. BERTHOUD† and Eric C. BEYER†

*Centro Interdisciplinario de Neurociencias de Valparaíso, Departamento de Neurociencias, Universidad de Vaparaíso, Valparaíso 2360102, Chile, and †Department of Pediatrics, University of Chicago, Chicago, IL, 60637, U.S.A.

Oligomerization of connexins is a critical step in gap junction channel formation. Some members of the connexin family can oligomerize with other members and form functional heteromeric hemichannels [e.g. Cx43 (connexin 43) and Cx45], but others are incompatible (e.g. Cx43 and Cx26). To find connexin domains important for oligomerization, we constructed chimaeras between Cx43 and Cx26 and studied their ability to oligomerize with wild-type Cx43, Cx45 or Cx26. HeLa cells co-expressing Cx43, Cx45 or Cx26 and individual chimaeric constructs were analysed for interactions between the chimaeras and the wild-type connexins using cell biological (subcellular localization by immunofluorescence), functional (intercellular diffusion of microinjected Lucifer yellow) and biochemical (sedimentation velocity through sucrose gradients) assays. All of the chimaeras containing the third transmembrane domain of Cx43 interacted with wild-type Cx43 on the basis of co-localization, dominant-negative inhibition of intercellular communication, and altered sedimentation velocity. The same chimaeras also interacted with co-expressed Cx45. In contrast, immunofluorescence and intracellular diffusion of tracer suggested that other domains influenced oligomerization compatibility when chimaeras were co-expressed with Cx26. Taken together, these results suggest that amino acids in the third transmembrane domain are critical for oligomerization with Cx43 and Cx45. However, motifs in different domains may determine oligomerization compatibility in members of different connexin subfamilies.

Key words: chimaera, connexin26, connexin43, gap junction, intercellular communication, oligomerization.

INTRODUCTION

Gap junction channels connect the cytoplasms of adjacent cells allowing intercellular passage of ions and molecules up to 1 kDa in size between coupled cells. Each gap junction channel is an oligomer composed of twelve protein subunits of connexin. The connexins are polytopic membrane proteins containing four TM (transmembrane) (TM1–TM4), three cytoplasmic [NT (N-terminal), IL (intracellular loop) and CT (C-terminal)], and two extracellular (E1 and E2) domains (reviewed in [1]). Six connexins oligomerize within the secretory pathway to form a hemichannel or connexon. Different connexins oligomerize in different intracellular compartments, as examples Cx43 (connexin 43) in the trans-Golgi network [2,3] and Cx32 in the ER (endoplasmic reticulum) [3]. After trafficking to the plasma membrane through the secretory pathway, connexons dock with complementary connexons of the adjacent cell, leading to the formation of gap junction channels.

Many cell types express more than one connexin subtype, making possible oligomerization between identical subunits to form homomeric connexons or between different subunits to form heteromeric connexons. Homomeric connexons may form homotypic gap junction channels (when docking takes place between two connexons made of the same connexin), or heterotypic gap junction channels (when docking involves two homomeric connexons made of different connexins). The resulting homotypic, heterotypic and heteromeric gap junction channels have specific and greatly diverse properties (e.g. single channel conductance, permeability, gating and regulation by kinases) [4–9].

We and others have shown that Cx43 forms heteromeric channels with Cx45, Cx40 and Cx37, but not with the 'β' connexins, Cx26 and Cx32 [4,10–14]. However, there is limited available information regarding the molecular determinants for connexin compatibility and oligomerization between different connexins. Recently, Maeda et al. [15] published the crystal structure of the gap junction channel formed by human Cx26 at 3.5 Å (1 Å = 0.1 nm) resolution. This structure confirms that the docking between the two adjoining connexons involves both E1 and E2, consistent with previous studies showing that amino acid sequences in E2 determine compatibility of connexins for heterotypic pairing [16]. This structure also indicates that the inter-protomer interactions that stabilize the hexameric connexon are mostly located in the extracellular half of transmembrane helices and in the extracellular loops [15]. However, the domains determining oligomerization compatibility remain unknown. Our previous studies suggest that the CT domain of Cx43 is not needed for oligomerization, since Cx43tr251, a truncated form of Cx43, forms functional homomeric channels [17]. When expressed alone, Cx43tr251 does not form gap junction plaques detectable by immunofluorescence; however, when co-expressed with wild-type Cx43 or Cx45, Cx43tr251 forms gap junction plaques [17]. To identify critical domains for connexin compatibility, we generated a series of chimaeric constructs in which domains were reciprocally exchanged between two incompatible connexins, Cx43 and Cx26, to determine whether they oligomerize with wild-type connexins.

Abbreviations used: Chi, chimaera; CT, C-terminal; Cx, connexin; E, extracellular loop; ER, endoplasmic reticulum; HA, haemagglutinin; IL, intracellular loop; LY, Lucifer yellow; NT, N-terminal; TM domain, transmembrane domain.

[1] To whom correspondence should be addressed (email agustin.martinez@uv.cl).

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 25, pp. 22203–22210, June 24, 2011

Homodimerization of the Src Homology 3 Domain of the Calcium Channel β-Subunit Drives Dynamin-dependent Endocytosis*[S]

Received for publication, November 10, 2010, and in revised form, April 6, 2011 Published, JBC Papers in Press, April 18, 2011, DOI 10.1074/jbc.M110.201871

Erick Miranda-Laferte[‡], Giovanni Gonzalez-Gutierrez[‡1], Silke Schmidt[‡], Andre Zeug[‡], Evgeni G. Ponimaskin[‡], Alan Neely[§], and Patricia Hidalgo[‡2]

From the[‡]Institut für Neurophysiologie, Medizinische Hochschule Hannover, 30625 Hannover, Germany and [§]Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso, Valparaíso 2349400, Chile

Voltage-dependent calcium channels constitute the main entry pathway for calcium into excitable cells. They are heteromultimers formed by an α_1 pore-forming subunit ($Ca_V\alpha_1$) and accessory subunits. To achieve a precise coordination of calcium signals, the expression and activity of these channels is tightly controlled. The accessory β-subunit ($Ca_V\beta$), a membrane associated guanylate kinase containing one guanylate kinase (β-GK) and one Src homology 3 (β-SH3) domain, has antagonistic effects on calcium currents by regulating different aspects of channel function. Although β-GK binds to a conserved site within the α_1-pore-forming subunit and facilitates channel opening, β-SH3 binds to dynamin and promotes endocytosis. Here, we investigated the molecular switch underlying the functional duality of this modular protein. We show that β-SH3 homodimerizes through a single disulfide bond. Substitution of the only cysteine residue abolishes dimerization and impairs internalization of L-type $Ca_V1.2$ channels expressed in *Xenopus* oocytes while preserving dynamin binding. Covalent linkage of the β-SH3 dimerization-deficient mutant yields a concatamer that binds to dynamin and restores endocytosis. Moreover, using FRET analysis, we show in living cells that $Ca_V\beta$ form oligomers and that this interaction is reduced by $Ca_V\alpha_1$. Association of $Ca_V\beta$ with a polypeptide encoding the binding motif in $Ca_V\alpha_1$ inhibited endocytosis. Together, these findings reveal that β-SH3 dimerization is crucial for endocytosis and suggest that channel activation and internalization are two mutually exclusive functions of $Ca_V\beta$. We propose that a change in the oligomeric state of $Ca_V\beta$ is the functional switch between channel activator and channel internalizer.

Voltage-dependent calcium channels link membrane depolarization to transient increases in cytosolic calcium concentration, which in turn mediate a variety of cellular processes, including gene expression, contraction, neurotransmission, and exocytosis (1). To direct the signal to a specific subset of an extensive family of effectors, cells are endowed with a complex network of regulators that constrain the timing and spreading of the calcium increase. The subset of voltage-dependent calcium channels that are activated by strong depolarization, also called high voltage-activated (HVA)[3] channels, are heteromultimers consisting of a central pore-forming subunit ($Ca_V\alpha_1$) that associates with one or more accessory subunits. Among them the accessory β-subunit ($Ca_V\beta$) has traditionally been recognized as one of the most important modulator of HVA channels (1–3). More recently, it has been shown that the same subunit mediates several other cellular processes (for review, see Ref. 4), including regulation of insulin secretion (5), gene transcription (6) and endocytosis (7). Four $Ca_V\beta$ isoforms ($Ca_V\beta_1$ to $Ca_V\beta_4$) have been cloned from different nonallelic genes. Crystallographic studies of three of these provided the molecular basis for its functional versatility by identifying two protein-protein interactions modules: an Src homology 3 (β-SH3) and a guanylate kinase (β-GK), which are typically found in members of the membrane-associated guanylate kinase family of scaffolding proteins (see Fig. 1*A*) (8–10). The functional multiplicity of $Ca_V\beta$ is achieved by establishing pairwise interactions with other protein partners beside $Ca_V\alpha_1$. How $Ca_V\beta$ sorts out the different molecular targets inside the cell remains elusive. One of these partners is dynamin, the GTPase that scisses newly formed vesicles from the plasma membrane during endocytosis (11, 12). Dynamin contains a proline-rich region with several P*xx*P consensus motifs that serve as docking sites for SH3 domains (13). SH3/proline-rich region-mediated interactions appear to recruit dynamin to areas of endocytosis (14, 15). SH3 domains are small modules found in a variety of proteins that mediate inter- and intramolecular interactions (16). Several conserved amino acids are critical for binding to the P*xx*P motif in the ligand proteins. These residues are also present in the SH3 domain of $Ca_V\beta$, but they appear occluded in the crystal structure (8–10). However,

* This work was supported by grants from the Deutsche Forschungsgemeinschaft Grants DFG HI 800/3-1 and 4-1 (to P. H.), Red de Anillo de Ciencia y Tecnologia Grant RED-24 (to A. N.), and the German-Chilean Scientific Cooperation Program Deutsche Forschungsgemeinschaft-Comisión Nacional de Investigación Científica y Tecnológica 2010 (to P. H. and A. N.).

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3, a movie, and additional references.

[1] Present address: Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL 61801.

[2] To whom correspondence should be addressed: Medizinische Hochschule Hannover, Institut für Neurophysiologie, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany. Tel.: 49-511/532-2883; Fax: 49-511/532-2776. E-mail: hidalgo.patricia@mh-hannover.de.

[3] The abbreviations used are: HVA, high voltage-activated channel(s); $Ca_V\alpha_1$, α_1 pore-forming subunit of HVA channel(s); $Ca_V\beta$, accessory β-subunit of HVA channel(s); SH3, Src homology 3; GK, guanylate kinase; AID, α_1-interaction domain; BN-PAGE, blue native PAGE; CMBI, charge-movement based internalization; Ef_D, apparent FRET efficiency; CFP, Cyan Fluorescent Protein; pC, pico Coulomb.

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 15, pp. 13382–13392, April 15, 2011

Iron Mediates *N*-Methyl-D-aspartate Receptor-dependent Stimulation of Calcium-induced Pathways and Hippocampal Synaptic Plasticity*[S]

Received for publication, December 17, 2010, and in revised form, February 1, 2011 Published, JBC Papers in Press, February 4, 2011, DOI 10.1074/jbc.M110.213785

Pablo Muñoz[‡§1,2], **Alexis Humeres**[¶2], **Claudio Elgueta**[‡], **Alfredo Kirkwood**[‖], **Cecilia Hidalgo**[¶**], **and Marco T. Núñez**[‡‡]

From the [‡]*Centro de Neurociencia, Universidad de Valparaíso, Valparaíso 2360102, Chile, the* [§]*Centro Regional de Estudios en Alimentos Saludables, Valparaíso 2362696, Chile,* [¶]*Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile, Santiago 8380453, Chile, the* [‖]*Department of Neurosciences, The Johns Hopkins University, Baltimore, Maryland 21218, the* [**]*Programa de Fisiología y Biofísica, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago 8389100, and the* [‡‡]*Departamento de Biología, Facultad de Ciencias, and Institute for Cell Dynamics and Biotechnology, Universidad de Chile, Santiago 7800024, Chile*

Iron deficiency hinders hippocampus-dependent learning processes and impairs cognitive performance, but current knowledge on the molecular mechanisms underlying the unique role of iron in neuronal function is sparse. Here, we investigated the participation of iron on calcium signal generation and ERK1/2 stimulation induced by the glutamate agonist *N*-methyl-D-aspartate (NMDA), and the effects of iron addition/chelation on hippocampal basal synaptic transmission and long-term potentiation (LTP). Addition of NMDA to primary hippocampal cultures elicited persistent calcium signals that required functional NMDA receptors and were independent of calcium influx through L-type calcium channels or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors; NMDA also promoted ERK1/2 phosphorylation and nuclear translocation. Iron chelation with desferrioxamine or inhibition of ryanodine receptor (RyR)-mediated calcium release with ryanodine reduced calcium signal duration and prevented NMDA-induced ERK1/2 activation. Iron addition to hippocampal neurons readily increased the intracellular labile iron pool and stimulated reactive oxygen species production; the antioxidant *N*-acetylcysteine or the hydroxyl radical trapper MCI-186 prevented these responses. Iron addition to primary hippocampal cultures kept in calcium-free medium elicited calcium signals and stimulated ERK1/2 phosphorylation; RyR inhibition abolished these effects. Iron chelation decreased basal synaptic transmission in hippocampal slices, inhibited iron-induced synaptic stimulation, and impaired sustained LTP in hippocampal CA1 neurons induced by strong stimulation. In contrast, iron addition facilitated sustained LTP induction after suboptimal tetanic stimulation. Together, these results suggest that hippocampal neurons require iron to generate RyR-mediated calcium signals after NMDA receptor stimulation, which in turn promotes ERK1/2 activation, an essential step of sustained LTP.

Iron deficiency during early life is associated with significantly lower cognitive and behavioral infant development (1–3), severe deterioration of hippocampal neuronal function (4–6), and poor memory and spatial learning capabilities (7–9). Current understanding of the relationship between neuronal function and brain iron status is sparse, and the molecular mechanisms underlying the essential role of iron in neuronal function remain mostly unidentified. Nonetheless, a role for iron in synaptic plasticity and the associated generation of postsynaptic Ca^{2+} signals has begun to emerge (10–12).

Neurons obtain iron via transferrin-dependent and -independent uptake pathways. The iron concentration in cerebrospinal fluid is sufficient to saturate the binding capacity of transferrin (13). This feature highlights the need for transferrin-independent iron uptake, which is likely to occur in neurons that express the iron transporter DMT1, such as hippocampal pyramidal and granule cells, cerebellar granule cells, pyramidal cells of the piriform cortex, *substantia nigra*, and the ventral portion of the anterior olfactory nucleus (14–16). The high DMT1 expression levels in these neurons suggest that DMT1-mediated iron uptake is necessary for their function.

Iron uptake into neurons stimulates the generation of reactive oxygen species (ROS)[3] and modifies the redox potential established by the intracellular levels of oxidized and reduced glutathione (17). Consequently, by modifying the cellular redox potential, iron is likely to modulate the balance between reduced and oxidized sulfhydryl groups in proteins. Iron, through the Haber-Weiss and Fenton reactions, is also a net generator of ROS, including the highly reactive hydroxyl radical (18, 19).

* This work was supported by Millennium Scientific Initiative Grant ICM-P05-001-F (to M. T. N.), Fondecyt-FONDAP Grant 15010006 (to C. H.), and Fondo Nacional de Desarrollo Científico y Tecnológico Grant FONDECYT 3080046 (to P. M.).

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S5.

[1] To whom correspondence should be addressed: Centro de Neurociencia, Universidad de Valparaíso, Valparaíso, Chile. Tel.: 56-32-2508186; Fax: 56-32-2508027; E-mail: pmunoz@cnv.cl.

[2] Both authors contributed equally to this work.

[3] The abbreviations used are: ROS, reactive oxygen species; NMDA, *N*-methyl-D-aspartate; RyR, ryanodine receptor; LTP, long-term potentiation; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; LIP, labile iron pool; Fe-NTA, $FeCl_3$-sodium nitrilotriacetate; DCDHF-DA, 2′,7′-dichlorodihydrofluorescein diacetate; DCF, 2′,7′-dichlorofluorescein; DFO, desferrioxamine; NAC, *N*-acetylcysteine; ACSF, artificial cerebrospinal fluid; TBS, theta burst stimulation; CREB, cAMP-response element-binding protein; fEPSP, field excitatory postsynaptic potential.

4962 • The Journal of Neuroscience, March 30, 2011 • 31(13):4962–4977

Cellular/Molecular

Amyloid β-Induced Death in Neurons Involves Glial and Neuronal Hemichannels

Juan A. Orellana,[1] Kenji F. Shoji,[1] Verónica Abudara,[2,3] Pascal Ezan,[2] Edwige Amigou,[2] Pablo J. Sáez,[1] Jean X. Jiang,[4] Christian C. Naus,[5] Juan C. Sáez,[1,6] and Christian Giaume[2]

[1]Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago 6513677, Chile, [2]Inserm, Unité 840, Collège de France, 75231 Paris Cedex 05, France, [3]Departamento de Fisiología, Facultad de Medicina, Universidad de la República, 11800 Montevideo, Uruguay, [4]Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas 78229, [5]Department of Cellular and Physiological Sciences, Life Sciences Institute, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada, and [6]Instituto Milenio, Centro Interdisciplinario de Neurociencias de Valparaíso, Valparaíso 2340000, Chile

The mechanisms involved in Alzheimer's disease are not completely understood and how glial cells contribute to this neurodegenerative disease remains to be elucidated. Because inflammatory treatments and products released from activated microglia increase glial hemichannel activity, we investigated whether amyloid-β peptide (Aβ) could regulate these channels in glial cells and affect neuronal viability. Microglia, astrocytes, or neuronal cultures as well as acute hippocampal slices made from GFAP-eGFP transgenic mice were treated with the active fragment of Aβ. Hemichannel activity was monitored by single-channel recordings and by time-lapse ethidium uptake, whereas neuronal death was assessed by Fluoro-Jade C staining. We report that low concentrations of $A\beta_{25-35}$ increased hemichannel activity in all three cell types and microglia initiate these effects triggered by Aβ. Finally, neuronal damage occurs by activation of neuronal hemichannels induced by ATP and glutamate released from $A\beta_{25-35}$-activated glia. These responses were observed in the presence of external calcium and were differently inhibited by hemichannel blockers, whereas the $A\beta_{25-35}$-induced neuronal damage was importantly reduced in acute slices made from Cx43 knock-out mice. Thus, Aβ leads to a cascade of hemichannel activation in which microglia promote the release of glutamate and ATP through glial (microglia and astrocytes) hemichannels that induces neuronal death by triggering hemichannels in neurons. Consequently, this work opens novel avenues for alternative treatments that target glial cells and neurons to maintain neuronal survival in the presence of Aβ.

Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disease that results in memory loss, behavior and personality changes, among other symptoms. The generation of amyloid plaques in the extracellular brain parenchyma composed by amyloid-β (Aβ) peptide (LaFerla et al., 2007) and reactive gliosis are among tissue manifestations. Aβ is a peptide generated by proteolytic cleavage of APP (amyloid precursor protein) (LaFerla et al., 2007) and its fragment, 25–35 ($A\beta_{25-35}$), contains the neurotoxic Aβ domain (Pike et al., 1995b). Although Aβ neurotoxicity involves activation of NMDA receptors, sustained elevations of $[Ca^{2+}]_i$, and oxidative stress (LaFerla et al., 2007), the full underlying mechanism associated with AD remains to be elucidated.

Brains from AD patients exhibit a reactive gliosis characterized by glial activation closely associated with amyloid plaques (Kalaria, 1999). Moreover, it has been reported that the immunoreactivity of connexin 43 (Cx43), a gap junction channel and hemichannel protein subunit (Sáez et al., 2003), is increased around amyloid plaques (Nagy et al., 1996; Mei et al., 2010). Gap junctions are membrane specializations that provide a direct cytoplasmic pathway between contacting cells by aggregates that contain a few tens to thousands of cell-to-cell channels, termed gap junction channels (Sáez et al., 2003). They are formed by the docking of two hemichannels, contributed by each contacting cell (Sáez et al., 2003). Each hemichannel is formed by oligomerization of connexins, which are expressed by astrocytes, microglia, and neurons (Orellana et al., 2009). A more recently described three-member protein family, termed pannexins (Panxs), can also form hemichannels at the cell surface of diverse mammalian cells (Scemes et al., 2009) and have been proposed to play a relevant role in inflammasome activation in astrocytes and neurons (Iglesias et al., 2009).

It has been proposed that, under chronic pathological conditions (e.g., AD), activated microglia release proinflammatory molecules that increase hemichannel opening and reduce gap junctional communication in astrocytes, depriving neurons of

Received Dec. 9, 2010; revised Jan. 17, 2011; accepted Jan. 30, 2011.

This work was partially supported by the Caisse de Retraite et de Prévoyance des Clercs et Employés de Notaires and Inserm (France) (C.G.), Comisión Nacional de Investigación Científica y Tecnológica Grant 24080055 (J.A.O.), Fondo Nacional de Desarrollo Científico y Tecnológico Grant 1070591 (J.C.S.), Fondo de Fomento al Desarrollo Científico y Tecnológico Grant DO7I1086 (J.C.S.), Anillo Grant ACT-71 (J.C.S.), Institut National de la Santé et de le Recherche Medicale (France) (Département des Relations Internationales) (J.A.O.), Heart and Stroke Foundation of British Columbia and Yukon (C.C.N.), and National Institutes of Health Grant AR46798 and Welch Foundation Grant AQ-1507 (J.X.J.). The data of this work were presented by Dr. Juan A. Orellana as partial fulfillment of the requirements to obtain the degree of PhD in Physiological Sciences at the Pontificia Universidad Católica de Chile.

Correspondence should be addressed to Dr. Juan A. Orellana, Departamento de Fisiología, Pontificia Universidad Católica de Chile, Alameda 340, Santiago 6513677, Chile. E-mail: jaorella@uc.cl.

DOI:10.1523/JNEUROSCI.6417-10.2011

 0270-6474/11/314962-16$15.00/0

Journal of Neurochemistry JNC

JOURNAL OF NEUROCHEMISTRY | 2011 | 118 | 826–840 doi: 10.1111/j.1471-4159.2011.07210.x

ORIGINAL ARTICLE

ATP and glutamate released via astroglial connexin 43 hemichannels mediate neuronal death through activation of pannexin 1 hemichannels

Juan A. Orellana,* Nicolas Froger,† Pascal Ezan,† Jean X. Jiang,‡ Michael V. L. Bennett,§ Christian C. Naus,¶ Christian Giaume† and Juan C. Sáez*,§,**

**Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago, Chile*
†INSERM U840, Collège de France, 11 place Marcelin Berthelot, Paris, Cedex 05, France
‡Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas, USA
§Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York, USA
¶Department of Cellular and Physiological Sciences, Life Sciences Institute, University of British Columbia, Vancouver, Canada
***Instituto Milenio, Centro Interdisciplinario de Neurociencias de Valparaíso, Valparaíso, Chile*

Abstract

Inflammation contributes to neurodegeneration in post-ischemic brain, diabetes, and Alzheimer's disease. Participants in this inflammatory response include activation of microglia and astrocytes. We studied the role of microglia treated with amyloid-β peptide (Aβ) on hemichannel activity of astrocytes subjected to hypoxia in high glucose. Reoxygenation after 3 h hypoxia in high glucose induced transient astroglial permeabilization via Cx43 hemichannels and reduction in intercellular communication via Cx43 cell-cell channels. Both responses were greater and longer lasting in astrocytes previously exposed for 24 h to conditioned medium from Aβ-treated microglia (CM-Aβ). The effects of CM-Aβ were mimicked by TNF-α and IL-1β and were abrogated by neutralizing TNF-α with soluble receptor and IL-1β with a receptor antagonist. Astrocytes under basal conditions protected neurons against hypoxia, but exposure to CM-Aβ made them toxic to neurons subjected to a sub-lethal hypoxia/reoxygenation episode, revealing the additive nature of the insults. Astrocytes exposed to CM-Aβ induced permeabilization of cortical neurons through activation of neuronal pannexin 1 (Panx1) hemichannels by ATP and glutamate released through astroglial Cx43 hemichannels. In agreement, inhibition of NMDA or P2X receptors only partially reduced the activation of neuronal Panx1 hemichannels and neuronal mortality, but simultaneous inhibition of both receptors completely prevented the neurotoxic response. Therefore, we suggest that responses to ATP and glutamate converge in activation of neuronal Panx1 hemichannels. Thus, we propose that blocking hemichannels expressed by astrocytes and/or neurons in the inflamed nervous system could represent a novel and alternative strategy to reduce neuronal loss in various pathological states including Alzheimer's disease, diabetes and ischemia.

Keywords: Alzheimer's disease, amyloid β-peptide, connexin, cytokines, diabetes mellitus, gap junctions, pannexin, stroke.
J. Neurochem. (2011) **118**, 826–840.

ORIGINAL ARTICLE

The most common acute brain insult is ischemic stroke, where transient or permanent reduction in cerebral blood flow deprives the tissue of oxygen and glucose and permits build-up of potentially toxic substances, effects that together lead to rapid or delayed cell death (Dirnagl *et al.* 1999). An association between Alzheimer's disease (AD) and ischemic stroke has been established. Indeed, patients that on autopsy show cerebral infarcts and AD pathology are more cognitively impaired than patients with AD pathology alone (Snowdon *et al.* 1997). Moreover, the presence of high levels

Received November 26, 2010; revised manuscript received January 26, 2011; accepted January 26, 2011.

Address correspondence and reprint requests to Juan A. Orellana, Departamento de Fisiología, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile. E-mail: jaorella@uc.cl

Abbreviations used: AD, Alzheimer's disease; AU, arbitrary units; BBG, brillian blue G; CM, conditioned medium; CM-Aβ, conditioned medium from Aβ-treated microglia; DM, diabetes mellitus; DMEM, Dulbecco's modified Eagle's medium; FCS, Fetal Calf Serum; F-Jade, Fluoro-Jade C; GFAP, glial fibrillary acidic protein; LY, Lucifer yellow; Panx1, pannexin 1; PBS, phosphate-buffered saline.

404 *CNS & Neurological Disorders - Drug Targets*, 2011, *10*, 404-414

Hemichannels in the Neurovascular Unit and White Matter Under Normal and Inflamed Conditions

Juan A. Orellana[1,*], Xavier F. Figueroa[1], Helmuth A. Sánchez[2], Susana Contreras-Duarte[1], Victoria Velarde[1] and Juan C. Sáez[1,2,3]

[1]*Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago, Chile*

[2]*Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, USA*

[3]*Instituto Milenio, Centro Interdisciplinario de Neurociencia de Valparaíso, Chile*

Abstract: In the normal brain, cellular types that compose the neurovascular unit, including neurons, astrocytes and endothelial cells express pannexins and connexins, which are protein subunits of two families that form plasma membrane channels. Most available evidence in mammals indicated that endogenously expressed pannexins only form hemichannels, and connexins form both gap junction channels and hemichannels. While gap junction channels connect the cytoplasm of contacting cells and coordinate electrical and metabolic activities, hemichannels communicate intra- and extracellular compartments and serve as diffusional pathways for ions and small molecules. Here, evidence supporting the functional role of hemichannels in the neurovascular unit and white matter under physiological and pathological conditions are reviewed. A sub-threshold acute pathological threatening condition (e.g., stroke and brain infection) leads to glial cell activation, which maintains an active defense and restores the normal function of the neurovascular unit. However, if the stimulus is deleterious, microglia and the endothelium become overactivated, both releasing bioactive molecules (e.g., glutamate, cytokines, prostaglandins and ATP) that increase the activity of astroglial hemichannels, reducing the astrocyte neuroprotective functions, and further reducing neuronal cell viability. Moreover, ATP is known to contribute to myelin degeneration of axons. Consequently, hemichannels might play a relevant role in the excitotoxic response of oligodendrocytes observed in ischemia and encephalomyelitis. Regulated changes in hemichannel permeability in healthy brain cells can have positive consequences in terms of paracrine/autocrine signaling, whereas persistent changes in cells affected by neurological disorders can be detrimental. Therefore, blocking hemichannels expressed by glial cells and/or neurons of the inflamed central nervous system might prevent neurovascular unit dysfunction and neurodegeneration.

Keywords: Cerebral vasculature, connexins, glial cells, inflammation, myelination, pannexins.

INTRODUCTION

Astroglial cells were long considered to be part of connective tissue or simple support cells in the central nervous system (CNS). However, with the advent of the *tripartite synapse* conception, they are now recognized as essential protagonists in brain processing as well as in neurodegeneration. The term *tripartite synapse* denotes that in addition to the information flow between pre- and postsynaptic neurons, astroglial processes that wrap the *synapses* exchange information with them and respond to synaptic activity and modulate synaptic transmission [1]. Neuron-glia communication not only occurs through the release of gliotransmitters and neurotransmitters by astrocytes and neurons, respectively, but could also take place by direct contact between neurons, or neurons and glia, *via* electrical and metabolic coupling through gap junctions [2, 3]. Moreover, astrocytes form extended networks along domains of the brain parenchyma by direct communication between each other. They also do so with oligodendrocytes mainly through gap junctions [4, 5], which protect neurons through the "spatial buffering" of neurotoxic molecules [6]. In fact, reduced gap junctional communication between astrocytes and/or oligodendrocytes is associated with neurotoxicity [7, 8].

Gap junctions are membrane specializations that provide cytoplasmic pathways between contacting cells, and are permeable to molecules smaller than ~1.4 nm of diameter. Generally, gap junctions are aggregates or plaques that contain a few tens to thousands of cell-cell channels. Each gap junction channel is formed by two hemichannels (also termed connexons) that are contributed by each adjacent cell [9] (Fig. **1**). Each hemichannel is composed of six protein subunits termed connexins, which belong to a highly conserved protein family encoded by 21 genes in human and 20 genes in mouse with orthologs in other vertebrate species [10]. Connexins are abundantly expressed in cells of the CNS [6], and are named after their predicted molecular mass expressed in kDa, so that connexin43 (Cx43) has a molecular mass of ~43 kDa.

In addition to providing a direct communication pathway for the intercellular exchange of small molecules, such as metabolites (e.g., ADP, glucose, glutamate, and glutathione) and second messengers (e.g., cAMP and inositol (1,4,5)-trisphosphate (IP_3) [11-16]), gap junctions also allow the intercellular spread of electrotonic potentials in excitable and non-excitable tissues [17-19]. In the last decade, the presence of functional connexin hemichannels in nonjunctional membranes has been demonstrated by several experimental approaches [20]. So far, most connexins expressed in exogenous systems generate non-selective current pathways attributed to hemichannel opening [21]. These channels allow cellular release of relevant quantities of autocrine/paracrine signaling molecules (e.g., ATP, glutamate, NAD^+, and prostaglandin E_2) to the extracellular milieu [22-25], as well as uptake of small molecules (e.g., glucose) [26].

An increasing body of evidence has situated the hemichannels as potential regulators of the initiation and maintenance of homeostatic imbalances present in diverse brain diseases [6]. Pioneering findings by Contreras *et al.* [27] showed that astroglial death induced by ischemia-like conditions is accelerated by the opening of Cx43 hemichannels. Because astrocytes provide metabolic and structural support to neurons and control the extracellular concentration of glutamate, K^+ and H^+, damage associated with hemichannel opening has been proposed to increase

*Address correspondence to this author at the Departamento de Fisiología, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile; Tel: +5626862860; Fax: +5622225515; E-mail: jaorella@uc.cl

1871-5273/11 $58.00+.00

10

Role of Connexin Hemichannels in Neurodegeneration

Juan A. Orellana[1,2], Christian Giaume[3] and Juan C. Sáez[1,4]
[1]*Departamento de Fisiología; Facultad de Ciencias Biológicas*
[2]*Departamento de Neurología; Facultad de Medicina*
Pontificia Universidad Católica de Chile, Santiago,
[3]*CIRB; UMR CNRS 7241/ INSERM U1050, Collège de France, Paris*
[4]*Instituto milenio, Centro Interdiciplinario de Neurociencias de Valparaiso, Valparaiso,*
[1,2,4]*Chile*
[3]*France*

1. Introduction

Progressive loss of neuronal structure and function occur in several neurodegenerative diseases. Cellular responses to brain injury depend on properties of the cells (e.g., hormonal nutritional status) and insult (e.g., duration, intensity, and quality), whereas, tissue responses depend on interactions between their constituent cells, including chemical and electrical transmission as well as paracrine and autocrine signaling. In vertebrate cells, autocrine and paracrine communication occur in part via release of chemical signals through connexin hemichannels (Sáez et al. 2010), the precursors of gap junction channels that are formed by two hemichannels provided by one of each apposed cells (Fig. 1). Each hemichannel is composed of six protein subunits termed connexins, which are highly conserved proteins encoded by 21 genes in human and 20 in mouse with orthologs in other vertebrate species (Cruciani and Mikalsen 2005). Connexins are abundantly expressed in cells of the central nervous system (CNS) (Orellana et al. 2009) (Fig. 2), and they are named after their predicted molecular mass expressed in kDa, so that connexin43 (Cx43) has a molecular mass of ~43 kDa.

For a long time the main function attributed to connexin hemichannels was the formation of gap junction channels. Nevertheless, in the last decade, the presence of functional connexin hemichannels in nonjunctional membranes has been demonstrated by several experimental approaches (Sáez et al. 2010). These channels allow cellular release of relevant quantities of autocrine/paracrine signaling molecules (e.g., ATP, glutamate, NAD^+ and PGE_2) to the extracellular milieu (Bruzzone et al. 2001; Cherian et al. 2005; Stout et al. 2002), as well as uptake of small molecules (e.g., glucose) (Retamal et al. 2007a). Recently, another gene family encoding a set of three membrane proteins, named pannexins (Panxs 1-3), has been identified (Bruzzone et al. 2003). Up to now, only Panx3 has been shown to form gap junctions in osteoblasts (Ishikawa et al. 2011) and further studies will be required to identify pannexin gap junctions in other cell types. Connexins and pannexins present similar membrane topology, with four α-helical transmembrane domains connected by two

Un recorrido junto a Francisco Varela

Adrián Palacios, Diego Cosmelli y Amy Cohen-Varela[1]

Sin duda, Francisco Varela (1946 - 2001) fue un explorador intrépido y curioso, en sus propias palabras, del "Fenómeno de la vida en todo su esplendor". ¿Pero quién fue Francisco Varela? ¿Cuál es la importancia de su obra?

Deseosos de responder a estas preguntas hemos querido recopilar en un escrito orientador y motivador, una suerte de cartografía o mapa, dirigido al público general, para dar cuenta de las principales rutas de trabajo que exploró Francisco Varela a lo largo de su vida. Sin pretender ser exhaustivos en una compilación como ésta, sí esperamos que la selección, que nos invita a una suerte de exploración de los caminos recorridos por Francisco, nos permita vislumbrar la magnitud y diversidad del trabajo de este gran pensador y científico de nuestra época. Al hacerlo queremos entregar una fuente de reflexión sobre el origen de las ideas sin perder de vista el tiempo histórico en el cual éstas vieron el día. Francisco publicó cerca de 200 artículos científicos, escribió varios libros y desarrolló su investigación y formación de estudiantes durante más de 30 años.

Francisco estuvo desde siempre interesado en la "biología del cosmos". Si bien esto pareciese a simple lectura una meta sin dimensión,

1. Amy Cohen-Varela, Psicóloga Clínica, Psicoanalista. Viuda de Francisco Varela, Paris, Francia.
   Adrián Palacios, Doctor en Neurociencia, Universidad de Pierre et Marie Curie, Paris, Francia. Profesor Universidad de Valparaíso, Investigador del Centro Interdisciplinario de Neurociencia de Valparaíso e Instituto de Sistemas Complejos de Valparaíso, Valparaíso, Chile.
   Diego Cosmelli, Doctor en Ciencias Cognitiva, Escuela Politécnica, Paris, Francia. Profesor Asociado, P Universidad Católica de Chile, Escuela de Psicología, Santiago, Chile.

Mar Biotechnol (2011) 13:142–150
DOI 10.1007/s10126-010-9274-2

ORIGINAL ARTICLE

Transgenic Expression of Walleye Dermal Sarcoma Virus rv-cyclin (orfA) in Zebrafish does not Result in Tissue Proliferation

Thomas A. Paul · Joel Rovnak · Sandra L. Quackenbush · Kathleen Whitlock · Huiqing Zhan · Zhiyuan Gong · Jan Spitsbergen · Paul R. Bowser · James W. Casey

Received: 14 October 2009 / Accepted: 19 January 2010 / Published online: 28 March 2010

Abstract Walleye dermal sarcoma (WDS) is a benign tumor of walleye fish that develops and completely regresses seasonally. The retrovirus associated with this disease, walleye dermal sarcoma virus, encodes three accessory genes, two of which, *rv-cyclin* (*orfA*) and *orfb*, are thought to play a role in tumor development. In this study, we attempted to recapitulate WDS development by expressing rv-cyclin in chimeric and stable transgenic zebrafish. Six stable transgenic lines expressing rv-cyclin from the constitutive CMVtk promoter were generated. Immunohistochemistry and quantitative reverse transcriptase polymerase chain reaction demonstrate that rv-cyclin is widely expressed in different tissues in these fish. These lines were viable and histologically normal for up to 2 years. No increase in tumors or tissue proliferation was observed following *N*-ethyl *N*-nitrosourea exposure or following tail wounding and subsequent tissue regeneration compared to controls. These data indicate that rv-cyclin is not independently sufficient for tumor induction in zebrafish.

Keywords Walleye dermal sarcoma virus · orfA · rv-cyclin · Fish retrovirus · Walleye dermal sarcoma · Zebrafish

T. A. Paul · P. R. Bowser · J. W. Casey (✉)
Department of Microbiology and Immunology,
Cornell University,
Ithaca, NY, USA
e-mail: jwc3@cornell.edu

J. Rovnak · S. L. Quackenbush
Department of Microbiology, Immunology and Pathology,
Colorado State University,
Fort Collins, CO, USA

K. Whitlock
Centro de Genómica de la Célula, Centro de Neurociencia,
Universidad de Valparaíso,
Valparaíso, Chile

H. Zhan · Z. Gong
Department of Biological Sciences,
National University of Singapore,
Singapore, Singapore

J. Spitsbergen
Department of Microbiology and Marine and Freshwater
Biomedical Sciences Center, Oregon State University,
Corvallis, OR, USA

Introduction

Walleye dermal sarcoma virus (WDSV), an *Epsilonretrovirus*, was first identified in association with benign cutaneous proliferative lesions in walleyes (*Sander vitreus*) (Martineau et al. 1992), known as walleye dermal sarcoma (WDS), and was found to be highly enriched within neoplastic cells (Poulet et al. 1995). Subsequent molecular characterization of WDSV identified three novel open reading frames: *rv-cyclin (orfA)*, *orfB*, and *orfC*. The accessory genes *rv-cyclin* and *orfb* are located downstream of *env* in the 3′ proximal region of the genome, and *orfC* lies between the 5′ long terminal repeat and *gag* (Holzschu et al. 1995).

Several observations suggest that rv-cyclin and OrfB may play a central role in inducing cell proliferation, leading to WDS. In developing WDS fall tumors, low levels of spliced viral transcripts are present that are capable of encoding rv-cyclin and OrfB exclusively (Quackenbush et al. 1997). The rv-cyclin protein contains a region homologous to the cyclin box fold (LaPierre et al. 1998). Functionally, rv-cyclin complements cyclin function in G1

5554 • The Journal of Neuroscience, April 13, 2011 • 31(15):5554–5556

Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Could an Allosteric Gating Model Explain the Role of TRPA1 in Cold Hypersensitivity?

Marcelo Salazar,[1,2] Hans Moldenhauer,[1,3] and David Baez-Nieto[1,3]

[1]Centro Interdisciplinario de Neurociencia Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Valparaiso 2349400, Chile, [2]PhD program in Cellular and Molecular Biology, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile, and [3]PhD program in Neuroscience, Facultad de Ciencias, Universidad de Valparaiso, Valparaiso 2349400, Chile

Review of del Camino et al.

Mammalian transient receptor potential (TRP) channels consist of at least seven families of related proteins. A subset belong to the TRP channel superfamily, and they are responsible for temperature detection in mammals. These TRP channels (thermoTRPs) are expressed primarily in the dorsal root ganglion neurons in both somatosensory and nociceptive fibers. They respond to a wide range of temperatures from extreme cold (<10°C) to extreme heat (>42°C) (Latorre et al., 2009). TRPA1 is a cold-activated thermoTRP, and it is also activated by a broad spectrum of endogenous agonists and exogenous reactive irritants. Several of its activators are related to inflammatory processes. Although TRPA1 has been reported as an extreme cold receptor, its role in acute response to low temperatures is still controversial.

Using a heterologous expression, del Camino et al. (2010) confirmed findings by other groups (Sawada et al., 2007; Karashima et al., 2009) that TRPA1 channels and the cold-mediated activation of TRPA1 current are Ca^{2+} independent. More importantly, del Camino et al. (2010) showed that TRPA1 elicited currents by allyl isothiocyanate (AITC) and other agonists were increased when the temperature was lowered from 25°C to 10°C. These results conflict with those obtained by Karashima et al. (2009), who found that agonist-induced currents were reduced at lower temperatures. del Camino et al. (2010) explained that this discrepancy was due to a slower cooling system and the presence of extracellular calcium in the Karashima et al. (2009) experiment.

Figure 3 in del Camino et al. (2010) demonstrates that TRPA1 currents show a stronger increment when stimulated by AITC than by extreme cold. The increment is even greater when both stimuli are combined. Furthermore, when TRPA1 was stimulated by a highly saturated concentration of agonist [del Camino et al. (2010), their Fig. 3*C*] and the temperature lowered from 30°C to 10–20°C, it produced comparable relative increases of currents. This result suggests that the equilibrium constant that governs the transition between the closed and open states is biased toward the open state by the presence of AITC.

The best model that can explain this bias is the allosteric kinetic model. This Journal Club paper aims to can clarify how TRPA1 might respond synergistically to different stimuli, such as temperature and agonists (Fig. 1*A*). Our model establishes that different sensors (temperature and chemical) are located in different structural domains of the protein. Each structural domain is allosterically coupled to the channel gate, thus influencing the channel open probability (P_o).

The allosteric model for temperature and agonist concentration predicts that P_o as a function of temperature and agonist is given by the following equation:

$$P_o = \frac{1}{1 + \frac{1 + K + Q + KQ}{L(1 + KC + QF + KCQF)}},$$

where L is the equilibrium constant between closed and open state, when all sensors are relaxed; K and Q are the constants that govern the temperature- and agonist-dependent transitions; and C and F are the allosteric constants that couple the temperature and agonist sensors, respectively, with the channel gate.

Loss of sensitivity to other stimuli at high concentrations of agonist has been reported for other thermoTRPs, including TRPM8 and TRPV1 (Matta and Ahern, 2007). Our model supports these findings and predicts that in nonsaturated agonist concentration the relative increase of P_o in response to thermal stimulus should be greater (Fig. 1*D*) as reported by del Camino et al. (2010) (their Fig. 3*C*). Keeping in mind that a chemical stimulus causes more TRPA1 activity than cold, introducing a saturated

Received Dec. 27, 2010; revised Feb. 1, 2011; accepted Feb. 4, 2011.

This work was supported by Fondo Nacional de Desarrollo Científico y Tecnológico Grant 1070049 and Comisión Nacional de Investigación Científica y Tecnológica fellowships. We thank to Drs. Ramon Latorre and Carlos Gonzalez for reading our manuscript, and for giving some suggestions that have been helpful to improve it. Centro Interdisciplinario de Neurociencia Valparaiso is a millenium institute.

Correspondence should be addressed to David Baez-Nieto, Centro Interdisciplinario de Neurociencias de Valparaiso, Gran Bretaña #1111, Playa Ancha, Valparaiso 2349400, Chile. E-mail: david.baez@cinv.cl.

DOI:10.1523/JNEUROSCI.6775-10.2011

 0270-6474/11/315554-03$15.00/0

ZEBRAFISH
Volume 8, Number 1, 2011

DOI: 10.1089/zeb.2010.0671

Original Article

Zebrafish Preference for Light or Dark Is Dependent on Ambient Light Levels and Olfactory Stimulation

Jessica F. Stephenson,[1,2] Kathleen E. Whitlock,[2] and Julian C. Partridge[1]

Abstract

Zebrafish have been shown to have preference for light or dark environments depending on the ambient light level and the presence or absence of food odor. We used a cylindrical tank, half of which was surrounded by a black surface and the other half by white, to elicit a choice from individual wild-type, adult zebrafish. One treatment group was exposed to food odor and the other to water (as a control) at the beginning of the trial. During 10-min trials, the light level was increased each minute over a fivefold range in steps from 1.34×10^{17} photons/s/m^2 at the beginning to a final light level of 8.31×10^{17} photons/s/m^2. We demonstrate that the preference of the zebrafish for the light or dark half of the cylinder is dependent upon ambient light levels as well as olfactory stimulation. These results provide a potential explanation for the contradictory observations that, when given a choice, adult zebrafish prefer brighter light environments (Gerlai *et al.*, 2000) or darker light environments (Serra *et al.*, 1999). Thus, we present data useful in designing more powerful and reliable behavioral assays for use with zebrafish as well as further information about the effect of olfactory stimulation on zebrafish visual behavior.

Introduction

Zebrafish are an important model species in studies of genetics, physiology, and developmental biology[1] and in medical science.[2–4] Because of its potential use in behavioral genetic studies,[5,6] as well as behavioral assays of the effects of drugs,[7,8] disease,[9] or genetic mutations,[10] zebrafish are also becoming more popular as a model for behavioral studies.[11–14]

Two studies, by Gerlai *et al.*[15] and Serra *et al.*,[16] tested the effectiveness of a simple behavioral assay in zebrafish: the preference for a light or dark environment. The fish tested by Gerlai *et al.*[15] appeared to prefer a light environment, whereas those tested by Serra *et al.*[16] demonstrated the opposite preference. The simplicity of this assay and the strength of the response make it a potentially attractive and widely applicable test, but first these contradictory results reported need explanation.

Zebrafish visual behavior has been well documented,[17] and several studies suggest that it is affected by olfactory stimulation[18–20] (Stephenson, Partridge, and Whitlock, Unpublished data). Behavioral tests of this hypothesis have been restricted to determining whether the threshold of light required for a response to a visual stimulus is lower after exposure to olfactory stimuli[20] (Stephenson, Partridge, and Whitlock, Unpublished data).

This experiment therefore tests two hypotheses: (1) Zebrafish exhibit a preference for a relatively light or dark environment depending on the ambient light level; (2) olfactory stimulation alters zebrafish light/dark preference behavior at light levels above the threshold required for a response to a visual stimulus.

Materials and Methods

Fish origin and maintenance

The zebrafish used in this study were from the breeding population at the University of Valparaíso, Chile, and were naive to any form of behavioral experiment. The genetically defined "New Wild-type" line, derived from the University of Oregon AB line (http://zfin.org/; Zebrafish International Resources Center [ZIRC]), was used throughout this work. Twenty wild-type zebrafish (10 females and 10 males) were kept at 26°C–27°C in 40-L aquaria filled with deionized (reverse osmosis) water to which Instant Ocean® Sea Salt was added to reach a conductivity of 400 micro-Siemens/cm^2 and sodium bicarbonate was added to reach pH 7.3 (hereafter referred to as "fish water"). Fluorescent room light provided a 13.5:10.5 (L:D) photoperiod (light phase: 9 am to 10.30 pm); light level at the surface of the water in the communal home tanks ranged from 619 to 705 lx. When fish were held in

[1]School of Biological Sciences, University of Bristol, Bristol, United Kingdom.
[2]Centro de Neurociencia, Centro de Genomicas Celular, Universidad de Valparaíso, Valparaíso, Chile.

Neurotox Res (2012) 22:59–68
DOI 10.1007/s12640-011-9304-5

Altered Voltage Dependent Calcium Currents in a Neuronal Cell Line Derived From the Cerebral Cortex of a Trisomy 16 Fetal Mouse, an Animal Model of Down Syndrome

Mario A. Acuña · Ramón Pérez-Nuñez · Jorge Noriega · Ana María Cárdenas · Juan Bacigalupo · Ricardo Delgado · Christian Arriagada · Juan Segura-Aguilar · Raúl Caviedes · Pablo Caviedes

Received: 16 August 2011 / Revised: 30 November 2011 / Accepted: 13 December 2011 / Published online: 28 December 2011

Abstract Human Down syndrome (DS) is determined by the trisomy of autosome 21 and is expressed by multiple abnormalities, being mental retardation the most striking feature. The condition results in altered electrical membrane properties (EMPs) of fetal neurons, which are qualitatively identical to those of trisomy 16 fetal mice (Ts16), an animal model of the human condition. Ts16 hippocampal cultured neurons reportedly exhibit increased voltage-dependent calcium currents (I_{Ca}) amplitude. Since Ts16 animals are unviable, we have established immortalized cell lines from the cerebral cortex of Ts16 (named CTb) and normal littermates (named CNh). Using the whole-cell patch-clamp technique, we have now studied I_{Ca} in CTb and CNh cells. Current activation occurs at −40 mV in both cell lines ($V_{holding} = -80$ mV). Trisomic cells exhibited a 2.4 fold increase in the maximal Ca^{2+} current density compared to normal cells (CNh = -6.3 ± 0.77 pA/pF, $n = 18$; CTb = -16.4 ± 2.423 pA/pF; $P < 0.01$, $n = 13$). Time dependent kinetics for activation and inactivation did not differ between the two cell types. However, steady state inactivation studies revealed a 15 mV shift toward more depolarized potentials in the trisomic condition, suggesting that altered voltage dependence of inactivation may underlie the increased current density. Further, the total charge movement across the membrane is increased in CTb cells, in agreement with that expected by the potential sensitivity shift. These results indicate that CTb cells present altered Ca^{2+} currents, similar to those of Ts16 primary cultured central neurons. The CTb cell line represents a model for studying DS-related impairments of EMPs.

Keywords Down syndrome · Calcium currents · Trisomy · Patch clamp

M. A. Acuña · R. Pérez-Nuñez · J. Noriega · J. Segura-Aguilar · R. Caviedes · P. Caviedes (✉)
Program of Molecular and Clinical Pharmacology, ICBM, Faculty of Medicine, University of Chile, Clasificador 7, Independencia 1027, 8389100 Independencia, Santiago, Chile
e-mail: pcaviede@med.uchile.cl

A. M. Cárdenas
Centro Interdisciplinario de Neurociencia de Valparaíso (CINV), Universidad de Valparaíso, Valparaíso, Chile

J. Bacigalupo · R. Delgado
Department of Biology and Millennium Institute, ICDB, Faculty of Science, University of Chile, Santiago, Chile

C. Arriagada
Program of Anatomy & Developmental Biology, ICBM, Faculty of Medicine, University of Chile, Santiago, Chile

Introduction

Down Syndrome (DS) in humans is caused by the presence of an extra copy of chromosome 21 (HSA21) (Jacobs et al. 1959; Lejeune et al. 1959). The condition represents the most common cause of mental retardation of genetic origin (Oster-Granite 1986; Loesch-Mdzewska 1968; Johnson and Abelson 1969; Scott et al. 1983). Other abnormalities include cardiac malformations, immunological disorders, a higher incidence of leukemia and patients also develop Alzheimer's disease (AD)-like pathology after the fourth decade of life (Epstein 1986a, b; Ault et al. 1989; Schapiro et al. 1988). 127 genes have been identified in this chromosome, along with 98 possible new genes, and 59 pseudogenes, all of which could be overexpressed in DS (Epstein 1986a; Hattori et al. 2000; Saud et al. 2006), determining an excess gene dosage effect.

JCTC Journal of Chemical Theory and Computation

Article

pubs.acs.org/JCTC

Insight into the Properties of Cardiolipin Containing Bilayers from Molecular Dynamics Simulations, Using a Hybrid All-Atom/United-Atom Force Field

Daniel Aguayo,[†,‡,§] Fernando D. González-Nilo,[‡,§] and Christophe Chipot*[,∥,⊥]

[†]Centro de Bioinformática y Simulación Molecular, Facultad de Ingeniería en Bioinformática, Universidad de Talca, 2 Norte 685, Casilla 721, Talca, Chile

[‡]Centro de Bioinformática y Biología Integrativa, Facultad de Ciencias Biológicas, Universidad Andrés Bello, República 239, Santiago, Chile

[§]Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso, Valparaíso, Chile

[∥]Theoretical and Computational Biophysics Group, Beckman Institute for Advanced Science and Engineering, University of Illinois at Urbana−Champaign, 405 North Mathews, Urbana, Illinois 61801, United States

[⊥]Équipe de dynamique des assemblages membranaires, UMR 7565, Université de Lorraine, BP 239, 54506 Vandoeuvre-lès-Nancy cedex, France

Supporting Information

ABSTRACT: Simulation of three models of cardiolipin (CL) containing membranes using a new set of parameters for tetramyristoyl and tetraoleoyl CLs has been developed in the framework of the united-atom CHARMM27-UA and the all-atom CHARMM36 force fields with the aim of performing molecular dynamics (MD) simulations of cardiolipin-containing mixed-lipid membranes. The new parameters use a hybrid representation of all-atom head groups in conjunction with implicit-hydrogen united-atom (UA) to describe the oleoyl and myristoyl chains of the CLs, in lieu of the fully atomistic description, thereby allowing longer simulations to be undertaken. The physicochemical properties of the bilayers were determined and compared with previously reported data. Furthermore, using tetramyristoyl CL mixed with POPG and POPE lipids, a mitochondrial membrane was simulated. The results presented here show the different behavior of the bilayers as a result of the lipid composition, where the length of the acyl chain and the conformation of the headgroup can be associated with the mitochondrial membrane properties. The new hybrid CL parameters prove to be well suited for the simulation of the molecular structure of CL-containing bilayers and can be extended to other lipid bilayers composed of CLs with different acyl chains or alternate head groups.

1. INTRODUCTION

Mitochondria are organelles that provide most of the chemical energy required by the cell, namely adenosine triphosphate (ATP), from oxidative metabolism. In the inner mitochondrial membrane (IMM) is found an unusual type of dimeric phospholipid, cardiolipin (CL), the structure of which consists of three glycerol backbones, four acyl chains and a divalent anionic headgroup (see Figure 1).[1] CLs play multiple roles related to energy transformation, apoptosis and membrane integrity.[2,3] Several mitochondrial proteins have been shown to require CLs for their optimal activity. Such is the case, among others, of cytochrome P-450$_{SCC}$,[4] mitochondrial creatine kinase,[5,6] mitochondrial L-glycerol-3-phosphate dehydrogenase[7] and mitochondrial carnitine acylcarnitine translocase.[8] A number of investigations have suggested that CLs are associated with the mitochondrial apoptotic pathway,[9] or different complexes of the respiratory chain, involved in the transduction of electrons and the synthesis of ATP in the IMM.[10,11] In addition, CLs are of paramount importance in the formation of contact sites between the inner and outer mitochondrial membrane by virtue of its ability to arrange spatially into hexagonal H_{II} phases.[12] They are also related to membrane lipid polarization in prokaryotic organisms in a process mediated by CL intrinsic curvature.[13,14]

Detailed structural information of CLs is admittedly scarce: only a handful of data on CL aggregates and IMM protein−CL interactions have been reported hitherto.[15] A number of structures deposited in the Protein Data Bank (PDB) contain CLs cocrystallized with IMM proteins.[16−19] Because CLs were cocrystallized with the protein, the conformation of these lipids is not necessarily representative of that found in a hydrated bilayer.

Computer simulations have proven to constitute a useful approach to study the properties of lipid aggregates.[20,21] Simulations can be employed to investigate the dynamics of individual molecules and the assembly thereof, with the potential to attain a much more detailed picture than would be obtained at the experimental level. Moreover, computer simulations allow theoretical and experimental parameters to be compared directly and are now sufficiently reliable to endeavor the investigation of molecular assemblies that can only be

Received: November 23, 2011
Published: April 16, 2012

dx.doi.org/10.1021/ct200849k | J. Chem. Theory Comput. 2012, 8, 1765−1773

Postsynaptic dysfunction is associated with spatial and object recognition memory loss in a natural model of Alzheimer's disease

Álvaro O. Ardiles[a], Cheril C. Tapia-Rojas[b], Madhuchhanda Mandal[c], Frédéric Alexandre[d], Alfredo Kirkwood[c,1], Nibaldo C. Inestrosa[b], and Adrian G. Palacios[a,1]

[a]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, 2360102 Valparaíso, Chile; [b]Centro de Envejecimiento y Regeneración, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, 8331010 Santiago, Chile; [c]Mind/Brain Institute and Department of Neurosciences, Johns Hopkins University, Baltimore, MD 21218; and [d]Institut des Maladies Neurodégénératives, Department Mnemosyne, Centre de Recherche Inria Bordeaux, F-33076 Bordeaux, France

Edited by Robert C. Malenka, Stanford University School of Medicine, Stanford, CA, and approved July 6, 2012 (received for review January 23, 2012)

Alzheimer's disease (AD) is an age-related neurodegenerative disorder associated with progressive memory loss, severe dementia, and hallmark neuropathological markers, such as deposition of amyloid-β (Aβ) peptides in senile plaques and accumulation of hyperphosphorylated tau proteins in neurofibrillary tangles. Recent evidence obtained from transgenic mouse models suggests that soluble, nonfibrillar Aβ oligomers may induce synaptic failure early in AD. Despite their undoubted value, these transgenic models rely on genetic manipulations that represent the inherited and familial, but not the most abundant, sporadic form of AD. A nontransgenic animal model that still develops hallmarks of AD would be an important step toward understanding how sporadic AD is initiated. Here we show that starting between 12 and 36 mo of age, the rodent *Octodon degus* naturally develops neuropathological signs of AD, such as accumulation of Aβ oligomers and phosphorylated tau proteins. Moreover, age-related changes in Aβ oligomers and tau phosphorylation levels are correlated with decreases in spatial and object recognition memory, postsynaptic function, and synaptic plasticity. These findings validate *O. degus* as a suitable natural model for studying how sporadic AD may be initiated.

memory dysfunction | neural plasticity | aging | T-maze | hippocampus

Alzheimer's disease (AD) is an age-related neurodegenerative disorder characterized by the accumulation of abnormally processed proteins in neurofibrillary tangles (NFTs) and senile plaques (1). These lesions are present in both familial and sporadic forms of AD. Familial AD is linked to inherited mutations in AD-related genes and represents a small percentage of AD cases, whereas sporadic AD represents the vast majority of cases and is not inherited. Results from transgenic mice bearing mutations in APP, PSEN1/2, and TAU show synaptic dysfunction in early stages of AD, before overt neurodegeneration (2, 3). More recent studies have demonstrated a critical role for soluble Aβ oligomers as an early trigger for AD, as well as associations with memory and neural plasticity loss (4–8).

Although transgenic mice have been extremely useful in elucidating the pathological mechanisms of AD, they have some substantial limitations. Examples include the absence of tau mutations linked to AD except for a triple transgenic mouse 3xTg-AD, bearing mutations for APP, PSEN1/2, and TAU (9); inability to develop the whole spectrum of the disease; overexpression of transgenes into a nonphysiological scenario; and the fact that the manipulated genes represent only familial, not sporadic forms of AD (10, 11). It would be highly desirable to have a nontransgenic model of AD to complement the existing models. Several species naturally develop features of AD with age; however, the usefulness of these species is limited, because none exhibits the full spectrum of AD-related alterations (12–14). For example, the Aβ peptide sequences of *Cavia porcellus* (guinea pig) and *Microcebus murinus* are similar to that of human (15, 16), but the first fails to develop senile plaques and NFTs (15), and experiments examining synaptic function and memory have not been carried out in such models. A promising candidate model for sporadic AD is the rodent *Octodon degus* (degus), which naturally develops the histochemical hallmarks of AD, including intracellular and extracellular accumulation of amyloid plaques, tau deposition in NFT (17), and hippocampal disconnection and brain parenchyma pathology (18). Prompted by these preliminary observations, we examined the neuropathological spectrum of AD in degus. Here we report that degus exhibits an age-related accumulation of soluble Aβ oligomers and tau protein phosphorylation that correlates with cognitive decline in spatial memory (T-maze) and object recognition memory (ORM), as well as synaptic and neural plasticity dysfunction. Based on these findings, we propose that (*i*) Aβ dodecamers (Aβ*56) may associate with phosphorylated tau proteins, constituting an early candidate for the neural toxicity and synaptic dysfunction that occurs before the appearance of fibrillar forms of Aβ, which are common to familial and sporadic forms of AD, and (*ii*) degus is a suitable nontransgenic model of sporadic AD.

Results

We evaluated the degree of neuropathology at the behavioral, synaptic, and molecular levels in degus at different ages. This approach allowed us to establish interpretative correlations to identify those animals suffering from AD (Table S1).

Age-Related Cognitive Decline with Aging in *O. degus*. We first evaluated memory capacity with ORM and T-maze tests in 6-, 12-, 36-, and 60-mo-old degus. The results showed no significant difference between 6- and 12-mo-old degus and between 36- and 60-mo-old degus, and thus we classified the animals as either young (6 and 12 mo-old) or aged (36 and 60 mo-old) (Fig. 1 *B* and *D*). In the T-maze, the aged degus had poorer performance than the young degus (Fig. 1 *A* and *B*; $P < 0.0001$, ANOVA). On the ORM test, the aged degus explored less (total time and number of visits) and had longer latency than the younger degus (Table S2). Moreover, unlike young degus, aged degus did not demonstrate a preference between new objects and familiar objects (Fig. 1 *C* and *D*; $P < 0.0001$, *t* test). In general, we observed an age-dependent decline in memory performance beginning at 36 mo-old and persisting through 60 mo-old (Fig. 1*B*).

Author contributions: A.O.A., C.C.T.-R., A.K., N.C.I., and A.G.P. designed research; A.O.A., C.C.T.-R., and M.M. performed research; A.K., N.C.I., and A.G.P. contributed new reagents/analytic tools; A.O.A., C.C.T.-R., F.A., A.K., N.C.I., and A.G.P. analyzed data; and A.O.A., A.K., and A.G.P. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence may be addressed. E-mail: kirkwood@jhu.edu or adrian.palacios@uv.cl.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1201209109/-/DCSupplemental.

The Journal of Experimental Biology 215, 1740-1748

doi:10.1242/jeb.067264

RESEARCH ARTICLE

Odorant tuning of olfactory crypt cells from juvenile and adult rainbow trout

Alejandra Bazáes and Oliver Schmachtenberg*

Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Avda Gran Bretaña 1111, 2360102 Playa Ancha, Valparaíso, Chile

*Author for correspondence (oliver.schmachtenberg@uv.cl)

Accepted 6 February 2012

SUMMARY

Teleost fish lack independent olfactory organs for odorant and pheromone detection. Instead, they have a single sensory epithelium with two populations of receptor neurons, ciliated and microvillous, that are conserved among vertebrates, and a unique receptor cell type named the olfactory crypt cell. Crypt cells were shown to be chemosensory neurons that project to specific areas in the olfactory bulb, but their odorant tuning and overall function remain unclear. Reproduction in fish is generally synchronized by sex pheromonal signaling between males and females, but the sensors responsible for pheromone detection remain unknown. In crucian carp, a seasonal variation in the population of olfactory crypt cells and their brain projections pathways, involved in reproduction, led to the hypothesis of a role as sex pheromone detectors. In the present study, morphology and localization of olfactory crypt cells were compared between juvenile and mature rainbow trout of both sexes, and calcium imaging was used to visualize responses of crypt cells from the three groups to common social and food-related odorants, sex hormones and conspecific tissue extracts. Crypt cells from mature trout were found to be larger than those of juvenile specimens, and preferentially localized to the apical surface of the olfactory epithelium. Although a fraction of crypt cells of all groups responded to common odorants such as amino acids and bile salts, cells from mature trout showed a characteristic preference for gonadal extracts and hormones from the opposite sex. These results support an involvement of olfactory crypt cells in reproduction-related olfactory signaling in fishes.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/215/10/1740/DC1

Key words: olfaction, teleost, pheromone, salmonid, chemical sense.

INTRODUCTION

The olfactory crypt cell was first identified and named in an ultrastructural study of teleost fishes (Hansen et al., 1997). Crypt cells were found in several common fishes, including zebrafish, catfish and goldfish, but they are apparently absent from other species, including two types of lungfishes (Hansen and Finger, 2000; Hansen et al., 1999). Their presence in salmonids was first demonstrated by Sandahl and co-workers (Sandahl et al., 2006). Subsequent immunohistochemical studies reported the presence of crypt-cell-like cells in the olfactory epithelium of cartilaginous fish, the elasmobranch *Scyliorhinus canicula* and the skate *Raja clavata* (Ferrando et al., 2006; Ferrando et al., 2007; Ferrando et al., 2010), suggesting that the evolutionary emergence of olfactory crypt cells represents an ancient feature in vertebrate development. Interestingly, thus far crypt cells have been found solely in fishes, as opposed to amphibians or other aquatic animals, suggesting that they are an exclusive attribute of this large and diverse animal group.

Olfactory crypt cells are oval to egg-shaped neurons that are completely surrounded by one or two supporting cells. The association of the crypt cell with its supporting cell(s) is sufficiently strong to withstand mechanical tissue dissociation, and the two cell types are physiologically coupled by gap junctions (Schmachtenberg, 2006). An apical invagination of up to 5 μm depth is the principal characteristic of the neuron. This invagination is bordered by microvilli from the crypt cell and its supporting cell(s), and usually contains both short cilia and microvilli in its inner part. Crypt cells are dispersed in the olfactory epithelium, and are rare compared with ciliated and microvillous olfactory receptor neurons (Hansen and Finger, 2000; Schmachtenberg, 2006).

To date, little is known about the molecular properties of crypt cells. Immunohistochemical studies in different species suggest that olfactory crypt cells express the G-proteins $G\alpha_o$ and $G\alpha_q$ as well as adenylate cyclase type-III and the glial marker protein S-100, but these proteins may not be expressed in all crypt cells, and are not exclusive markers of this receptor neuron type (Belanger et al., 2003; Germana et al., 2004; Hansen et al., 2004; Hansen et al., 2003; Vielma et al., 2008). An interesting recent study in zebrafish showed that nearly all crypt cells express *ora4*, a single member of the V1R receptor-like *ora* genes, and the G-protein Gi_{1b} (Oka et al., 2012; see also Saraiva and Korsching, 2007). However, the physiological implications of this intriguing finding remain unclear.

Crypt cells were shown to project their thin unmyelinated axons to small, restricted patches of the ventral olfactory bulb in catfish (Hansen et al., 2003) and crucian carp (Hamdani and Døving, 2006). The latter study also reported that second-order olfactory bulb neurons, which make synaptic contacts with crypt cells, project to the olfactory cortex primarily through the lateral bundle of the medial olfactory tract, which conveys olfactory information related to reproduction in crucian carp (Hamdani and Døving, 2006; Hamdani and Døving, 2007; Weltzien et al., 2003). Similarly, the medial olfactory tract of male goldfish responds to sex hormones released by females of this species, coordinating spawning between both sexes (Sorensen et al., 1991). Further studies in crucian carp reported a striking dependence of crypt cell density and apical localization

BRAIN RESEARCH 1487 (2012) 3–15

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Review

Connexin and pannexin hemichannels in inflammatory responses of glia and neurons

Michael V.L. Bennett[a,*], Juan M. Garré[a,1], Juan A. Orellana[b,2], Felix F. Bukauskas[a], Maiken Nedergaard[c], Juan C. Sáez[a,b,d,e]

[a]Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[b]Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago, Chile
[c]Division of Glial Disease and Therapeutics, Center for Translational Neuromedicine, Department of Neurosurgery, University of Rochester, Rochester, NY 14642, USA
[d]Instituto Milenio, Centro Interdisciplinario de Neurociencias de Valparaíso, Valparaíso, Chile
[e]Center of Interdisciplinary Research in Biology, Collège de France, Paris, France

ARTICLE INFO

Article history:
Accepted 22 August 2012
Available online 10 September 2012

Keywords:
Connexin
Pannexin
Hemichannel
FGF-1
Astrocyte
Microglia
Inflammation
Neuronal death
Connexon
Pannexon

ABSTRACT

Mammals express ~20 different connexins, the main gap junction forming proteins in mammals, and 3 pannexins, homologs of innexins, the main gap junction forming proteins in invertebrates. In both classes of gap junction, each channel is formed by two hemichannels, one contributed by each of the coupled cells. There is now general, if not universal, agreement that hemichannels of both classes can open in response to various physiological and pathological stimuli when they are not apposed to another hemichannels and face the external milieu. Connexin (and likely pannexin) hemichannel permeability is consistent with that of the cell–cell channels and open hemichannels can be a release site for relatively large molecules such as ATP and glutamate, which can serve as transmitters between cells. Here we describe three experimental paradigms in which connexin and pannexin hemichannel signaling occurs. (1) In cultures of spinal astrocytes FGF-1 causes the release of ATP, and ATP causes opening of pannexin hemichannels, which then release further ATP. Subsequently, several hours later, connexin hemichannels are also opened by an unknown mechanism. Release of ATP appears to become self sustaining through action of P2X7 receptors to open pannexin hemichannels and then connexin hemichannels, both of which are ATP permeable. (2) Spinal cord injury by dropping a small weight on the exposed cord is followed by release of ATP in the region surrounding the primary lesion. This release is greatly reduced in a mouse in which Cx43 is knocked down in the astrocytes. Application of FGF-1 causes a similar release of ATP in the uninjured spinal cord, and an inhibitor of the FGF-1 receptor, PD173074, inhibits both FGF-1 and injury-induced release. Reduction in ATP release is associated with reduced inflammation and less secondary expansion of the lesion. (3) Cortical astrocytes in culture are permeabilized by hypoxia, and this effect is increased by high or zero glucose. The mechanism of permeabilization is opening of Cx43 hemichannels, which can lead to cell

*Corresponding author. Fax: +1 718 430 8932.
E-mail address: michael.bennett@einstein.yu.edu (M.V.L. Bennett).
[1] Present address: Department of Anesthesiology, New York University School of Medicine, New York, NY 10016, USA.
[2] Departamento de Neurología, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile

0006-8993/$ - see front matter
http://dx.doi.org/10.1016/j.brainres.2012.08.042

J Neural Transm (2012) 119:173–195
DOI 10.1007/s00702-011-0731-5

DEMENTIAS - REVIEW ARTICLE

Recent rodent models for Alzheimer's disease: clinical implications and basic research

Nady Braidy · Pablo Muñoz · Adrian G. Palacios · Gloria Castellano-Gonzalez · Nibaldo C. Inestrosa · Roger S. Chung · Perminder Sachdev · Gilles J. Guillemin

Received: 26 May 2011 / Accepted: 24 October 2011 / Published online: 16 November 2011

Abstract Alzheimer's disease (AD) is the most common origin of dementia in the elderly. Although the cause of AD remains unknown, several factors have been identified that appear to play a critical role in the development of this debilitating disorder. In particular, amyloid precursor protein (APP), tau hyperphosphorylation, and the secretase enzymes, have become the focal point of recent research. Over the last two decades, several transgenic and non-transgenic animal models have been developed to elucidate the mechanistic aspects of AD and to validate potential therapeutic targets. Transgenic rodent models over-expressing human β-amyloid precursor protein (β-APP) and mutant forms of tau have become precious tools to study and understand the pathogenesis of AD at the molecular, cellular and behavioural levels, and to test new therapeutic agents. Nevertheless, none of the transgenic models of AD recapitulate fully all of the pathological features of the disease. *Octodon degu*, a South American rodent has been recently found to spontaneously develop neuropathological signs of AD in old age. This review aims to address the limitations and clinical relevance of transgenic rodent models in AD, and to highlight the potential for *O. degu* as a natural model for the study of AD neuropathology.

Keywords Alzheimer's disease · Animal models · *Octodon degu* · Amyloid-β · Tau phosphorylation · Transgenic models

N. Braidy · G. J. Guillemin (✉)
Department of Pharmacology, University of New South Wales, Sydney, NSW 2052, Australia
e-mail: g.guillemin@unsw.edu.au

N. Braidy · P. Sachdev
School of Psychiatry, University of New South Wales, Sydney, Australia

P. Muñoz · A. G. Palacios
Centro Interdiciplinario de Neurociencia de Valparaiso (CINV), Facultad de Ciencias, Universidad de Valparaiso, Valparaiso, Chile

G. Castellano-Gonzalez · G. J. Guillemin
St Vincent's Centre for Applied Medical Research, Sydney, Australia

N. C. Inestrosa
Centre for Aging and Regeneration (CARE), Faculty of Biological Sciences, P. Catholic University of Chile, Santiago, Chile

R. S. Chung
Menzies Research Institute, University of Tasmania, Hobart, Australia

P. Sachdev
Neuropsychiatric Institute, Prince of Wales Hospital, Sydney, Australia

Introduction

Alzheimer's disease (AD) is the most common cause of dementia, characterised by progressive memory loss and neurodegeneration in the cerebral cortex (Maccioni et al. 2001). The two pathological hallmarks of AD are neurofilament tangles (NFT) and neuritic plaques. NFT are intracellular twisted nerve cell fibers composed of hyperphosphorylated tau, a low molecular weight microtubule-associated protein (Glenner and Wong 1984). The core component of plaques is β amyloid (Aβ) (Glenner and Wong 1984).

Aβ peptides are typically $\sim$4 kDa β-pleated sheet peptides with different N- and C-terminal endings that are

ELSEVIER

Archives of Medical Research 43 (2012) 645–654

Archives of Medical Research

REVIEW ARTICLE

Role of Tau Protein in Neuronal Damage in Alzheimer's Disease and Down Syndrome

Ana M. Cárdenas,[a] Alvaro O. Ardiles,[a] Natalia Barraza,[a] Ximena Baéz-Matus,[a] and Pablo Caviedes[b]

[a]*Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso, Valparaíso, Chile*
[b]*Programa de Farmacología Molecular y Clínica, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile*

Received for publication October 9, 2012; accepted October 22, 2012 (ARCMED-D-12-00565).

Neurodegenerative disorders constitute a growing concern worldwide. Their incidence has increased steadily, in particular among the elderly, a high-risk population that is becoming an important segment of society. Neurodegenerative mechanisms underlie many ailments such as Parkinson's disease, Huntington's disease, Alzheimer's disease (AD) and Down syndrome (DS, trisomy 21). Interestingly, there is increasing evidence suggesting that many such diseases share pathogenic mechanisms at the cellular and subcellular levels. These include altered protein misfolding, impaired autophagy, mitochondrial dysfunction, membrane damage, and altered axonal transport. Regarding AD and DS, the first common link comes from observations that DS patients undergo AD-like pathology early in adulthood. Also, the gene encoding for the amyloid precursor protein is present in human autosome 21 and in murine chromosome 16, an animal model of DS. Important functions related to preservation of normal neuronal architecture are impaired in both conditions. In particular, the stable assembly of microtubules, which is critical for the cytoskeleton, is impaired in AD and DS. In this process, tau protein plays a pivotal role in controlling microtubule stability. Abnormal tau expression and hyperphosphorylation are common features in both conditions, yet the mechanisms leading to these phenomena remain obscure. In the present report we review possible common mechanisms that may alter tau expression and function, in particular in relation to the effect of certain overexpressed DS-related genes, using cellular models of human DS. The latter contributes to the identification of possible therapeutic targets that could aid in the treatment of both AD and DS. © 2012 IMSS. Published by Elsevier Inc.

Key Words: Down syndrome, Alzheimer's disease, Tau protein, Amyloid precursor protein, Dyrk1A, Rcan.

Introduction

In neurons, the cytoskeleton constitutes a complex, dynamic and pivotal structure that not only shapes the neuronal architecture, but also plays an essential role in different functions and properties of neurons. These include vesicle transport, transmitter release, neurite elongation, synapse formation, and cone growth as well as regulation of plasticity. Thus, malfunction of this important cytoskeleton network results in common pathophysiological mechanisms that underlie severe diseases such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD) and Down syndrome (DS). A critical process in such functions depends on the correct, stable assembly of microtubules where tau protein, a microtubule-associated protein (MAP) abundantly expressed in axons, plays a critical role. In the present paper we present evidence of abnormal tau expression and function as a possible mechanism underlying these ailments.

Address reprint requests to: Ana M. Cárdenas, Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso, Gran Bretaña 1111, Playa Ancha, Valparaíso, Chile; Phone: 56-32-250 8052; FAX: 56-32-250 8027; E-mail: ana.cardenas@uv.cl

Tau Structure and Its Role in Microtubule Dynamics

Tau is a cytosolic protein encoded by a gene located on the long arm of human chromosome 17 in band 17q21. The

0188-4409/$ - see front matter. Copyright © 2012 IMSS. Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.arcmed.2012.10.012

J Membrane Biol (2012) 245:423–436
DOI 10.1007/s00232-012-9485-8

REVIEW ARTICLES

Connexin- and Pannexin-Based Channels in Normal Skeletal Muscles and Their Possible Role in Muscle Atrophy

Luis A. Cea · Manuel A. Riquelme · Bruno A. Cisterna · Carlos Puebla · José L. Vega · Maximiliano Rovegno · Juan C. Sáez

Received: 27 April 2012 / Accepted: 28 June 2012 / Published online: 1 August 2012

Abstract Precursor cells of skeletal muscles express connexins 39, 43 and 45 and pannexin1. In these cells, most connexins form two types of membrane channels, gap junction channels and hemichannels, whereas pannexin1 forms only hemichannels. All these channels are low-resistance pathways permeable to ions and small molecules that coordinate developmental events. During late stages of skeletal muscle differentiation, myofibers become innervated and stop expressing connexins but still express pannexin1 hemichannels that are potential pathways for the ATP release required for potentiation of the contraction response. Adult injured muscles undergo regeneration, and connexins are reexpressed and form membrane channels. In vivo, connexin reexpression occurs in undifferentiated cells that form new myofibers, favoring the healing process of injured muscle. However, differentiated myofibers maintained in culture for 48 h or treated with proinflammatory cytokines for less than 3 h also reexpress connexins and only form functional hemichannels at the cell surface. We propose that opening of these hemichannels contributes to drastic changes in electrochemical gradients, including reduction of membrane potential, increases in intracellular free Ca^{2+} concentration and release of diverse metabolites (e.g., NAD^{+} and ATP) to the extracellular milieu, contributing to multiple metabolic and physiologic alterations that characterize muscles undergoing atrophy in several acquired and genetic human diseases. Consequently, inhibition of connexin hemichannels expressed by injured or denervated skeletal muscles might reduce or prevent deleterious changes triggered by conditions that promote muscle atrophy.

Keywords Gap junction · Cell–cell channel · Physiology of calcium channels in muscle · Pharmacology of muscle diseases

L. A. Cea · M. A. Riquelme · B. A. Cisterna · C. Puebla · J. L. Vega · M. Rovegno · J. C. Sáez (✉)
Departamento de Fisiología, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile
e-mail: jcsaezc@gmail.com

L. A. Cea
e-mail: lacea@uc.cl

C. Puebla · J. C. Sáez
Centro Interdisciplinario de Neurociencias de Valparaíso, Valparaiso, Chile

J. L. Vega
Laboratorio de Fisiología Experimental, Universidad de Antofagasta, Antofagasta, Chile

Connexin- and Pannexin-Based Channels

Connexins (Cxs) and pannexins (Panxs) constitute two families of integral membrane proteins that, in mammals, are composed of about 20 and 3 members, respectively. In most cells studied thus far, the pattern of Cx expression varies according to the species, cell type and physiological state (Gorbe et al. 2005; Račkauskas et al. 2010; Bedner et al. 2011). Similarly, Panxs are expressed in many different cell types, but Panx2 has been detected preferentially in the nervous system of vertebrate animals (Bruzzone et al. 2003; Li et al. 2011; Ray et al. 2006).

Since several investigators have been unable to find evidence of gap junctions formed by Panxs, it was recently proposed that Panxs only form hemichannels (HCs); thus, it was recommended to call them Panx channels (Sosinsky et al. 2011). However, Panx gap junction channels (GJCs) have been observed in exogenous expression systems, including *Xenopus* oocytes and mammalian cells, as well as

Chapter 1
Spike Train Statistics From Empirical Facts To Theory: The Case Of The Retina.

Bruno Cessac and Adrian Palacios

Abstract This chapter focuses on methods from statistical physics and probability theory allowing the analysis of spike trains in neural networks. Taking as an example the retina we present recent works attempting to understand how retina ganglion cells encode the information transmitted to the visual cortex via the optical nerve, by analyzing their spike train statistics. We compare the maximal entropy models used in the literature of retina spike train analysis to rigorous results establishing the exact form of spike train statistics in conductance-based Integrate-and-Fire neural networks.

This chapter is done in the spirit of the course "Neuronal dynamics", given at the Master of Computational Biology, University of Nice, aiming at showing how a specific problem in neuroscience can be addressed on theoretical grounds, and how it can be related to experimental methods and results. As a consequence, this chapter contains both recent biological results and mathematical developments.

1.1 Introduction

Given a stimulus from the external world (visual scene, sound, smell, ...) biological sensors at the periphery of the nervous system are able to transduce the physical manifestations of this stimulus (light emission, air pressure variations, chemical concentrations) into sequences of action potentials (spike trains), which propagate through the nervous system. Then, the brain is able to *analyze* those spike trains and infer crucial information on the nature of the stimulus. Critical - yet unsolved - questions in neuroscience are How is the physical signal encoded by the nervous system ? How does the brain analyze the spike trains ? What are the underlying computational *coding* principles ? At the current stage of scientific knowledge, answering those questions is still a challenge for biology and computational neuroscience.

Among sensory systems the retina provides functionality such as detection of movement, orientation, temporal and spatial prediction, response to flash omissions and contrast, that were up to recently viewed as the exclusive duty of higher brain centers [22]. The retina is an accessible part of the brain [13] and a prominent system to study the neurobiology and the underlying computational capacity of the neural coding. As a matter of fact, there is currently a wide research activity in understanding how the retina encodes visual information. However, basic questions are still open, such as: Are the ganglion cells (which send spikes from the eyes to the brain via the optical nerve), independent signal-encoders or are neural correlations important for coding a visual scene, and how to interpret them ?

B. Cessac
INRIA NeuroMathComp, 2004 Route des Lucioles, 06902 Sophia-Antipolis, France. e-mail: bruno.cessac@inria.fr

A. G. Palacios
CINV-Centro Interdisciplinario de Neurociencia de Valparaiso, Universidad de Valparaiso, Harrington 287, Valparaiso 2360102, Chile. e-mail: adrian.palacios@uv.cl

Modulation of BK channel voltage gating by different auxiliary β subunits

Gustavo F. Contreras[a,b], Alan Neely[a], Osvaldo Alvarez[a,c], Carlos Gonzalez[a,1], and Ramon Latorre[a,1]

[a]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, and [b]Doctorado en Ciencias mención Neurociencia, Universidad de Valparaíso, Valparaíso 2366103, Chile; and [c]Facultad de Ciencias, Universidad de Chile, Santiago 7800003, Chile

Contributed by Ramon Latorre, October 1, 2012 (sent for review September 5, 2012)

Calcium- and voltage-activated potassium channels (BK) are regulated by a multiplicity of signals. The prevailing view is that different BK gating mechanisms converge to determine channel opening and that these gating mechanisms are allosterically coupled. In most instances the pore forming α subunit of BK is associated with one of four alternative β subunits that appear to target specific gating mechanisms to regulate the channel activity. In particular, β1 stabilizes the active configuration of the BK voltage sensor having a large effect on BK Ca^{2+} sensitivity. To determine the extent to which β subunits regulate the BK voltage sensor, we measured gating currents induced by the pore-forming BK α subunit alone and with the different β subunits expressed in *Xenopus* oocytes (β1, β2IR, β3b, and β4). We found that β1, β2, and β4 stabilize the BK voltage sensor in the active conformation. β3 has no effect on voltage sensor equilibrium. In addition, β4 decreases the apparent number of charges per voltage sensor. The decrease in the charge associated with the voltage sensor in α β4 channels explains most of their biophysical properties. For channels composed of the α subunit alone, gating charge increases slowly with pulse duration as expected if a significant fraction of this charge develops with a time course comparable to that of K^+ current activation. In the presence of β1, β2, and β4 this slow component develops in advance of and much more rapidly than ion current activation, suggesting that BK channel opening proceeds in two steps.

kinetic model | modulatory beta subunits

The open probability of large conductance Ca^{2+}- and voltage-activated K^+ (BK) channels increases when confronted with a membrane depolarization or an increase in the intracellular Ca^{2+} concentration (1–3). The BK pore-forming α subunit is coded by a single gene (*Slo1; KCNMA1*) and yet, it displays a variety of phenotypes in different cells and tissues as a consequence of alternative splicing, metabolic regulation, and modulation by β subunits. This great diversity of BK channels is fundamental to the adequate function of many tissues. In particular, β subunits are associated with BK channels in most tissues where they are present and dramatically modify their gating properties (4).

At present, four β subunits have been cloned in mammals (β1–β4) (4–10). BK β subunits have two transmembrane segments joined together by a loop (~148-aa residues). The external loop, and N and C termini are intracellular. Sequence similarities are major between β1–β2 and β2–β3, respectively. β4 is the most distantly related of all β subunits. β1 and β2 subunits induce an increase of the apparent Ca^{2+} sensitivity and a slowing of the macroscopic kinetics (4, 7, 8). β2 also induces fast and complete inactivation (6, 10, 11) and an instantaneous outward rectification that suggests that the β2 external loop approaches the BK pore as to alter ion conduction (12). Four splice variants of β3 have been identified, β3a–c. β3b induces fast and partial inactivation of BK currents and also produces an outward rectification of the open channel currents (10, 13). Outward rectification is regulated by the extracellular segment of this subunit (14). β4 has a complex Ca^{2+} concentration-dependent effect on BK channel gating. This subunit decreases apparent Ca^{2+} sensitivity at low Ca^{2+} concentrations but induces an increase in the apparent sensitivity at high Ca^{2+} concentrations (8, 14–18). β4 also slows down activation and deactivation kinetics (7, 8).

Despite the fact that the BK phenotype produced by each of the β subunits has been well characterized, controversies exist regarding the biophysical mechanisms by means of which these auxiliary subunits modify BK channel gating (19–23). Since the finding of Bao and Cox (19) that the main effect of the β1 subunit is to alter the equilibrium between the resting and active configurations of the voltage sensor, several important questions remain to be answered: To what extent do β2 and β4 alter BK channel voltage sensing? Does the slow component of the OFF gating charge detected by Horrigan et al. (24) in channels formed by the α subunit alone parallel the slowing down in the I_K activation induced by β1, β2, and β4 as predicted by the two-tiered allosteric model? To assay potential effects of the β subunits on the workings of the voltage sensor, we measured gating currents (I_g) induced by channels formed by the pore-forming BK α subunit alone and with the different β subunits expressed on *Xenopus* oocytes (β1, β2IR, β3b, and β4).

Results

Characterization of BK Gating Currents in the Presence of β Subunits. We first measured the macroscopic K^+ currents (I_K) in the cell-attached configuration to confirm that BK channels were formed by the expected α or α/β complex (Fig. S1). In cell-attached configuration the large size of the ionic currents prevented adequate voltage clamp. Therefore, we lowered K^+ to record current in symmetrical 1-mM K^+ (Fig. 1, *Left*) in excised patches. Notice in Fig. 1 *B*, *C*, and *E* that the time course of the K^+ currents of channels formed by α/β1, α/β2, and α/β4 were much slower than the ones of channels formed by the α subunit alone (Fig. 1*A*). The slowing of the K^+ current by coexpression of the these β subunits is one of the hallmarks of the phenotype of channels formed by α/β*x* (*x* = 1,2,4). For β3, (α/β3) BK channels were identified by a small, fast, and incomplete inactivation process (Fig. 1*D*).

After patch excision, I_g currents were measured in conditions of 0 K^+ and 0 Ca^{2+} by profusely perfusing the internal side of the macropatch with a K^+-free solution (Fig. 1, *Right*). Gating current recordings reveal fast time courses with time constants, which were not very different between the different subunit combinations. In addition, the gating currents develop fully before channel opening (e.g., Fig. S1). These results show that in BK channels voltage sensor activation occurs before channel opening, as previously reported for the cases of α and α/β1 BK channels (19, 25, 26). In other words, in the absence of Ca^{2+}, BK gating charge movement takes place between several closed states.

Families of I_g as those shown in Fig. 1, *Right* evoked at different voltages (−90 to 350 mV) were integrated between the beginning and the end of the pulse to obtain the gating charge activation relationships, Q_{ON}–V and Q_{OFF}–V. For all of the different types of channels tested and for voltage pulses of 1 ms in

Author contributions: G.F.C., A.N., C.G., and R.L. designed research; G.F.C. and C.G. performed research; G.F.C., A.N., O.A., C.G., and R.L. analyzed data; and O.A., C.G., and R.L. wrote the paper.

The authors declare no conflict of interest.

[1]To whom correspondence may be addressed. E-mail: ramon.latorre@uv.cl or carlos.gonzalezl@uv.cl.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1216953109/-/DCSupplemental.

PHYSIOLOGY

THYROID
Volume 22, Number 9, 2012

DOI: 10.1089/thy.2010.0400

Hypothyroidism in the Adult Rat Causes Incremental Changes in Brain-Derived Neurotrophic Factor, Neuronal and Astrocyte Apoptosis, Gliosis, and Deterioration of Postsynaptic Density

Claudia Cortés,[1] Eliseo Eugenin,[2] Esteban Aliaga,[3] Leandro J. Carreño,[4] Susan M. Bueno,[4] Pablo A. Gonzalez,[4] Silvina Gayol,[5] David Naranjo,[5] Verónica Noches,[1] Michelle P. Marassi,[6] Doris Rosenthal,[6] Cindy Jadue,[1] Paula Ibarra,[1] Cecilia Keitel,[7] Nelson Wohllk,[7,8] Felipe Court,[9] Alexis M. Kalergis,[4,10] and Claudia A. Riedel[1]

Background: Adult hypothyroidism is a highly prevalent condition that impairs processes, such as learning and memory. Even though tetra-iodothyronine (T_4) treatment can overcome the hypothyroidism in the majority of cases, it cannot fully recover the patient's learning capacity and memory. In this work, we analyzed the cellular and molecular changes in the adult brain occurring with the development of experimental hypothyroidism.
Methods: Adult male Sprague-Dawley rats were treated with 6-propyl-2-thiouracil (PTU) for 20 days to induce hypothyroidism. Neuronal and astrocyte apoptosis were analyzed in the hippocampus of control and hypothyroid adult rats by confocal microscopy. The content of brain-derived neurotrophic factor (BDNF) was analyzed using enzyme-linked immunosorbent assay (ELISA) and *in situ* hybridization. The glutamatergic synapse and the postsynaptic density (PSD) were analyzed by electron microscopy. The content of PSD proteins like tyrosine receptor kinase B (TrkB), p75, and N-methyl-D-aspartate receptor (NMDAr) were analyzed by immunoblot.
Results: We observed that the hippocampus of hypothyroid adult rats displayed increased apoptosis levels in neurons and astrocyte and reactive gliosis compared with controls. Moreover, we found that the amount of BDNF mRNA was higher in the hippocampus of hypothyroid rats and the content of TrkB, the receptor for BDNF, was reduced at the PSD of the CA3 region of hypothyroid rats, compared with controls. We also observed that the glutamatergic synapses from the stratum radiatum of CA3 from hypothyroid rats, contained thinner PSDs than control rats. This observation was in agreement with a reduced content of NMDAr subunits at the PSD in hypothyroid animals.
Conclusions: Our data suggest that adult hypothyroidism affects the hippocampus by a mechanism that alters the composition of PSD, reduces neuronal and astrocyte survival, and alters the content of the signaling neurotrophic factors, such as BDNF.

Introduction

THYROID HORMONES TRI-IODOTHYRONINE (T_3) and tetra-iodothyronine (T_4) are essential for appropriate brain development and function (1–5). Hypothyroidism is highly prevalent worldwide and is characterized by low plasma levels of T_4 and high plasma levels of thyroid stimulating hormone (TSH). Hypothyroidism in the adult reduces cell metabolism in almost all tissues of the body (6). At the brain level, adult hypothyroid patients show cognitive

[1]Millennium Institute on Immunology and Immunotherapy, Department of Biological Sciences, Faculty of Biological Sciences and Faculty of Medicine, Andrés Bello University, Santiago, Chile.
[2]Department of Pathology, Albert Einstein College of Medicine, Bronx, New York.
[3]Neurobiology and Plasticity Development Center, Department of Physiology; [5]Interdisciplinary Center of Neurosciences; University of Valparaíso, Valparaíso, Chile.
[4]Millennium Institute on Immunology and Immunotherapy, Department of Molecular Genetics and Microbiology, Faculty of Biological Sciences; [9]Millennium Nucleus for Regenerative Biology, Faculty of Biological Sciences; [10]Department of Rheumatology, Faculty of Medicine; Pontifical Catholic Universty of Chile, Santiago, Chile.
[6]Carlos Chagas Filho Institute of Biophysics, University of Rio de Janeiro, Rio de Janeiro, Brazil.
[7]Institute of Advanced Medical Studies, Santiago, Chile.
[8]Salvador Hospital, University of Chile, Santiago, Chile.

RESEARCH REPORTS
Biological

E. Couve[1,3]*, R. Osorio[1,3], and O. Schmachtenberg[2,3]

[1]Departamento de Biología y Cs. Ambientales, Laboratorio de Microscopía Electrónica; [2]Centro Interdisciplinario de Neurociencia de Valparaíso (CINV), Chile; and [3]Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile; *corresponding author, eduardo.couve@uv.cl

J Dent Res 91(7):696-701, 2012

Mitochondrial Autophagy and Lipofuscin Accumulation in Aging Odontoblasts

ABSTRACT

Aging of long-lived post-mitotic cells is characterized as a progressive and irreversible reduction of functional activity. In such cells, mitochondria are organelles critical for bioenergetic supply, whose turnover is mediated by an autophagic-lysosomal pathway. In human teeth, odontoblasts are post-mitotic cells responsible for sensory function and dentin preservation. Here, human odontoblasts were processed for immunohistochemistry with antibodies against mitochondrial (MTCO2) and lysosomal (LAMP2) markers, and comparatively analyzed in two age groups (young-adult and adult) with light and electron microscopy. Selective engulfment of mitochondrial profiles into autophagic vacuoles is common in young-adult odontoblasts, suggesting a microautophagic pathway. With age, the odontoblast layer is reduced in width, and mitochondrial elements converge around large clusters of autofluorescent lipofuscin deposits. Age-related changes in odontoblasts are observed as a long-term process in which the progressive accumulation of intralysosomal debris limits the autophagic turnover of mitochondrial components, causing an eventual decline in physiological cell functions, which leads to increased vulnerability under stress conditions.

KEY WORDS: microautophagy, dentin, human teeth, dental pulp, mitophagy, mitochondria.

DOI: 10.1177/0022034512449347

Received January 23, 2012; Last revision April 24, 2012; Accepted May 1, 2012

INTRODUCTION

Dentin tissue developed as a complex mechano- and thermosensory system early in vertebrate evolution (Smith and Sansom, 2000; Farahani *et al*., 2011). The cell responsible for dentin formation and maintenance is the odontoblast, a long-lived post-mitotic cell like neurons or cardiomyocytes (Terman *et al*., 2010). Odontoblasts are thought to participate in sensory transduction and transmit the fluid movements caused by mechano- and thermosensory stimuli within the dentin sensory system to nerve endings of the Raschkow plexus (Byers, 1984; Brannström, 1986). However, the precise role of odontoblasts in dentin sensory function remains unresolved (Son *et al*., 2009; Magloire *et al*., 2010; El Karim *et al*., 2011). In healthy human teeth, the prolonged existence of odontoblasts is dependent on organelle turnover by autophagy and is associated with an age-related decrease of dentin secretory activity (Murray *et al*., 2002; Couve and Schmachtenberg, 2011), but the molecular and cell physiological changes underlying this process are poorly understood.

The aging process of post-mitotic cells is generally accompanied by a diminished activity of the autophagic-lysosomal pathway, decreasing organelle turnover (Cuervo and Dice, 2000; Cuervo *et al*., 2005). In human odontoblasts, the aging process was shown to consist of a reduction of cell size, and a slow but progressive accumulation of undegradable lipofuscin deposits with time (Couve and Schmachtenberg, 2011). These changes are thought to decrease dentin formation, reduce functional responses to exogenous stimuli like caries, and altogether increase the probability of cell death (Bjorndal and Darvann, 1999; Murray *et al*., 2002; Mitsiadis *et al*., 2008). Mitochondria and lysosomes have been described as the main organelles relevant to age-related changes in post-mitotic cells, and the constitutive turnover of mitochondrial components by autophagy implies the irreversible accumulation of undegradable lipofuscin deposits in autophagic vacuoles (Brunk and Terman, 2002b; Terman *et al*., 2010).

During the past decade, it has become increasingly clear that mitochondria are not isolated functional compartments; instead, they form an intricate and dynamic functional network in most eukaryotic cells (Benard and Rossignol, 2008). Mitochondrial dynamics have an important impact on cellular physiology, homeostasis, and bioenergetics and may be especially important in long-lived post-mitotic cells due to an elevated oxygen demand (Benard *et al*., 2007). The maintenance of a bioenergetically efficient mitochondrial system depends on the autophagy of selective organelle domains to ensure the clearance of dysfunctional components, allowing for an adequate energy metabolism and cell survival (Terman *et al*., 2010).

Autophagy is a ubiquitous degradative process that is regulated through a lysosomal pathway (Klionsky and Emr, 2000; Levine and Yuan, 2005; Eskelinen and Saftig, 2009) and is strongly implicated within cellular survival mechanisms and aging processes (Cuervo *et al*., 2005; Lemasters, 2005; Terman *et al*.,

OPEN ACCESS Freely available online

PLoS one

Splicing of the rSlo Gene Affects the Molecular Composition and Drug Response of Ca^{2+}-Activated K^+ Channels in Skeletal Muscle

Maria Maddalena Dinardo[1☯], Giulia Camerino[1☯], Antonietta Mele[1], Ramon Latorre[2], Diana Conte Camerino[1], Domenico Tricarico[1]*

1 Departments of Pharmacobiology, Faculty of Pharmacy, University of Bari, Bari, Italy, **2** Centro Interdisciplinario de Neurociencias de Valparaíso, Facultad de Ciencias, Universidad de Valparaiso, Valparaiso, Chile

Abstract

The molecular composition and drug responses of calcium-activated K^+ (BK) channels of skeletal muscle are unknown. Patch-clamp experiments combined with transcript scanning of the Kcnma1 gene encoding the alpha subunit of the BK channel were performed in rat slow-twitch soleus (Sol) and fast-twitch flexor digitorum brevis (FDB) skeletal muscles. Five splicing products of the Kcnma1 gene were isolated from Sol and FDB: the e17, e22, +29 aa, Slo27 and Slo0 variants. RT-PCR analysis demonstrated that the expression of e22 and Slo0 were 80–90% higher in FDB than Sol, whereas the expression of Slo27 was 60% higher in Sol than FDB, and the +29 aa variant was equally expressed in both muscle types. No beta 1-4 subunits were detected. In Sol, a large BK current with low Ca^{2+} sensitivity was recorded. The BK channel of Sol also showed a reduced response to BK channel openers, such as NS1619, acetazolamide and related drugs. In FDB, a reduced BK current with high Ca^{2+} sensitivity and an enhanced drug response was recorded. The total BK RNA content, which was 200% higher in Sol than in FDB, correlated with the BK currents in both muscles. Drug responses primarily correlated with e22 and Slo0 expression levels in FDB and to Slo27 expression in Sol muscle. In conclusion, phenotype-dependent BK channel biophysical and pharmacological properties correlated with the expression levels of the variants in muscles. These findings may be relevant to conditions affecting postural muscles, such as prolonged bed-rest, and to diseases affecting fast-twitch muscles, such as periodic paralysis. Down-regulation or up-regulation of the variants associated with pathological conditions may affect channel composition and drug responses.

Citation: Dinardo MM, Camerino G, Mele A, Latorre R, Conte Camerino D, et al. (2012) Splicing of the rSlo Gene Affects the Molecular Composition and Drug Response of Ca^{2+}-Activated K^+ Channels in Skeletal Muscle. PLoS ONE 7(7): e40235. doi:10.1371/journal.pone.0040235

Editor: Bradley Steven Launikonis, University of Queensland, Australia

Received January 9, 2012; **Accepted** June 3, 2012; **Published** July 10, 2012

Funding: This work was supported by Telethon GGP10101 (Pi of grant); grant Fondecyt 1110430 and the Centro Interdisciplinario de Neurociencia de Valparaíso is a Millennium Scientific Institute. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: dtricarico@farmbiol.uniba.it

☯ These authors contributed equally to this work.

Introduction

Ca^{2+}-activated K^+ channels (BK), which are present in virtually every cell, couple chemical signaling to electrical signaling [1 2]. All BK channels are activated by increases in the concentration of intracellular Ca^{2+} ions, and many can be modulated by other messengers, such as protein kinases, phosphatases, and G proteins [3 8]. By damping excitatory stimuli mediated by the entry and/or the release of Ca^{2+} from internal stores, BK channels control diverse physiological processes, including the regulation of vascular tone [9 12], neuronal excitability [13 14], neurotransmitter release [15 16], endocrine function [17 19], innate immunity [20], and hearing [21 22].

BK channels in native tissues exhibit a physiologically diverse array of phenotypes. At least three major post-transcriptional mechanisms are involved in generating such functional diversity: the alternative pre-mRNA splicing of the BK channel pore-forming alpha-subunit; the assembly of alpha-subunits with a family of modulatory beta-subunits; and metabolic regulation (e.g., phosphorylation). A BK channel assembles as tetramers of the pore-forming alpha -subunit and is encoded by a single gene (Kcnma1) [23].

Electrophysiological recordings in native cells have revealed Slo1 channels with different calcium sensitivities. However, the Slo1 channel is encoded by a single gene in mammals. This channel diversity is possibly due to the alternative processing of introns, which produce at least 11 splice variants expressed in different tissues and cell types [24]. This feature is evolutionarily conserved and is observed in mammals, reptiles, birds and insects [23 31]. When expressed in heterologous expression systems, channels formed by these splice variants present different calcium sensitivities and gating kinetics, resembling those found in native cells. Alternative splicing is responsible in part for the great variety of calcium sensitivities among Slo1 channels. Several of these splice variants are produced by "insertions" at the C-terminus, and one of the most studied variants is expressed under the activation of the hypothalamic-pituitary-adrenal axis (HP) [32 33]. Two splice variants produce dominant-negative subunits, which retain the channel in subcellular compartments [34 35]. One of these variants

J Neuroimmune Pharmacol (2012) 7:499–518
DOI 10.1007/s11481-012-9352-5

INVITED REVIEW

The Role of Gap Junction Channels During Physiologic and Pathologic Conditions of the Human Central Nervous System

Eliseo A. Eugenin · Daniel Basilio · Juan C. Sáez · Juan A. Orellana · Cedric S. Raine · Feliksas Bukauskas · Michael V. L. Bennett · Joan W. Berman

Received: 23 February 2012 / Accepted: 28 February 2012 / Published online: 23 March 2012

Abstract Gap junctions (GJs) are expressed in most cell types of the nervous system, including neuronal stem cells, neurons, astrocytes, oligodendrocytes, cells of the blood brain barrier (endothelial cells and astrocytes) and under inflammatory conditions in microglia/macrophages. GJs connect cells by the docking of two hemichannels, one from each cell with each hemichannel being formed by 6 proteins named connexins (Cx). Unapposed hemichannels (uHC) also can be open on the surface of the cells allowing the release of different intracellular factors to the extracellular space. GJs provide a mechanism of cell-to-cell communication between adjacent cells that enables the direct exchange of intracellular messengers, such as calcium, nucleotides, IP_3, and diverse metabolites, as well as electrical signals that ultimately coordinate tissue homeostasis, proliferation, differentiation, metabolism, cell survival and death. Despite their essential functions in physiological conditions, relatively little is known about the role of GJs and uHC in human diseases, especially within the nervous system. The focus of this review is to summarize recent findings related to the role of GJs and uHC in physiologic and pathologic conditions of the central nervous system.

Keywords Connexin · Hemichannels · NeuroAIDS · HIV · Alzheimer · Disease

Electronic supplementary material The online version of this article (doi:10.1007/s11481-012-9352-5) contains supplementary material, which is available to authorized users.

E. A. Eugenin (✉) · C. S. Raine · J. W. Berman
Department of Pathology,
F727, Albert Einstein College of Medicine,
1300 Morris Park Ave.,
Bronx, NY 10461, USA
e-mail: eliseo.eugenin@einstein.yu.edu

D. Basilio · F. Bukauskas · M. V. L. Bennett
Department of Neuroscience, Albert Einstein College of Medicine,
Bronx, NY, USA

J. C. Sáez
Centro Interdisciplinario de Neurociencias de Valparaíso,
Instituto Milenio,
Valparaíso, Chile

J. A. Orellana
Departamento de Neurología, Facultad de Medicina,
Pontificia Universidad Católica de Chile,
Santiago, Chile

J. W. Berman
Department of Microbiology and Immunology,
Albert Einstein College of Medicine,
Bronx, NY, USA

C. S. Raine
Department of Neurology, Albert Einstein College of Medicine,
Bronx, NY, USA

E. A. Eugenin
Public Health Research Institute (PHRI) and Department
of Microbiology and Molecular Genetics, UMDNJ,
Newark, USA

J. C. Sáez
Departmento de Fisiología,
Pontificia Universidad Católica de Chile,
Santiago, Chile

JBC Papers in Press. Published on October 9, 2012 as Manuscript M112.383281
The latest version is at http://www.jbc.org/cgi/doi/10.1074/jbc.M112.383281

Permeation of calcium through purified connexin 26 hemichannels

Mariana C. Fiori[1], Vania Figueroa[2], Maria E. Zoghbi[1], Juan C. Saéz[2], Luis Reuss[1], and Guillermo A. Altenberg[1,*]

[1]Department of Cell Physiology and Molecular Biophysics, and Center for Membrane Protein Research Texas Tech Health Sciences Center, Lubbock, TX 79430-6551
[2]Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago, Chile, and Instituto Núcleo Milenio, Centro Interdisciplinario de Neurociencias de Valparaiso, Valparaiso, Chile

Running title: *Hemichannel calcium permeability*

[*]To whom correspondence should be addressed: Guillermo A Altenberg, Department of Cell Physiology and Molecular Biophysics, Texas Tech Health Sciences Center, Lubbock, TX 79430-6551, USA, Tel.: (806) 743-2531; E-mail: g.altenberg@ttuhsc.edu

Keywords: gap-junction channel; fluorescence; deafness; connexon; liposomes; sodium

Background: Indirect evidence suggests that connexin hemichannels are permeable to Ca^{2+}, but direct demonstration is lacking.
Results: Calcium moves into liposomes containing purified Cx26 in response to a concentration gradient.
Conclusion: Cx26 hemichannels are permeable to Ca^{2+}.
Significance: Cx26 hemichannels may play a role in Ca^{2+} influx into cells under conditions that lead to hemichannel activation, such as ischemic damage.

Gap-junction channels communicate the cytoplasms of two cells and are formed by head-to-head association of two hemichannels, one from each of the cells. Gap-junction channels and hemichannels are permeable to ions and hydrophilic molecules of up to *Mr* 1,000, including second messengers and metabolites. Intercellular Ca^{2+} signaling can occur by movement of a number of second messengers, including Ca^{2+}, through GJCs, or by a paracrine pathway that involves activation of purinergic receptors in neighboring cells following ATP release through hemichannels. Understanding Ca^{2+} permeation through Cx26 hemichannels is important to assess the role of gap-junction channels and hemichannels in health and disease. In this context, it is possible that increased Ca^{2+} influx through hemichannels under ischemic conditions contributes to cell damage. Previous studies suggest Ca^{2+} permeation through hemichannels, based on indirect arguments. Here, we demonstrate for the first time hemichannel permeability to Ca^{2+} by measuring Ca^{2+} transport through purified Cx26 hemichannels reconstituted in liposomes. We trapped the low-affinity Ca^{2+}-sensitive fluorescent probe Fluo-5N into the liposomes and followed the increases in intraliposomal $[Ca^{2+}]$ in response to an imposed $[Ca^{2+}]$ gradient. We show that Ca^{2+} does move through Cx26 hemichannels, and that the permeability of the hemichannels to Ca^{2+} is high, similar to that for Na^{+}. We suggest that HCs can be a significant pathway for Ca^{2+} influx into cells under conditions such as ischemia.

Gap-junction channels (GJCs)[4] are aqueous channels that communicate the cytoplasms of adjacent cells (1-3). They are formed by head-to-head association of hemichannels (HCs, connexin hexamers, connexons), one from each of the neighboring cells, that are permeable to ions and hydrophilic molecules of up to *Mr* 1,000 (1-3).

In addition to their known permeability to inorganic monovalent ions, it is well established that the GJC and HC pores are sufficiently large to allow permeation of many larger compounds including cAMP, cGMP, IP_3, ATP and glucose, as well as Ca^{2+} (4-8). GJCs and HCs display

COMPREHENSIVE PHYSIOLOGY

K^+ Channels: Function-Structural Overview

Carlos González,[1] David Baez-Nieto,[1] Ignacio Valencia,[1] Ingrid Oyarzún,[1] Patricio Rojas,[2] David Naranjo,[1] and Ramón Latorre[1]*

ABSTRACT

Potassium channels are particularly important in determining the shape and duration of the action potential, controlling the membrane potential, modulating hormone secretion, epithelial function and, in the case of those K^+ channels activated by Ca^{2+}, damping excitatory signals. The multiplicity of roles played by K^+ channels is only possible to their mammoth diversity that includes at present 70 K^+ channels encoding genes in mammals. Today, thanks to the use of cloning, mutagenesis, and the more recent structural studies using x-ray crystallography, we are in a unique position to understand the origins of the enormous diversity of this superfamily of ion channels, the roles they play in different cell types, and the relations that exist between structure and function. With the exception of two-pore K^+ channels that are dimers, voltage-dependent K^+ channels are tetrameric assemblies and share an extremely well conserved pore region, in which the ion-selectivity filter resides. In the present overview, we discuss in the function, localization, and the relations between function and structure of the five different subfamilies of K^+ channels: (a) inward rectifiers, Kir; (b) four transmembrane segments-2 pores, K_{2P}; (c) voltage-gated, Kv; (d) the Slo family; and (e) Ca^{2+}-activated SK family, SKCa. © 2012 American Physiological Society. *Compr Physiol* 2:2087-2149, 2012.

Introduction

It is most probable that K^+ channels started to evolve from the moment that life appeared on earth, as the presence of more than 200 potassium channel-related proteins in archea and bacteria attest. Once K^+ channels were identified in bacteria (485), the dream of many biophysicists, to have large quantities of channel protein to produce crystals amenable to x-ray analysis, became a reality. This feat was performed by MacKinnon's group (115) when they crystallized the K^+ channel (KcsA) from the bacterium *Streptomyces lividans*. This primitive K^+ channel is a tetramer composed of four identical subunits consisting in two transmembrane (TM) domains connected by a pore region, in which the ion-selectivity filter resides. The exquisite K^+ selectivity of this class of ion channels is conferred by amino acids located in the pore region, the signature sequence T/SXGXGX (193).

This structure of the pore present in KcsA channels is retained in all the K^+ channels known to date, including those present in fungi, protozoans, and metazoans but although the pore structure did not evolved considerably, other parts of the channel sequence show considerable structural diversity. Thus, we have organized the present overview by dividing K^+ channels in three structural classes (157, 181, 280, 377, 467, 569) (Fig. 1): (i) the inward rectifier (Kir) family that follows the same structural pattern of the KcsA channel, their subunits contain two TM segments flanking the pore-forming domain and they assemble as tetramers. In mammals, Kir channels are encoded by 15 different genes grouped into 7 subfamilies, Kir1.x to Kir7.x and this diversity has been greatly increased by the identification of 6 alternative splicing isoforms in the case of Kir1.1 and the ability of the proteins inside the subfamilies to form heteromultimers (203,436); (ii) the two-pore four TM segments K^+ channels (K_{2P}) family, which in contrast to the other families we discuss in the present article, their subunits assemble as dimers. Fifteen different genes of this family has been found in mammals and surprisingly this class of channels has 46 genes in the worm *Caenorhabditis elegans*; (iii) the six TM (S1-S6) segments K^+ channels with one pore domain (S5-P-S6) that include the subfamily of voltage-gated channels, Kv1.x to Kv4.x (corresponding to Shaker, Shab, Shaw, and Shal channels, respectively, in *Drosophila*). Consisting of eight different genes the Kv1.x (*Shaker*) subfamily is the largest in this structural class of K^+ channels. Voltage-dependent K^+ channels are characterized by containing a voltage-sensor domain (VSD; S1-S4) in which the S4 contains positively charged amino acids that constitute the voltage-sensing elements. The six TM domains class also includes the KCNQ (Kv7.x), ether-a-go-go (Kv10.x; gated by voltage and cyclic nucleotides), erg (Kv11.x), and elk (Kv12.2) subfamilies.

Despite the fact that Kv5, Kv6, Kv8, and Kv9 share the same general structure with other members of the Kv

*Correspondence to ramon.latorre@uv.cl

[1]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile

[2]Departamento de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile

Published online, July 2012 (*comprehensivephysiology.com*)

DOI: 10.1002/cphy.c110047

Biophys Rev
DOI 10.1007/s12551-011-0061-8

REVIEW

Voltage sensor of ion channels and enzymes

Carlos Gonzalez · Gustavo F. Contreras · Alexander Peyser · Peter Larsson · Alan Neely · Ramón Latorre

Received: 21 September 2011 / Accepted: 17 November 2011

Abstract Placed in the cell membrane (a two-dimensional environment), ion channels and enzymes are able to sense voltage. How these proteins are able to detect the difference in the voltage across membranes has attracted much attention, and at times, heated debate during the last few years. Sodium, Ca^{2+} and K^+ voltage-dependent channels have a conserved positively charged transmembrane (S4) segment that moves in response to changes in membrane voltage. In voltage-dependent channels, S4 forms part of a domain that crystallizes as a well-defined structure consisting of the first four transmembrane (S1–S4) segments of the channel-forming protein, which is defined as the voltage sensor domain (VSD). The VSD is tied to a pore domain and VSD movements are allosterically coupled to the pore opening to various degrees, depending on the type of channel. How many charges are moved during channel activation, how much they move, and which are the molecular determinants that mediate the electromechanical coupling between the VSD and the pore domains are some of the questions that we discuss here. The VSD can function, however, as a bona fide proton channel itself, and, furthermore, the VSD can also be a functional part of a voltage-dependent phosphatase.

Keywords Voltage sensor · Kv channels · Cav Channels · BK channels · Proton channels and VSP

C. Gonzalez · G. F. Contreras · A. Neely · R. Latorre (✉)
Centro Interdisciplinario de Neurociencia de Valparaíso,
Facultad de Ciencias, Universidad de Valparaíso,
Pasaje Harrington 287,
Valparaíso 2360103, Chile
e-mail: ramon.latorre@uv.cl

A. Peyser · P. Larsson
Department of Physiology and Biophysics, University of Miami,
Miami, FL, USA

Published online: 16 December 2011

Introduction

The fascinating story of the voltage sensor of voltage-dependent channels started in 1952, and is summarized in two visionary sentences appearing in the last paper of the series by Hodgkin and Huxley (1952) predicting both the existence of a voltage sensor and the gating currents. The voltage sensor predicted: "…it seems difficult to escape to the conclusion that the changes in ionic permeability depend on the movement of some component of the membrane which behaves as though it has a large charge or dipole moment". The gating charge predicted: "For the movement of any charged particle in the membrane should contribute to the total current…".

It took 21 years to demonstrate the existence of Na^+ channel gating currents (Armstrong and Bezanilla 1973; Keynes and Rojas 1973; Bezanilla 2000), small (~1% of the ionic currents) transient currents appearing before the onset of the Na^+ current. Ten years later, with the introduction of the molecular biology techniques and the cloning and characterization of the primary structure and functional expression of the voltage-dependent Na^+ channel, we had the first hint about the structural determinants of the voltage sensor (Noda et al. 1984, 1986). The sodium channel protein was found to consist of four domains (I–IV), each containing six transmembrane (TM) segments (S1–S6). The fourth TM segment (S4) contains positively charged residues periodically separated by two hydrophobic residues. Numa and coworkers (Noda et al. 1984) proposed that this structure, S4, hosts the determinants for voltage-sensitivity and, together with Stühmer's group, gave some of the first electrophysiological evidence that the positive charges in S4 were involved in voltage-sensing (Stuhmer et al. 1989)

The actual "molecular biophysics" of voltage-dependent ion channels started, however, with the cloning of the

BJ www.biochemj.org

Biochem. J. (2012) **442**, 57–63 (Printed in Great Britain) doi:10.1042/BJ20111498

BJ Plant

The pH sensor of the plant K^+-uptake channel KAT1 is built from a sensory cloud rather than from single key amino acids

Wendy GONZÁLEZ*†[1,2], Janin RIEDELSBERGER†[1], Samuel E. MORALES-NAVARRO*, Julio CABALLERO*, Jans H. ALZATE-MORALES*, Fernando D. GONZÁLEZ-NILO*‡ and Ingo DREYER†§[2]

*Centro de Bioinformática y Simulación Molecular, Universidad de Talca, 2 Norte # 685, 3465548, Talca, Chile, †Universität Potsdam, Institut für Biochemie und Biologie, Heisenberg-Group BPMPB, Karl-Liebknecht-Strasse 24/25, Haus 20, D-14476 Golm, Germany, ‡Centro Interdisciplinario de Neurociencia de Valparaíso, Valparaíso, Chile, and §Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid, Campus de Montegancedo, Carretera M-40, km 37.7, E-28223-Pozuelo de Alarcón (Madrid) Spain

The uptake of potassium ions (K^+) accompanied by an acidification of the apoplasm is a prerequisite for stomatal opening. The acidification (approximately 2–2.5 pH units) is perceived by voltage-gated inward potassium channels (K_{in}) that then can open their pores with lower energy cost. The sensory units for extracellular pH in stomatal K_{in} channels are proposed to be histidines exposed to the apoplasm. However, in the *Arabidopsis thaliana* stomatal K_{in} channel KAT1, mutations in the unique histidine exposed to the solvent (His^{267}) do not affect the pH dependency. We demonstrate in the present study that His^{267} of the KAT1 channel cannot sense pH changes since the neighbouring residue Phe^{266} shifts its pK_a to undetectable values through a cation–π interaction. Instead, we show that Glu^{240} placed in the extracellular loop between transmembrane segments S5 and S6 is involved in the extracellular acid activation mechanism. Based on structural models we propose that this region may serve as a molecular link between the pH- and the voltage-sensor. Like Glu^{240}, several other titratable residues could contribute to the pH-sensor of KAT1, interact with each other and even connect such residues far away from the voltage-sensor with the gating machinery of the channel.

Key words: *Arabidopsis thaliana*, channel protein structure, channel protein–proton interaction, KAT1, pH regulation, potassium chanel.

INTRODUCTION

Stomata are pore structures found in the epidermis of plants. They optimize the uptake of CO_2 and loss of water vapour. Stomatal opening and closure is regulated by the turgor pressure of two guard cells that surround the stomatal pore [1]. Potassium (K^+) flux across the membrane is fundamental for the turgor-driven volume changes in guard cells. Specifically, K^+ uptake through inwardly rectifying channels (K_{in} channels) is essential for stomatal opening; a process accompanied by the acidification of the apoplast [2,3]. K^+ uptake depends on H^+-ATPase activity generating a sufficiently hyperpolarized membrane voltage, which provides an inward-directed electrochemical driving force for K^+ and opens the K_{in} channel gate [4]. K_{in} channels, besides opening upon hyperpolarization, sense through a protein-intrinsic pH-sensor the extracellular proton increase generated by H^+-ATPases [2,5,6]. Extracellular protons shift the voltage-dependence of guard cell K_{in} channels to more positive voltages and thereby facilitate K^+ uptake [7].

K_{in} channels display high sequence homology with animal *Shaker* channels. Each subunit possesses a six-segment (S1–S6) membrane-spanning topology. A selectivity filter segment (comprising the triad of GYG residues) [8], linking the S5 and S6 segments, forms the outer portion of a pore and confers the potassium-selectivity characteristics on the channel. Functional channels comprise four of these subunits, each one containing an intrinsic voltage sensor, which is composed primarily of charged amino acid residues of the S2, S3 and S4 segments [9].

Mutational analysis by Hoth et al. [6] allowed relating acid activation in KST1, a K_{in} channel from *Solanum tuberosum* (potato) guard cells, to two extracellular histidines. One histidine is located within the linker between the transmembrane helices S3 and S4 (His^{160}), and the other histidine is three amino acids outside the selectivity filter triad (His^{271}). The histidine from the GYGDXH motif is conserved among K_{in} channels. The conservation of this amino acid suggests that it represents a common entity of an extracellular pH-sensor [6] of plant K^+-uptake channels. To test this hypothesis, the guard cell K_{in} channel KAT1 from *Arabidopsis thaliana* was studied with respect to the structural basis for its acid activation. Surprisingly, the pH-dependent gating of KAT1 was not affected by mutation of this highly conserved histidine residue, suggesting that the two guard cell K_{in} channels KAT1 and KST1 have distinct molecular bases for acid activation [7].

In the present study, we came back to the structural aspects of extracellular acid activation of KAT1. Using site-directed mutagenesis, electrophysiology, yeast complementation, molecular simulation and quantum mechanics, we have shown that a glutamic acid residue (Glu^{240}) is involved in the extracellular acid activation mechanism. Furthermore, we have shown that the histidine of the GYGDXH motif cannot work as pH-sensor in KAT1 because it is interacting with the neighbouring phenylalanine (X position in the motif). From our combined results we conclude that the pH-sensor of KAT1 is built from a sensory cloud connecting different functional parts of the protein rather than single key amino acids.

EXPERIMENTAL

Molecular genetics and expression

KAT1 mutation, expression and analysis used standard molecular genetic methods. Site-directed mutations were generated as

Abbreviations used: CHARMM, Chemistry at HARvard Macromolecular Mechanics; HERG, human *ether-a-go-go*-related gene; K_{in} channel, inwardly rectifying potassium channel; SCF, self-consistent reaction field.

[1] These authors contributed equally to this work.

[2] Correspondence may be addressed to either of these authors (email wgonzalez@utalca.cl or ingo.dreyer@upm.es).

DEVELOPMENTAL DYNAMICS 241:1143–1154, 2012

RESEARCH ARTICLE

Close Association of Olfactory Placode Precursors and Cranial Neural Crest Cells Does Not Predestine Cell Mixing

Maegan V. Harden,[1] Luisa Pereiro,[2] Mirana Ramialison,[3†] Jochen Wittbrodt,[3] Megana K. Prasad,[4] Andrew S. McCallion,[4] and Kathleen E. Whitlock[2]*

Vertebrate sensory organs originate from both cranial neural crest cells (CNCCs) and placodes. Previously, we have shown that the olfactory placode (OP) forms from a large field of cells extending caudally to the pre-migratory neural crest domain, and that OPs form through cell movements and not cell division. Concurrent with OP formation, CNCCs migrate rostrally to populate the frontal mass. However, little is known about the interactions between CNCCs and the placodes that form the olfactory sensory system. Previous reports suggest that the OP can generate cell types more typical of neural crest lineages such as neuroendocrine cells and glia, thus marking the OP as an unusual sensory placode. One possible explanation for this exception is that the neural crest origin of glia and neurons has been overlooked due to the intimate association of these two fields during migration. Using molecular markers and live imaging, we followed the development of OP precursors and of dorsally migrating CNCCs in zebrafish embryos. We generated a *six4b:mCherry* line (OP precursors) that, with a *sox10:EGFP* line (CNCCs), was used to follow cell migration. Our analyses showed that CNCCs associate with and eventually surround the forming OP with limited cell mixing occurring during this process. *Developmental Dynamics 241:1143–1154, 2012.* © 2012 Wiley Periodicals, Inc.

Key words: sox10; dlx3b; six4b

Key findings:

- The *six4b:mCherry* line expresses mCherry in the forming olfactory placodes.
- mCherry expressing cells move caudally, away from the anterior midline, to form the olfactory placodes.
- Sox10:GFP positive cranial neural crest cells migrate rostrally to surround the forming olfactory placodes.
- Little cell-mixing is observed during the migation of Six4b:mCherry and Sox10:GFP expressing cells.

Accepted 9 April 2012

INTRODUCTION

The highly specialized structures of the vertebrate head, including the sensory organs, appeared concurrently with the neural crest and neurogenic placodes during the evolution of craniates (Northcutt and Gans, 1983; Northcutt, 1996). Neural crest cells are multipotent cells that contribute to a wide variety of cell types including neurons, glia, endocrine cells, and melanocytes, with the cranial neural crest cells (CNCCs) giving rise to cartilage, bone, cranial neurons, glia, and connective tissues of the face (Le Douarin and Kalcheim, 1999). To contribute to a variety of cell types in the frontal mass, CNCCs follow defined migratory

Additional Supporting Information may be found in the online version of this article.

[1]Department of Molecular Biology and Genetics, 445/449 Biotechnology Building, Cornell University, Ithaca, New York
[2]Centro Interdisciplinario de Neurociencia, Facultad de Ciencia, Universidad de Valparaiso, Valparaíso, Chile
[3]Centre for Organismal Studies, Heidelberg University, Heidelberg, Germany
[4]McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland
†Dr. Ramialison's present address is The Victor Chang Cardiac Research Institute, 405 Liverpool Street, Darlinghurst NSW 2010, Australia

Grant sponsor: FONDECYT; Grant numbers: 3095008, 1071071, 1111046; Grant sponsor: NIH; Grant numbers: R01DC0421802, R01HD50820, NIGMS: R01GM071648; Grant sponsor: ICM; Grant number: P06-039F.

***Correspondence to: Kathleen E. Whitlock, Centro Interdisciplinario de Neurociencia, Facultad de Ciencia, Universidad de Valparaiso, Avenida Gran Bretaň a 1111, Valparaíso, Chile. E-mail: kathleen.whitlock@uv.cl**

DOI 10.1002/dvdy.23797
Published online 22 May 2012 in Wiley Online Library (wileyonlinelibrary.com).

Neuron
Article

Pull-Push Neuromodulation of LTP and LTD Enables Bidirectional Experience-Induced Synaptic Scaling in Visual Cortex

ShiYong Huang,[1,5] Mario Treviño,[1,5] Kaiwen He,[1,5] Alvaro Ardiles,[1,2] Roberto de Pasquale,[1] Yatu Guo,[1,3] Adrian Palacios,[2] Richard Huganir,[4] and Alfredo Kirkwood[1,4,*]
[1]The Mind/Brain Institute, Johns Hopkins University, Baltimore, MD 21218, USA
[2]Centro Interdisciplinario de Neurociencias, Universidad de Valparaiso, Valparaíso, Chile
[3]Tianjin Eye Institute, Tianjin Eye Hospital, Tianjin Medical University, Tianjin, China
[4]Department of Neuroscience, Johns Hopkins University, Baltimore, MD 21205, USA
[5]These authors contributed equally to this work
*Correspondence: kirkwood@jhu.edu
DOI 10.1016/j.neuron.2011.11.023

SUMMARY

Neuromodulatory input, acting on G protein-coupled receptors, is essential for the induction of experience-dependent cortical plasticity. Here we report that G-coupled receptors in layer II/III of visual cortex control the polarity of synaptic plasticity through a pull-push regulation of LTP and LTD. In slices, receptors coupled to Gs promote LTP while suppressing LTD; conversely, receptors coupled to Gq11 promote LTD and suppress LTP. In vivo, the selective stimulation of Gs- or Gq11-coupled receptors brings the cortex into LTP-only or LTD-only states, which allows the potentiation or depression of targeted synapses with visual stimulation. The pull-push regulation of LTP/LTD occurs via direct control of the synaptic plasticity machinery and it is independent of changes in NMDAR activation or neuronal excitability. We propose these simple rules governing the pull-push control of LTP/LTD form a general metaplasticity mechanism that may contribute to neuromodulation of plasticity in other cortical circuits.

INTRODUCTION

Mechanisms for bidirectional synaptic plasticity such as NMDAR-dependent forms of long-term potentiation (LTP) and depression (LTD) are essential for experience-dependent modification of cortical function (Buonomano and Merzenich, 1998). A widespread consensus model states that the patterns of NMDAR activation and the ensuing increase in intracellular Ca are sufficient to encode the polarity of synaptic changes: changes in Ca above or below a modification threshold resulting in LTP or LTD respectively (Malenka and Bear, 2004). Indeed, in support of this idea, alterations in LTP and LTD induction are often accounted for by changes in NMDAR function. Recent studies indicate, however, that neuromodulators also play a role in determining the polarity of NMDAR-dependent synaptic plasticity through mechanisms that are not fully understood (see Pawlak et al., 2010).

Experience-induced plasticity depends not only on the patterns of sensory input, but also on neuromodulatory signals related to the behavioral and emotional state of the animal (Bear and Singer, 1986; Conner et al., 2003; Gu, 2002; Hu et al., 2007; Kilgard and Merzenich, 1998). Indeed, visual cortical plasticity depends crucially on the integrity of the cholinergic, adrenergic and serotonergic systems (Bear and Singer, 1986; Gu and Singer, 1995). This permissive function was originally attributed to increased neural excitability and sensory responsiveness (Bear and Singer, 1986; Thomas et al., 1996). However, neuromodulatory systems have only modest effects on the tuning and signal-to-noise ratio of visual responses (Ego-Stengel et al., 2002; Zinke et al., 2006), and most plausibly they gate experience-induced plasticity by directly controlling synaptic plasticity mechanisms such as LTP and LTD. Hence understanding the neuromodulation of LTP and LTD is of great significance.

Previous research on the neuromodulation of plasticity uncovered the simple principle that receptors coupled to the Gs-protein selectively gate and promote LTP, whereas the receptors coupled to Gq11 promote LTD (Choi et al., 2005; Kirkwood et al., 1999; Scheiderer et al., 2004; Seol et al., 2007). Importantly, although individually Gs- and Gq11-coupled receptors respectively enable LTP or LTD only, when coapplied they enable spike-timing dependent bidirectional changes (Seol et al., 2007). This suggests that the interaction between the signaling of these two types of receptors is not simply additive. Here we show that receptors coupled to different G-proteins can also selectively suppress LTP or LTD. As a consequence of these opposite actions, G protein-coupled receptors (GPCRs) regulate LTP and LTD in a pull-push manner: receptors coupled to the adenylyl cyclase signaling pathway via Gs promote LTP and suppress LTD, whereas receptors coupled to phospholipase C via Gq11 promote LTD and suppress LTP. We propose that this neuromodulator-based metaplasticity allows rapid dynamic control of the polarity and gain of NMDAR-dependent synaptic

MBoC | ARTICLE

Critical role of the first transmembrane domain of Cx26 in regulating oligomerization and function

Oscar Jara[a], Rodrigo Acuña[a], Isaac E. García[a], Jaime Maripillán[a], Vania Figueroa[a,b], Juan C. Sáez[a,b], Raúl Araya-Secchi[c], Carlos F. Lagos[c,d], Tomas Pérez-Acle[a,c,e], Viviana M. Berthoud[f], Eric C. Beyer[f], and Agustín D. Martínez[a]

[a]Centro Interdisciplinario de Neurociencias de Valparaíso, Universidad de Valparaíso, Valparaíso, Chile; [b]Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago, Chile; [c]Computational Biology Laboratory, Centro de Modelamiento Matemático, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Santiago, Chile; [d]Departamento de Farmacia, Facultad de Química, Pontificia Universidad Católica de Chile, Santiago, Chile; [e]Fundación Ciencia y Vida, Santiago, Chile; [f]Department of Pediatrics, University of Chicago, Chicago, IL 60637

ABSTRACT To identify motifs involved in oligomerization of the gap junction protein Cx26, we studied individual transmembrane (TM) domains and the full-length protein. Using the TOXCAT assay for interactions of isolated TM α-helices, we found that TM1, a Cx26 pore domain, had a strong propensity to homodimerize. We identified amino acids Val-37–Ala-40 (VVAA) as the TM1 motif required for homodimerization. Two deafness-associated Cx26 mutations localized in this region, Cx26V37I and Cx26A40G, differentially affected dimerization. TM1-V37I dimerized only weakly, whereas TM1-A40G did not dimerize. When the full-length mutants were expressed in HeLa cells, both Cx26V37I and Cx26A40G formed oligomers less efficiently than wild-type Cx26. A Cx26 cysteine substitution mutant, Cx26V37C formed dithiothreitol-sensitive dimers. Substitution mutants of Val-37 formed intercellular channels with reduced function, while mutants of Ala-40 did not form functional gap junction channels. Unlike wild-type Cx26, neither Cx26V37I nor Cx26A40G formed functional hemichannels in low extracellular calcium. Thus the VVAA motif of Cx26 is critical for TM1 dimerization, hexamer formation, and channel function. The differential effects of VVAA mutants on hemichannels and gap junction channels imply that inter-TM interactions can differ in unapposed and docked hemichannels. Moreover, Cx26 oligomerization appears dependent on transient TM1 dimerization as an intermediate step.

Monitoring Editor
Asma Nusrat
Emory University

Received: Jan 3, 2012
Revised: Jun 5, 2012
Accepted: Jul 5, 2012

INTRODUCTION

Gap junction channels constitute major pathways for direct cellular interactions because they allow the passage of metabolites, second messengers, and ions between neighboring cells. Opening of hemichannels can allow exchange of such small molecules between the cytoplasm and the extracellular compartment. Gap junction function is critical for many cellular processes; indeed, mutations in gap junction proteins are causally associated with a large spectrum of diseases, including deafness, skin disease, arrhythmias, neuropathies, and cataracts (reviewed by Harris, 2001; Willecke *et al.*, 2002; Saez *et al.*, 2003; Martinez *et al.*, 2009).

A gap junction channel is formed by the docking of two hemichannels or connexons. Each connexon is formed by the oligomerization of six subunit proteins called connexins (Cx). Each member of the connexin family has a similar topology, containing four transmembrane α-helices (TM1–TM4), cytoplasmic amino and carboxyl termini, two extracellular loops (ECLs), and one intracellular loop. The membrane-spanning regions of the connexins are important structural elements that participate in intraprotomer interactions

This article was published online ahead of print in MBoC in Press (http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E11-12-1058) on July 11, 2012.

Address correspondence to: Agustín D. Martínez (agustin.martinez@uv.cl).

Abbreviations used: 18-βGA, 18-β-glycyrrhetinic acid; a.u., arbitrary units; Cx, connexin; DTT, dithiothreitol; ECL, extracellular loop; EGFP, enhanced green fluorescent protein; ER, endoplasmic reticulum; GFP, green fluorescent protein; GPCR, G protein–coupled receptor; HA, hemagglutinin; HBSS, Hanks' balanced salt solution; IgG, immunoglobulin G; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; TBST, 0.1% Tween-20 in Tris-buffered saline; TM, transmembrane; ToxR, transactivation domain; TPC, two-pore channel.

Biochimica et Biophysica Acta 1818 (2012) 2048–2057

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Glial connexin expression and function in the context of Alzheimer's disease☆

Annette Koulakoff [a,b,c], Xin Mei [a,b,c], Juan A. Orellana [d], Juan C. Sáez [e,f], Christian Giaume [a,b,c,*]

[a] Collège de France, Center for Interdisciplinary Research in Biology (CIRB), Centre National de la Recherche Scientifique, Unité Mixte de Recherche 7241/Institut National de la Santé et de la Recherche Médicale U1050, 75231 Paris Cedex 05, France
[b] University Pierre et Marie Curie, ED, N°158, 75005 Paris, France
[c] MEMOLIFE Laboratory of Excellence and Paris Science Lettre Research University, 75005 Paris, France
[d] Departamento de Neurología, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
[e] Departamento de Fisiología, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile
[f] Departamento Centro Interdiciplinario de Neurociencias de Valparaíso, Valparaíso, Chile

ARTICLE INFO

Article history:
Received 13 May 2011
Received in revised form 20 September 2011
Accepted 3 October 2011
Available online 7 October 2011

Keywords:
Connexin
Astrocyte
Microglia
Hemichannel
Gap junction

ABSTRACT

A hallmark of neurodegenerative diseases is the reactive gliosis characterized by a phenotypic change in astrocytes and microglia. This glial response is associated with modifications in the expression and function of connexins (Cxs), the proteins forming gap junction channels and hemichannels. Increased Cx expression is detected in most reactive astrocytes located at amyloid plaques, the histopathological lesions typically present in the brain of Alzheimer's patients and animal models of the disease. The activity of Cx channels analyzed *in vivo* as well as *in vitro* after treatment with the amyloid β peptide is also modified and, in particular, hemichannel activation may contribute to neuronal damage. In this review, we summarize and discuss recent data that suggest glial Cx channels participate in the neurodegenerative process of Alzheimer's disease. This article is part of a Special Issue entitled: The Communicating junctions, composition, structure and characteristics.

Contents

1. Introduction

In the brain, neurons receive, integrate, process and propagate information. However, to achieve properly these functions and to survive, neurons need to interact with glial cells, in particular with astrocytes. Indeed, astrocytes provide trophic and metabolic support to neurons. They contribute to hyperemia, the blood flow regulation in response to neuronal activity [1], and to the maintenance of the extracellular medium homeostasis [2]. Moreover, they modulate synaptic activity and recent evidence suggests their involvement in brain functions as complex as sleep and sensory processing [3]. In pathological situations, astrocytes together with microglial cells, the resident immune cells of the brain, undergo striking changes referred to as reactive gliosis. During this long-lasting process, microglial cells become activated and astrocytes acquire progressively a reactive phenotype characterized by

☆ This article is part of a Special Issue entitled: The Communicating junctions, composition, structure and characteristics.

* Corresponding author. Tel.: +33 1 44 27 12 22; fax: +33 1 44 27 12 68.
E-mail address: christian.giaume@college-de-france.fr (C. Giaume).

0005-2736/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2011.10.001

J Mol Model (2012) 18:2055–2064
DOI 10.1007/s00894-011-1224-z

ORIGINAL PAPER

Molecular modeling of *Trypanosoma cruzi* glutamate cysteine ligase and investigation of its interactions with glutathione

Carlos F. Lagos · Raul Araya-Secchi · Pablo Thomas · Tomás Pérez-Acle · Ricardo A. Tapia · Cristian O. Salas

Received: 17 June 2011 / Accepted: 11 August 2011 / Published online: 6 September 2011

Abstract *Trypanosoma cruzi* glutamate cysteine ligase (TcGCL) is considered a potential drug target to develop novel antichagasic drugs. We have used a variety of computational methods to investigate the interactions between TcGCL with Glutathione (GSH). The three-dimensional structure of TcGCL was constructed by comparative modeling methods using the *Saccharomyces cerevisiae* glutamate cysteine ligase as template. Molecular dynamics simulations were used to validate the TcGCL model and to analyze the molecular interactions with GSH. Using RMSD clustering, the most prevalent GSH binding modes were identified paying attention to the residues involved in the molecular interactions. The GSH binding modes were used to propose pharmacophore models that can be exploited in further studies to identify novel antichagasic compounds.

Keywords Comparative modeling · Glutamate cysteine ligase · Molecular dynamics · Pharmacophore · *Trypanosoma cruzi*

Introduction

Chagas disease represents the leading cause of cardiac lesions in young, economically productive adults in Latin American countries where this disease is endemic [1]. *Trypanosoma cruzi*, the eukaryotic protozoan responsible for Chagas disease, has a redox metabolism based on trypanothione, a glutathionyl spermidine derivative. In *Trypanosoma cruzi*, glutathione (GSH) is synthesized from its constituent amino acids by the consecutive actions of

Carlos F. Lagos and Raul Araya-Secchi have contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00894-011-1224-z) contains supplementary material, which is available to authorized users.

C. F. Lagos
Departamento de Farmacia, Facultad de Química,
P. Universidad Catolica de Chile,
Av Vicuña Mackenna 4860,
Macul-Santiago, Chile

C. F. Lagos · R. Araya-Secchi
Departamento de Ciencias Biologicas, Facultad de Ciencias Biologicas, Universidad Andres Bello,
Av Republica 217,
Santiago, Chile

R. Araya-Secchi · T. Pérez-Acle
Computational Biology Lab (DLab), Centro de Modelamiento Matematico (CMM), Facultad de Ciencias Fisicas y Matematicas,
Universidad de Chile,
Santiago, Chile

T. Pérez-Acle
Fundacion Ciencia para la Vida,
Zañartu 1482, Ñuñoa Santiago, Chile

T. Pérez-Acle
Centro Interdisciplinario de Neurociencias de Valparaiso (CINV),
Pasaje Harrington 287, Playa Ancha Valparaiso, Chile

P. Thomas · R. A. Tapia · C. O. Salas (✉)
Departamento de Química Organica, Facultad de Química,
P. Universidad Catolica de Chile,
Av Vicuña Mackenna 4860,
Macul- Santiago, Chile
e-mail: cosalas@uc.cl

The Journal of Neuroscience, May 16, 2012 · 32(20):6819–6829 · 6819

Cellular/Molecular

Genetic Analysis of Ecdysis Behavior in *Drosophila* Reveals Partially Overlapping Functions of Two Unrelated Neuropeptides

Eleanor C. Lahr,[1] Derek Dean,[1] and John Ewer[2]

[1]Department of Entomology, Cornell University, Ithaca, New York 14853, [2]Instituto Milenio, Centro Interdisciplinario de Neurociencias de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, 2360102 Valparaíso, Chile

Ecdysis behavior allows insects to shed their old exoskeleton at the end of every molt. It is controlled by a suite of interacting hormones and neuropeptides, and has served as a useful behavior for understanding how bioactive peptides regulate CNS function. Previous findings suggest that crustacean cardioactive peptide (CCAP) activates the ecdysis motor program; the hormone bursicon is believed to then act downstream of CCAP to inflate, pigment, and harden the exoskeleton of the next stage. However, the exact roles of these signaling molecules in regulating ecdysis remain unclear. Here we use a genetic approach to investigate the functions of CCAP and bursicon in *Drosophila* ecdysis. We show that null mutants in CCAP express no apparent defects in ecdysis and postecdysis, producing normal adults. By contrast, a substantial fraction of flies genetically null for one of the two subunits of bursicon [encoded by the *partner of bursicon* gene (*pburs*)] show severe defects in ecdysis, with escaper adults exhibiting the expected failures in wing expansion and exoskeleton pigmentation and hardening. Furthermore, flies lacking both CCAP and bursicon show much more severe defects at ecdysis than do animals null for either neuropeptide alone. Our results show that the functions thought to be subserved by CCAP are partially effected by bursicon, and that bursicon plays an important and heretofore undescribed role in ecdysis behavior itself. These findings have important implications for understanding the regulation of this vital insect behavior and the mechanisms by which hormones and neuropeptides control the physiology and behavior of animals.

Introduction

Neuropeptides are small signaling molecules that regulate animal development, physiology, and behavior (Strand, 1999). The ancient association of neuropeptides with nervous system function is accompanied by a diverse and complex spectrum of actions. In insects, an emblematic case of neuropeptide action is the control of ecdysis, a precisely timed series of behaviors that enables insects to shed the remains of the old exoskeleton at the end of every molt. Research conducted over the last 40 years has revealed that several hormones and neuropeptides regulate the precise order and timing of the different ecdysial behavioral subroutines (for review, see Ewer and Reynolds, 2002; Zitnan and Adams, 2004).

Received Oct. 20, 2011; revised March 19, 2012; accepted March 21, 2012.

Author contributions: E.C.L., D.D., and J.E. designed research; E.C.L., D.D., and J.E. performed research; E.C.L., D.D., and J.E. analyzed data; E.C.L., D.D., and J.E. wrote the paper.

Funding was provided by National Science Foundation Grant IBN-0343699000 and Fondo Nacional de Desarollo Cientifico y Tecnólogico Grants 1071079 and 1111023. We thank Gunisha Kauer, Tashana Williams, Chloe Ota, and Bianca Chiang for assistance during various stages of this work. We are grateful to Hans Agricola for anti-CCAP antiserum, Aaron Hsueh for anti-PBURS antiserum, and Benjamin White for anti-BURS antiserum. We thank John Roote for *pupal* alleles. We appreciate comments on the manuscript from Ralph Greenspan and Hans-Willi Honegger. This project was initiated by J.E. in the Department of Entomology, Cornell University, Ithaca, NY 14853.

Correspondence should be addressed to John Ewer, Instituto Milenio Centro Interdisciplinario de Neurociencias de Valparaíso (CINV), Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile. E-mail: john.ewer@uv.cl.

Eleanor Lahr's present address: Division of Biological Sciences, The University of Montana, Missoula, MT 59812.

Derek Dean's present address: Department of Biology, Williams College, 59 Lab Campus Drive, Williamstown, MA 01267.

DOI:10.1523/JNEUROSCI.5301-11.2012

 0270-6474/12/326819-11$15.00/0

The main endocrine signal that commits the animal to executing ecdysis is the phasic release of ecdysis triggering hormone (ETH) that occurs at the end of the molt. Crustacean cardioactive peptide (CCAP) has long been considered the neuropeptide that acts downstream of ETH to turn on the motor program that causes the old exoskeleton to be shed (ecdysis proper). Indeed, adding CCAP peptide to an isolated *Manduca* CNS activates this motor program and turns off the preparatory motor program of preecdysis (Gammie and Truman, 1997). Also, RNA interference of CCAP signaling in *Tribolium* causes a failure in ecdysis (Arakane et al., 2008; Li et al., 2011). Finally, *Drosophila* bearing targeted ablations of CCAP-expressing neurons do not exhibit pupal ecdysis behavior (Park et al., 2003). However, additional studies have implied a more complex model. In *Drosophila* and other insects, subsets of CCAP neurons express additional neuropeptides (Luo et al., 2005; Kim et al., 2006a,b; Luan et al., 2006; Woodruff et al., 2008), suggesting that some of the functions assigned to CCAP through targeted cell-killing experiments (Park et al., 2003) could be effected by other coexpressed neuropeptides, acting alone or in combination with CCAP. In particular, although bursicon (the so-called tanning hormone) has traditionally been associated with postecdysial functions (Cottrell, 1962; Fraenkel and Hsiao, 1962; Honegger et al., 2008), recent work suggests that it may play a role at ecdysis itself (Loveall and Deitcher, 2010; Veverytsa and Allan, 2011).

To further elucidate the specific role that CCAP plays at ecdysis in *Drosophila*, we isolated a mutant lacking *CCAP* function; we

C

Ca^{2+} Activation of K^+ Channels: RCK Domains

Ramon Latorre and David Baez-Nieto
Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso, Valparaíso, Chile

Introduction

Some K^+ channels can be activated by a rise in cytosolic Ca^{2+} and, in two cases (the K^+ channels from the archeon *Methanobacterium thermoautotrophicum* (MthK) and the large conductance voltage- and Ca^{2+}-activated K^+ (BK) channel), the Ca^{2+}-binding sites are contained within the *regulatory domains for K^+ conductance* (RCK). The MthK channel is a tetramer formed by subunits containing two transmembrane domains and a C-terminal domain containing a single RCK domain (Jiang et al. 2002). The functional MthK channel, however, contains eight RCK domains, and the green domains in Fig. 1a come from the cytoplasmic milieu.

The structure of the RCK domain of a six transmembrane domain K^+ channel from *E. coli* (solved at 2.4 Å resolution) has a Rossmann-fold topology, which is a very common structural motif of enzymes and ligand-binding proteins (see ▶ Structural Motifs). Rossmann-fold secondary structures are organized into two linked β-α-β-α-β units (see Fig. 1b) and were first identified in a number of NAD^+-dependent dehydrogenases. This is the type of structure present in the MthK and in the *Drosophila*, mouse, and human Slo1 (commonly known as BK; see ▶ Potassium Channels (Kv, Kir, KCa, and K(2P) Channels) channels; e.g. Fig. 1b). The cloning of the BK channel from *Drosophila* showed that it is a member of the S4 superfamily encompassing voltage-dependent K^+ (Kv), Na^+, and Ca^{2+} channels. The gene coding for BK was called *Slowpoke* or *Slo* (later renamed *Slo1* after the cloning and expression of *Slo2* and *Slo3* (Salkoff et al. 2006)). In the case of Kv channels, the channel-forming protein possesses six transmembrane domains (S1–S6) containing the pore-forming domain S5-P-S6, and an S4 voltage-sensing element (S1-S4). As Kv channels, the BK channel is a tetramer; however, unlike Kv channels, the BK channel protein consists of seven transmembrane (S0–S6) domains with an exoplasmic N-terminus (Fig. 1c). The intracellular C-terminal domain, comprising two thirds of the protein, contains four hydrophobic segments (S7–S10) and the Ca^{2+}- and Mg^{2+}-binding sites. The C-terminus of BK channels consists of two tandem RCK domains (only RCK1 is shown in Fig. 1b). The RCK domain in the BK channel was initially unveiled by MacKinnon's group (Jiang et al. 2001) by multiple sequence alignment of the BK channel with prokaryotic K^+ channels and other proteins known to possess the RCK domain motif. Based on their primary sequence, the C-terminal domains of K^+ channels *Slo2* and *Slo3* are believed to be structured as two RCK domains in tandem (Salkoff et al. 2006). Unlike *Slo1*, however, *Slo2* is activated by internal Na^+ and Cl^-, and *Slo3* is activated by protons. The actual structural nature of the C-termini of these two channels is unknown at present and will not be discussed further here.

G.C.K. Roberts (ed.), *Encyclopedia of Biophysics*, DOI 10.1007/978-3-642-16712-6,

Signal Transduction–Dependent Channels 4

Ramon Latorre, Carlos González, and Patricio Rojas

Brief History

From the moment the first member of this ion channel family, Slo1, was discovered, the scientific world was confronted with a molecular Pandora's box: once opened, its electrical language left scientists bewitched. They were fascinated with this "monster" of a single-channel conductance (250 pS in symmetrical 100 mM K^+) close to the ceiling imposed by simple diffusion combined with an exquisite K^+ selectivity. Slo1 channels are essentially impermeable to Na^+ and conduct K^+ 10- and 200-fold more effectively than Rb^+ and Cs^+, respectively, though it was previously thought that large conductance channels were not supposed to be so selective. At the same time, the channel was activated by voltage and cytoplasmic Ca^{2+}. This latter property led Meech in 1978 to hypothesize that this conductance system was perfect link between cell metabolism and electrical activity, and he was right on the mark. Because of its large conductance, this voltage- and calcium-activated K^+ channel was christened "maxi-K" or "BK" (for big K^+). This high single-channel

R. Latorre (✉)
Centro de Neurociencia, Universidad de Valparaíso, Valparaiso, Chile
e-mail: ramon.latorre@uv.cl

C. González
Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso Facultad de Ciencias, Valparaíso, Chile
e-mail: carlos.gonzalez@cinv.cl

P. Rojas
Departamento de Biología, Facultad de Química y Biología Universidad de Santiago de Chile, La Serena, Chile
e-mail: patricio.rojas.m@usach.cl

D.W. Pfaff (ed.), *Neuroscience in the 21st Century*,
DOI 10.1007/978-1-4614-1997-6_5,

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 39, pp. 32588–32597, September 21, 2012

A Short Polybasic Segment between the Two Conserved Domains of the β_{2a}-Subunit Modulates the Rate of Inactivation of R-type Calcium Channel*[S]

Received for publication, March 15, 2012, and July 26, 2012 Published, JBC Papers in Press, July 31, 2012, DOI 10.1074/jbc.M112.362509

Erick Miranda-Laferte[‡1], **Silke Schmidt**[‡], **Antonella C. Jara**[§], **Alan Neely**[§2], **and Patricia Hidalgo**[‡1,3]

From the [‡]*Institut für Neurophysiologie, Medizinische Hochschule Hannover, 30625 Hannover, Germany and the* [§]*Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso, 2349400 Valparaíso, Chile*

Background: Membrane anchoring underlies inhibition of voltage-dependent inactivation (VDI) of calcium channels by the β_{2a}-subunit.
Results: A polybasic segment of β_{2a} slows VDI without membrane association. This effect is abolished by charge neutralization.
Conclusion: VDI inhibition by the β_{2a}-subunit can occur without membrane anchoring by a mechanism that relies on positively charged residues.
Significance: A novel mechanism underlying inhibition of VDI in calcium channels is revealed.

Besides opening and closing, high voltage-activated calcium channels transit to a nonconducting inactivated state from which they do not re-open unless the plasma membrane is repolarized. Inactivation is critical for temporal regulation of intracellular calcium signaling and prevention of a deleterious rise in calcium concentration. R-type high voltage-activated channels inactivate fully in a few hundred milliseconds when expressed alone. However, when co-expressed with a particular β-subunit isoform, β_{2a}, inactivation is partial and develops in several seconds. Palmitoylation of a unique di-cysteine motif at the N terminus anchors β_{2a} to the plasma membrane. The current view is that membrane-anchored β_{2a} immobilizes the channel inactivation machinery and confers slow inactivation phenotype. β-Subunits contain one Src homology 3 and one guanylate kinase domain, flanked by variable regions with unknown structures. Here, we identified a short polybasic segment at the boundary of the guanylate kinase domain that slows down channel inactivation without relocating a palmitoylation-deficient β_{2a} to the plasma membrane. Substitution of the positively charged residues within this segment by alanine abolishes its slow inactivation-conferring phenotype. The linker upstream from the polybasic segment, but not the N- and C-terminal variable regions, masks the effect of this determinant. These results reveal a novel mechanism for inhibiting voltage-dependent inactivation of R-type calcium channels by the β_{2a}-subunit that might involve electrostatic interactions with an unknown target on the channel's inactivation machinery or its modulatory components. They also suggest that intralinker interactions occlude the action of the polybasic segment and that its functional availability is regulated by the palmitoylated state of the β_{2a}-subunit.

The entry of calcium ions into the cell triggers a multitude of cellular responses that rely on a tight spatio-temporal regulation of the calcium transient spreading within the cell for their coordination (1, 2). High voltage-activated (HVA)[4] calcium channels open in response to large membrane depolarization and constitute the major entry pathway for calcium into excitable cells. The largest component of HVA channels, α_1-subunit ($Ca_V\alpha_1$), contains the ion conduction pore, the voltage sensor, and multiple intracellular domains that regulate calcium influx and provide interaction sites for regulatory proteins. Two subfamilies of HVA $Ca_V\alpha_1$ are recognized as follows: $Ca_V1.x$ or L-type channels and $Ca_V2.x$, also referred as to neuronal channels ($Ca_V2.1$ encoding P/Q-type; $Ca_V2.2$ encoding N-type; and $Ca_V2.3$ encoding R-type) (3). Following strong depolarization and calcium permeation, HVA channels enter into an inactivated nonconducting state that constrains the amount of calcium influx and protects the cells from the cytotoxic effects of an excessive calcium rise. Inactivation of HVA channels is triggered by both calcium increase and prolonged membrane depolarization, referred as to calcium-dependent inactivation and voltage-dependent inactivation (VDI), respectively (4). Although calcium-dependent inactivation depends on the binding of calmodulin to a conserved site among HVA channels (5), VDI is an intrinsic property of $Ca_V\alpha_1$, but it is strongly modulated by the regulatory β-subunit ($Ca_V\beta$) (6–11) that binds to a site highly conserved among HVA channels, termed the α-interaction domain (12).

Crystallographic studies from three of the four known $Ca_V\beta$ isoforms ($Ca_V\beta_1$ to $Ca_V\beta_4$) revealed the molecular aspects of this interaction. $Ca_V\beta$ shares a common structural arrangement with members of the membrane-associated guanylate kinase family (MAGUK), a highly conserved Src homology 3 (SH3) and guanylate kinase (GK) domain flanked and joined by variable segments (13–15). The binding motif in the pore-

* This work was supported by Deutsche Forschung Gemeinschaft Grants DFG Hi 800/3-1 and 4-1 (to P. H.), Fondo Nacional de Ciencia y Tecnología Grant FONDECYT 1090635 (to A. N.), and the German-Chilean Scientific Cooperation Program Grant DFG-CONICYT 2010 (to P. H. and A. N.).
[S] This article contains supplemental Figs. S1–S4, Table S1, and Methods.
[1] Present address: Institute of Complex Systems 4 (ICS-4) Zelluläre Biophysik, Forschungszentrum Jülich, 52425 Jülich, Germany.
[2] To whom correspondence may be addressed. E-mail: alan.neely@cinv.cl.
[3] To whom correspondence may be addressed. E-mail: hidalgo.patricia@mh-hannover.de.

[4] The abbreviations used are: HVA, high voltage-activated channels; VDI, voltage-dependent inactivation; PBLK, polybasic linker segment; PSLK, polyserine linker segment; SH3, Src homology 3 domain; GK, guanylate kinase domain; NT, N terminus; CT, C terminus.

FEBS Letters 586 (2012) 2287–2293

ELSEVIER

FEBS Letters

journal homepage: www.FEBSLetters.org

The first transmembrane domain (TM1) of β2-subunit binds to the transmembrane domain S1 of α-subunit in BK potassium channels

Francisco J. Morera [a,b], Abderrahmane Alioua [c,1], Pallob Kundu [c,2], Marcelo Salazar [a], Carlos Gonzalez [a], Agustin D. Martinez [a], Enrico Stefani [c,d], Ligia Toro [c,e], Ramon Latorre [a,*]

[a] Centro Interdisciplinario de Neurociencia de Valparaiso, Facultad de Ciencias, Universidad de Valparaíso, Valparaiso, Chile
[b] Institute of Pharmacology and Morphophysiology, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile
[c] Division of Molecular Medicine, Department of Anesthesiology, Brain Research Institute and Cardiovascular Research Laboratories, University of California, Los Angeles, USA
[d] Department of Physiology, University of California, Los Angeles, USA
[e] Department of Molecular and Medical Pharmacology, University of California, Los Angeles, USA

ARTICLE INFO

Article history:
Received 22 March 2012
Revised 31 May 2012
Accepted 31 May 2012
Available online 16 June 2012

Edited by Maurice Montal

Keywords:
BK channels
β2-Subunit
TOXCAT assay
Transmembrane domain TM1
BK α and β-subunits interactions

ABSTRACT

The BK channel is one of the most broadly expressed ion channels in mammals. In many tissues, the BK channel pore-forming α-subunit is associated to an auxiliary β-subunit that modulates the voltage- and Ca^{2+}-dependent activation of the channel. Structural components present in β-subunits that are important for the physical association with the α-subunit are yet unknown. Here, we show through co-immunoprecipitation that the intracellular C-terminus, the second transmembrane domain (TM2) and the extracellular loop of the β2-subunit are dispensable for association with the α-subunit pointing transmembrane domain 1 (TM1) as responsible for the interaction. Indeed, the TOXCAT assay for transmembrane protein–protein interactions demonstrated for the first time that TM1 of the β2-subunit physically binds to the transmembrane S1 domain of the α-subunit.

Structured summary of protein interactions:
BK channel subunit alpha physically interacts with **BK channel subunit beta-2** by anti tag coimmunoprecipitation(View interaction)

1. Introduction

The high-conductance voltage- and Ca^{2+}-activated K^+ channel is one of the most ubiquitously expressed potassium channels in mammals [1]. This channel is named BK for "big K", given its single-channel conductance that can be as large as 250 pS in 100 mM, symmetrical KCl [2–4]. BK channels increase their activity by membrane depolarization or rises in cytosolic Ca^{2+} levels [2,5]. As its activation leads to membrane hyperpolarization, it serves as a negative-feedback mechanism for the excitatory events that lead to increases in calcium concentration or membrane depolarization.

The BK channel is a homotetramer of its pore-forming α-subunit, which is encoded by the gene Slo1 (*kcnma1*) and is a member of the voltage-dependent potassium (K_v) channels superfamily. As in all other K_v channels, the S4 transmembrane segment is part of an intrinsic voltage sensor [6,7]. Gating and ionic currents in BK channels can be elicited by membrane depolarization in the absence of calcium, suggesting that this is a voltage-dependent channel [8,9].

In many tissues, the BK channel α-subunit is associated to an auxiliary β-subunit. The β-subunits are intrinsic membrane proteins consisting of two transmembrane domains (TM1 and TM2) and a large (~120 residues) external loop. There are four types of β-subunits, β1–β4, that have tissue-specific distributions and impart unique effects on voltage- and Ca^{2+}-dependent activation of BK channels (reviewed in [10]).

The functional coupling between α- and β1-subunits requires the transmembrane segment S0 of the α-subunit [11]. However, Morrow et al. (2006) reported that the physical association between the α-subunit and the β1-subunit does not require the S0

Abbreviations: BK, high-conductance voltage- and Ca^{2+}-activated K^+ channel; TM, transmembrane; WB, western blot; K_v, voltage-dependent potassium channels; HEK, human embryonic kidney cells; Co-IP, co-immunoprecipitation

* Corresponding author. Address: Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Gran Bretaña 1111, Valparaíso, Chile.
E-mail address: ramon.latorre@uv.cl (R. Latorre).
[1] Present address: Ronald Reagan UCLA Medical Center, Department of Pathology and Laboratory Medicine, Los Angeles, USA.
[2] Present address: Division of Plant Biology, Bose Institute, Kolkata 700 054, West Bengal, India.

0014-5793/$36.00 © 2012 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.febslet.2012.05.066

1198 Biophysical Journal Volume 103 September 2012 1198–1207

K^+ Conduction and Mg^{2+} Blockade in a *Shaker* Kv-Channel Single Point Mutant with an Unusually High Conductance

Cristian Moscoso,[†‡] Ariela Vergara-Jaque,[¶] Valeria Márquez-Miranda,[‖] Romina V. Sepúlveda,[‖] Ignacio Valencia,[§] Ignacio Díaz-Franulic,[§] Fernando González-Nilo,[†‖] and David Naranjo[†‡*]

[†]Centro Interdisciplinario de Neurociencias de Valparaíso, [‡]Departamento de Neurociencia, and [§]Doctorado en Ciencias mención Neurociencia, Universidad de Valparaíso, Valparaíso, Chile; [¶]Centro de Bioinformática y Simulación Molecular Universidad de Talca, Talca, Chile; and [‖]Center for Bioinformatics and Integrative Biology, Facultad en Ciencias Biológicas, Universidad de Andres Bello, Santiago, Chile

ABSTRACT Potassium channels exhibit a large diversity of single-channel conductances. *Shaker* is a low-conductance K-channel in which Pro475→Asp, a single-point mutation near the internal pore entrance, promotes 6- to 8-fold higher unitary current. To assess the mechanism for this higher conductance, we measured *Shaker*-P475D single-channel current in a wide range of symmetrical K^+ concentrations and voltages. Below 300 mM K^+, the current-to-voltage relations (*i*-V) showed inward rectification that disappeared at 1000 mM K^+. Single-channel conductance reached a maximum of ~190 pS at saturating [K^+], a value 4- to 5-fold larger than that estimated for the native channel. Intracellular Mg^{2+} blocked this variant with ~100-fold higher affinity. Near zero voltage, blockade was competitively antagonized by K^+; however, at voltages >100 mV, it was enhanced by K^+. This result is consistent with a lock-in effect in a single-file diffusion regime of Mg^{2+} and K^+ along the pore. Molecular-dynamics simulations revealed higher K^+ density in the pore, especially near the Asp-475 side chains, as in the high-conductance MthK bacterial channel. The molecular dynamics also showed that K^+ ions bound distally can coexist with other K^+ or Mg^{2+} in the cavity, supporting a lock-in mechanism. The maximal K^+ transport rate and higher occupancy could be due to a decrease in the electrostatic energy profile for K^+ throughout the pore, reducing the energy wells and barriers differentially by ~0.7 and ~2 *k*T, respectively.

INTRODUCTION

Distinct K-channels are endowed with a highly K^+-selective pore that allows fluxes of 10^6–10^8 K^+ ions per second in equivalent experimental conditions. The diversity in the ion transport rate is congruent with the fact that K-channels constitute one of the most diverse membrane protein families (1). Potassium channels contain a highly conserved signature sequence of seven amino acid residues that shape the K^+ selectivity filter, the functional equivalent of the enzyme's active site (2,3). In spite of this, K-channels have very distinct single-channel conductances. For example, K-channels that have the same selectivity filter sequence (TTVGYGD: Kv1.1–Kv1.8, Kv2.2, as well as KcsA and KvAP bacterial channels), have single-channel conductance ranging from 5 to 170 pS (4) (see Table S1 in the Supporting Material). The origin of such diverse conductances is not resolved yet, but the sequence identity suggests that K^+ translocation across the selectivity filter is not the rate-limiting step in low-conductance K-channels. In these channels, the slowest step would be K^+ translocation toward or away from the selectivity filter.

High-conductance Ca-gated K-channels (BK channels) carry two conserved glutamate residues at the internal end of S6 (residues 321 and 324 in mSlo1; Table S1), suggesting an important role in large conductance. These residues, which are not present in low-conductance K-channels, form two negatively charged rings at the internal pore entrance. Charge neutralization of the rings reduced outward currents by ~50%, with modest effects on the inward currents, and significantly, but not dramatically, reduced blockade by Mg^{2+} (5,6). These effects faded away at high ionic strength, as would be predicted if the charged rings only produce an electrostatic accumulation of permeant and blocking ions in the vicinity of the internal mouth. Similarly, addition of negatively charged residues to position 108 in the KcsA channel, aligning with mSlo1's 320 (Table S1), doubled the outward currents and had minimal effects on the inward currents (7).

In contrast to BK channels, removal of either of the two charged rings of MthK (residues 92 and 96 aligning with mSlo1 320 and 324) reduced both inward and outward currents (8). This bidirectional effect on the current may not be accounted for by an electrostatic accumulation of K^+ ions near the internal entrance of the native MthK channel. Also in contrast to BK, charge neutralization in the ring decreased the divalent affinity dramatically. Therefore, the charged rings in the MthK channel may sustain a cation-binding site in the pore that can increase single-channel conductance and divalent affinity (8). In a similar fashion, a single-point mutation (Pro475Asp) at the internal entrance of the low-conductance *Shaker* K-channel produces a large (6- to 8-fold) increase in inward unitary currents (9). *Shaker*'s 475 aligns with 92 in MthK, 108 in KcsA, and 320 in mSlo1 (Table S1) (5,7,8), but in contrast to the case with KcsA and mSlo1, the inward

Submitted January 27, 2012, and accepted for publication August 1, 2012.

*Correspondence: david.naranjo@uv.cl

Editor: Chris Lingle.

0006-3495/12/09/1198/10 $2.00

http://dx.doi.org/10.1016/j.bpj.2012.08.015

Biometals (2012) 25:825–835
DOI 10.1007/s10534-012-9550-x

Iron deficiency on neuronal function

Pablo Muñoz · Alexis Humeres

Received: 31 December 2011 / Accepted: 6 April 2012 / Published online: 26 May 2012

Abstract Because of the intrinsic ability of iron to catalyze the formation of reactive oxygen species, it has been associated with oxidative stress and neurodegenerative diseases. However, iron deficiency (ID) also negatively impacts various functions of the brain, suggesting that iron plays an important physiological role in neuronal processes such as myelination, synaptogenesis, behavior and synaptic plasticity (SP). ID not only produces changes in the hippocampus, striatum, amygdale or prefrontal cortex, it also affects the interaction among these systems. In both humans and rodents, the perturbations of these structures are associated to cognitive deficits. These cognitive alterations have been well correlated with changes in neural plasticity, the possible cellular substrate of memory and learning. Given that SP is strongly affected by early ID and the lasting-neurological consequences remain even after ID has been corrected, it is important to prevent ID as well as to seek effective therapeutic interventions that reduce or reverse the long-term effects of the ID in the nervous system. This review will give an overview of the literature on the effects of iron deficit in neuronal functions such as behavior, neurotransmission and SP. We also discuss our recent data about the possible oxidative effect of iron on the mechanisms involved in neural plasticity.

Keywords Iron · Synaptic plasticity · Calcium signaling · Hippocampus · Cognitive impartment

Abbreviations

ROS	Reactive oxygen species
ID	Iron deficiency
SP	Synaptic plasticity
CNS	Central nervous system
PFC	Prefrontal cortex
DMT1	Divalent metal transporter 1
DFO	Desferrioxamine
LTP	Long-term potentiation
PPF	Paired-pulse facilitation
ISO	Isoproterenol
NMDAR	*N*-methyl-D-aspartate receptor
Ry	Ryanodine
RyR	Ry receptor
CICR	Calcium-induced calcium release
mEPSCs	Miniature excitatory postsynaptic currents

P. Muñoz (✉)
Centro Interdisciplinario de Neurociencias de Valparaíso, Universidad de Valparaíso, Valparaiso, Chile
e-mail: pablo.munoz@cinv.cl

A. Humeres
CEMC, Facultad de Medicina, Universidad de Chile, Santiago, Chile

A. Humeres
IBALAB, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

A. Humeres
Departamento de Morfofunción, Facultad de Medicina, Universidad Diego Portales, Ubicación, Chile

Communicative & Integrative Biology 5:2, 166–168; March/April 2012; © 2012 Landes Bioscience

Iron-mediated redox modulation in neural plasticity

Pablo Muñoz
Centro Interdisciplinario de Neurociencias de Valparaíso; Universidad de Valparaíso; Valparaíso, Chile

The role of iron in brain physiology has focused on the neuropathological, effects due to iron-induced oxidative stress. However, our recent work has established a physiological relationship between the iron-mediated oxidative modification and normal neuronal function. Our results obtained from hippocampal neurons, suggest that iron-generated reactive species oxygen (ROS) are involved in calcium signaling initiated by stimulation of NMDA receptors. This signal is amplified by ryanodine receptors (RyR), a redox- sensitive calcium channel, allowing the phosphorylation and nuclear translocation of ERK1/2. Furthermore, using electrophysiological approaches, we showed that iron is required for basal synaptic transmission and full expression of long-term potentiation, a type of synaptic plasticity. Our data combined suggest that the oxidative effect of iron is critical to activate processes that are downstream of NMDAR activation. Finally, due to the high reactivity of DNA with iron-generated ROS, we hypothesize an additional function of iron in gene regulation.

Keywords: iron, ryanodine receptor, synaptic plasticity, redox signaling, gene expression

Submitted: 11/07/11

Accepted: 11/08/11

http://dx.doi.org/10.4161/cib.18710

Correspondence to: Pablo Muñoz;
Email: pablo.munoz@cinv.cl

Addendum to: Muñoz P, Humeres A, Elgueta C, Kirkwood A, Hidalgo C, Núñez MT. Iron mediates N-methyl-D-aspartate receptor-dependent stimulation of calcium-induced pathways and hippocampal synaptic plasticity. J Biol Chem 2011; 286:13382–92; PMID:21296883; http://dx.doi.org/10.1074/jbc.M110.213785

Due to its ability to accept or transfer electrons, iron participates in a series of redox reactions[1,2] such as the Fenton reaction that generates hydroxyl free radicals or the Haber-Weiss reaction that combines the reduction of Fe^{3+} by superoxide plus oxygen to produce Fe^{2+}.

When iron accumulates, it can promote oxidative stress which in turn triggers neuronal death.[3] As a result of iron-mediated reactions, the study of iron in the brain has been focused on its neuropathological role.[4,5] However, our recent work has established a physiological relationship between the iron-mediated oxidative modification and normal neuronal function.[6] This work also suggests that iron-generated reactive species oxygen (ROS) could be a new class of molecules that act as second messengers in signaling cascades related to synaptic plasticity (SP), the putative cellular substrate of memory.

Consistent with a potential physiological role of iron, the activation of N-Methyl-D-aspartate (NMDA) receptors (NMDAR) induces iron uptake in cultured cortical neurons (**Fig. 1**), which in turn induces the production of the hydroxyl free radical- mediated Fenton reaction.[2] Similarly, in cultured hippocampal neurons, we showed that the entry of iron rapidly increased labile iron for the Fenton reaction.[6]

Why are there different mechanisms to incorporate iron, after neuronal activity? We propose that iron uptake is needed to provide a neuronal oxidative tone necessary for the proper functioning and operation of components sensitive to this tone. In agreement with this idea, ryanodine receptors (RyR), a channel involved in calcium- induced calcium release (CICR) which activates various signaling pathways involved in synaptic plasticity,[7] has a group of oxidation-sensitive cysteines[8] that could be one of the putative targets of iron-mediated oxidative attacks, as proposed by earlier

work in PC12 cells.[9] Our latest work in hippocampal neurons suggests that iron-generated ROS are involved in calcium signaling initiated by stimulation of NMDA receptors and amplified by the RyR.[6] An iron chelator, deferrioxamine, prevents CICR as well as the phosphorylation and nuclear translocation of ERK 1/2 necessary to establish synaptic plasticity (SP) and gene expression-dependent

A Rule-based Model of a Hypothetical Zombie Outbreak: Insights on the role of emotional factors during behavioral adaptation of an artificial population

Felipe Nuñez[1], Cesar Ravello[1], Hector Urbina[1], Tomas Perez-Acle[1,2*]
1 Computational Biology Lab (DLab), Fundación Ciencia & Vida
Avenida Zañartu 1482, Ñuñoa, Santiago, Chile
2 Centro Interdisciplinario de Neurociencias de Valparaíso, Universidad de Valparíso
Valparaíso, Chile
* **Corresponding author: tomas@dlab.cl**

Abstract

Models of infectious diseases have been developed since the first half of the twentieth century. There are different approaches to model an infectious outbreak, especially in terms of how individuals and their interactions are defined and treated. Most models haven't considered the role that emotional factors of the individual may play on the population's behavioral adaptation during the spread of a pandemic disease. Considering that local interactions among individuals generate patterns that -at a large scale- govern the action of masses, we have studied the behavioral adaptation of a population induced by the spread of an infectious disease. Therefore, we have developed a rule-based model of a hypothetical zombie outbreak, written in *Kappa* language, and simulated using Guillespie's stochastic approach. Our study addresses the specificity and heterogeneity of the system at the individual level, a highly desirable characteristic, mostly overlooked in classic epidemic models. Together with the basic elements of a typical epidemiological model, our model includes an individual representation of the disease progression and the traveling of agents among cities being affected. It also introduces an approximation to measure the effect of panic in the population as a function of the individual situational awareness. In addition, the effect of two possible countermeasures to overcome the zombie threat is considered: the availability of medical treatment and the deployment of special armed forces. However, due to the special characteristics of this hypothetical infectious disease, even using exaggerated numbers of countermeasures, only a small percentage of the population can be saved at the end of the simulations. As expected from a rule-based model approach, the global dynamics of our model resulted primarily governed by the mechanistic description of local interactions occurring at the individual level. As a whole, people's situational awareness resulted essential to modulate the inner dynamics of the system.

Introduction

Zombies are fictitious entities described in tales throughout history as human beings that, through various methods, have passed from a cataleptic state to a pseudo-life, lacking self control [1,2]. The etymological origin of the Zombie word can be traced to the Voodoo cult, in which, according to the controversial work of Davis [3,4], a cataleptic person -induced by a toxin or venom- could be raised from the grave by a wizard to be turned into his slave. However, the actual and more popular concept is from films of George A. Romero, among others, in which a zombie is a human affected by a highly contagious disease -typically a virus- that turns him into a mindless and wandering being with an insatiable hunger for human flesh [5,6]. According to pop culture, zombies represent entities that generate social chaos, similar to large scale outbreaks of infectious diseases, leading to a state of catastrophe, affecting people physical and emotionally [2,6,7]. Thus, the imaginary zombie scenario dictates that societies suffering an outbreak are inevitably driven to a loss of control and misrule. In this setting, decisions are taken by considering only local and immediate information elements regarding the situation to be surpassed, mainly focused on surviving [6,7]. At the governmental rank, decisions are taken by considering the

GLIA

GLIA 60:53–68 (2012)

Glucose Increases Intracellular Free Ca^{2+} in Tanycytes via ATP Released Through Connexin 43 Hemichannels

JUAN A. ORELLANA,[1] PABLO J. SÁEZ,[1] CHRISTIAN CORTÉS-CAMPOS,[2] ROBERTO J. ELIZONDO,[2] KENJI F. SHOJI,[1] SUSANA CONTRERAS-DUARTE,[1] VANIA FIGUEROA,[1] VICTORIA VELARDE,[1] JEAN X. JIANG,[3] FRANCISCO NUALART,[2] JUAN C. SÁEZ,[1,4] AND MARÍA A. GARCÍA[2]*

[1]*Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago, Chile*
[2]*Departamento de Biología Celular, Universidad de Concepción, Concepción, Chile*
[3]*Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas*
[4]*Instituto Milenio, Centro Interdisciplinario de Neurociencias de Valparaíso, Valparaíso, Chile*

KEY WORDS
glucosensing; hypothalamus; glucokinase; connexons

ABSTRACT
The ventromedial hypothalamus is involved in regulating feeding and satiety behavior, and its neurons interact with specialized ependymal-glial cells, termed tanycytes. The latter express glucose-sensing proteins, including glucose transporter 2, glucokinase, and ATP-sensitive K^+ (K_{ATP}) channels, suggesting their involvement in hypothalamic glucosensing. Here, the transduction mechanism involved in the glucose-induced rise of intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in cultured β-tanycytes was examined. Fura-2AM time-lapse fluorescence images revealed that glucose increases the intracellular Ca^{2+} signal in a concentration-dependent manner. Glucose transportation, primarily via glucose transporters, and metabolism via anaerobic glycolysis increased connexin 43 (Cx43) hemichannel activity, evaluated by ethidium uptake and whole cell patch clamp recordings, through a K_{ATP} channel-dependent pathway. Consequently, ATP export to the extracellular milieu was enhanced, resulting in activation of purinergic $P2Y_1$ receptors followed by inositol trisphosphate receptor activation and Ca^{2+} release from intracellular stores. The present study identifies the mechanism by which glucose increases $[Ca^{2+}]_i$ in tanycytes. It also establishes that Cx43 hemichannels can be rapidly activated under physiological conditions by the sequential activation of glucosensing proteins in normal tanycytes.

INTRODUCTION

The ventromedial hypothalamus (VMH) is involved in regulating feeding and satiety behaviors through their capacity to detect changes in glucose concentrations (Levin et al., 2004). The arcuate nucleus (AN) and the ventromedial nucleus (VMN) form the VMH; their neurons are in close contact with highly elongated ependymal-glial cells known as tanycytes (Akmayev and Popov, 1977; Chauvet et al., 1995; Flament-Durand and Brion, 1985). Tanycytes are the main glial cells present in the basal hypothalamus (García et al., 2001, 2003; Millan et al., 2010) and are classified into four types, according to their localization in the III–V ventricle and biochemical and molecular properties: α1, α2, β1, and β2 (Akmayev and Fidelina, 1974; Akmayev and Popov, 1977). α1- and α2-tanycytes are localized beside the VMN, while β1-tanycytes are localized within the lower lateral wall of the third ventricle, contacting neurons through cell processes in the AN as well as capillaries in the hypothalamus (García et al., 2001). β2-tanycytes are found in the floor of third ventricle lining the median eminence (García et al., 2001).

Both α and β tanycytes express several glucose-sensing molecules, including glucose transporter 2 (GLUT2) and ATP-sensitive K^+ (K_{ATP}) channels, suggesting their possible involvement in hypothalamus-mediated glucosensing (Alvarez et al., 1996; García et al., 2003; Millan et al., 2010; Navarro et al., 1996). Notably, unlike α-tanycytes, high expression of GLUT2 and glucokinase (GK) in the proximal pole has been observed in β1-tanycytes *in vivo* (García et al., 2003; Millan et al., 2010). The specific localization of β1 tanycytes in direct contact with cerebral spinal fluid and their prominent GLUT2/GK expression strongly support the idea that these cells have a high glucose uptake capacity. In fact, it has been proposed that β1-tanycytes could uptake and metabolize glucose to lactate through the glycolytic pathway, and subsequently export lactate to neurons of the AN through monocarboxylate transporters (MCTs) 1 and/or 4 (Cortés-Campos et al., 2011; García et al., 2003; Millan et al., 2010).

In support of the putative brain glucosensor role of tanycytes, it was recently demonstrated that extracellular glucose increases the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in α1- and α2-tanycytes (Frayling et al., 2011). Although expression of glucosensing proteins by different tanycyte subtypes is well-established, the

Additional Supporting Information may be found in the online version of this article.

Grant sponsor: CONICYT; Grant number: 24080055; Grant sponsor: FONDECYT; Grant number: 1100705; Grant sponsor: FONDECYT; Grant numbers: 1111033, 1100396; Grant sponsor: FONDEF; Grant number: DO7I1086; Grant sponsor: Anillo; Grant number: ACT-71; Grant sponsor: NIH; Grant number: ARO46798; Grant sponsor: Welch Foundation; Grant number: AQ-1507.

*Correspondence to: María A. García, Departamento de Biología Celular, Universidad de Concepción, Concepción, Chile. E-mail: mgarcia@udec.cl or Juan A. Orellana, Departamento de Fisiología, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile. E-mail: jaorella@uc.cl

Received 10 June 2011; Accepted 31 August 2011

DOI 10.1002/glia.21246

Published online 10 October 2011 in Wiley Online Library (wileyonlinelibrary.com).

Rev. Neurosci., Vol. 23(2): 163–177, 2012 • Copyright © by Walter de Gruyter • Berlin • Boston. DOI 10.1515/revneuro-2011-0065

Glial hemichannels and their involvement in aging and neurodegenerative diseases

Juan A. Orellana[1,*], Rommy von Bernhardi[1], Christian Giaume[2–4] and Juan C. Sáez[5,6]

[1]Facultad de Medicina, Departamento de Neurología, Pontificia Universidad Católica de Chile, Santiago, Chile
[2]Center for Interdisciplinary Research in Biology (CIRB), CNRS UMR 7241/INSERM U1050, Collège de France, 75005 Paris, France
[3]University Pierre et Marie Curie, ED, N° 158, 75005 Paris, France
[4]MEMOLIFE Laboratory of Excellence and Paris Science Lettre, Collège de France, 75005 Paris, France
[5]Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago, Chile
[6]Instituto Milenio, Centro Interdisciplinario de Neurociencias de Valparaíso, Valparaíso, Chile

*Corresponding author
e-mail: jaorella@uc.cl

Abstract

During the last two decades, it became increasingly evident that glial cells accomplish a more important role in brain function than previously thought. Glial cells express pannexins and connexins, which are member subunits of two protein families that form membrane channels termed hemichannels. These channels communicate intra- and extracellular compartments and allow the release of autocrine/paracrine signaling molecules [e.g., adenosine triphosphate (ATP), glutamate, nicotinamide adenine dinucleotide, and prostaglandin E_2] to the extracellular milieu, as well as the uptake of small molecules (e.g., glucose). An increasing body of evidence has situated glial hemichannels as potential regulators of the beginning and maintenance of homeostatic imbalances observed in diverse brain diseases. Here, we review and discuss the current evidence about the possible role of glial hemichannels on neurodegenerative diseases. A subthreshold pathological threatening condition leads to microglial activation, which keeps active defense and restores the normal function of the central nervous system. However, if the stimulus is deleterious, microglial cells and the endothelium become overactivated, both releasing bioactive molecules (e.g., glutamate, cytokines, prostaglandins, and ATP), which increase the activity of glial hemichannels, reducing the astroglial neuroprotective functions, and further reducing neuronal viability. Because ATP and glutamate are released via glial hemichannels in neurodegenerative conditions, it is expected that they contribute to neurotoxicity. More importantly, toxic molecules released via glial hemichannels could increase the Ca^{2+} entry in neurons also via neuronal hemichannels, leading to neuronal death. Therefore, blockade of hemichannels expressed by glial cells and/or neurons during neuroinflammation might prevent neurodegeneration.

Keywords: astrocytes; brain; connexins; inflammation; microglia; pannexins.

Introduction

In the central nervous system (CNS), the two main cellular components are glial cells and neurons. The former constitute ~90% of CNS cells, and the latter represent just ~10%. However, both cellular types contribute in equal form (50% and 50%) to the total cell mass of the brain (Verkhratsky and Toescu, 2006). Glial cells are divided into macroglia (oligodendrocytes, astrocytes, and ependymoglial cells) and microglia, which are from neuroectodermal and mesenchymal origin, respectively. Whereas neurons and macroglial cells are endogenous cells of the brain, microglia invade the CNS early during embryonic development (Carson et al., 2006). For a long time, glial cells were considered as part of the brain connective tissue that provides support to neurons. Nevertheless, during the last two decades, it became evident that glial cells have more significant roles in brain function than previously thought.

The brain performs exceptionally complex and dynamic tasks that depend on the coordinated interaction of endothelial cells, macroglial cells, microglia, and neurons. For instance, hundreds of astrocytes can be connected with each other (Giaume et al., 2010). Such astrocyte-to-astrocyte intercellular communication is attained by sharing cytoplasmic content through membrane specializations termed gap junctions. These cell junctions are aggregates of few tens to thousands of intercellular conduits termed gap junction channels (GJCs) that allow direct but selective cytoplasmic continuity between contacting cells. Through GJCs, the intercellular exchange of metabolites (e.g., ADP, glucose, glutamate, and glutathione), second messengers (e.g., cyclic adenosine monophosphate and inositol triphosphate), and the intercellular spread of electrotonic potentials in excitable and non-excitable tissues (Sáez et al., 2003; Sohl and Willecke, 2004; Evans et al., 2006) are possible. Whereas a GJC is formed by the serial docking of two hemichannels (each one contributed by one of two adjacent cells), each hemichannel is composed of six protein subunits termed connexins (Cxs). The latter belong to a highly conserved protein family encoded by 21 genes in humans and 20 in mice with orthologs in other vertebrate species (Cruciani and Mikalsen, 2005).

J Neurophysiol 108: 3009–3023, 2012.
First published September 5, 2012; doi:10.1152/jn.01033.2011.

Role of I_h in the firing pattern of mammalian cold thermoreceptor endings

Patricio Orio,[1] Andrés Parra,[2] Rodolfo Madrid,[3] Omar González,[2,4] Carlos Belmonte,[2] and Félix Viana[2]

[1]*Centro Interdisciplinario de Neurociencia de Valparaíso (CINV) and Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile;* [2]*Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-CSIC, Alicante, Spain;* [3]*Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile; and* [4]*Fundación de Investigación Oftalmológica, Instituto Fernandez-Vega, Oviedo, Spain*

Submitted 11 November 2011; accepted in final form 30 August 2012

Orio P, Parra A, Madrid R, González O, Belmonte C, Viana F. Role of I_h in the firing pattern of mammalian cold thermoreceptor endings. *J Neurophysiol* 108: 3009–3023, 2012. First published September 5, 2012; doi:10.1152/jn.01033.2011.—Mammalian peripheral cold thermoreceptors respond to cooling of their sensory endings with an increase in firing rate and modification of their discharge pattern. We recently showed that cultured trigeminal cold-sensitive (CS) neurons express a prominent hyperpolarization-activated current (I_h), mainly carried by HCN1 channels, supporting subthreshold resonance in the soma without participating in the response to acute cooling. However, peripheral pharmacological blockade of I_h, or characterization of HCN1$^{-/-}$ mice, reveals a deficit in acute cold detection. Here we investigated the role of I_h in CS nerve endings, where cold sensory transduction actually takes place. Corneal CS nerve endings in mice show a rhythmic spiking activity at neutral skin temperature that switches to bursting mode when the temperature is lowered. I_h blockers ZD7288 and ivabradine alter firing patterns of CS nerve endings, lengthening interspike intervals and inducing bursts at neutral skin temperature. We characterized the CS nerve endings from HCN1$^{-/-}$ mouse corneas and found that they behave similar to wild type, although with a lower slope in the firing frequency vs. temperature relationship, thus explaining the deficit in cold perception of HCN1$^{-/-}$ mice. The firing pattern of nerve endings from HCN1$^{-/-}$ mice was also affected by ZD7288, which we attribute to the presence of HCN2 channels in the place of HCN1. Mathematical modeling shows that the firing phenotype of CS nerve endings from HCN1$^{-/-}$ mice can be reproduced by replacing HCN1 channels with the slower HCN2 channels rather than by abolishing I_h. We propose that I_h carried by HCN1 channels helps tune the frequency of the oscillation and the length of bursts underlying regular spiking in cold thermoreceptors, having important implications for neural coding of cold sensation.

hyperpolarization-activated current; HCN channel; subthreshold oscillation; bursting; cold thermoreceptors

MAMMALIAN COLD THERMORECEPTORS are sensory terminals specialized in the detection of innocuous cold/cool temperatures impinging on the skin (Hensel 1981). The transduction of cold stimuli takes place in the free nerve endings of the peripheral axons of cold thermoreceptor neurons from trigeminal and dorsal root ganglia. They display spontaneous, tonic or bursting, spike activity at neutral skin temperatures (34°C) that is accelerated by cooling the receptive field and suppressed by warming (Braun et al. 1980; Brock et al. 2001; Dykes 1975; Iggo 1969). The average frequency of their static discharge has a bell-shaped relationship with respect to skin temperature, attaining the maximum frequency at temperatures ranging from 18°C to 34°C. Therefore, the action potential rate cannot encode unambiguously the peripheral temperature under static conditions (Bade et al. 1979; Dykes 1975; Hensel and Wurster 1970), suggesting that discrimination depends on the temporal structure of the impulse sequence (Braun et al. 1980; Hensel 1981). At static temperatures above ~30°C the activity consists mainly in regularly fired single spikes, while at lower temperatures the bursting activity prevails (Iggo 1969). The transitions between these different spiking patterns are continuous, and their temporal structure suggests that all the different patterns can be attributed to slow oscillations of the membrane potential that are systematically altered by temperature (Braun et al. 1980, 1998; Schafer et al. 1991). Acute cooling leads to a transient rise in mean frequency caused by an immediate increase in the frequency of bursts followed by an increase in burst duration and number of impulses per burst while the frequency of bursts decreases (Braun et al. 1980). There is growing experimental evidence showing that bursting is involved in the transmission of behaviorally relevant information in other sensory systems (Krahe and Gabbiani 2004), and it has been proposed that bursting enhances the sensitivity and working range of cold receptors (Iggo 1969). However, the contribution of this temporal firing code to the psychophysical capacities for cold discrimination in vivo has not been established.

The cellular and molecular mechanisms that confer specificity for thermal detection at cold nerve endings have been unveiled in recent times. Cooling and cooling compounds, like menthol, activate TRPM8, a nonselective, calcium-permeable cation channel of the transient receptor potential family (reviewed in Babes et al. 2011 and Latorre et al. 2011; McKemy et al. 2002; Peier et al. 2002). This channel is selectively expressed in cold-sensitive (CS) neurons and is a major determinant of their cold sensitivity (Bautista et al. 2007; Colburn et al. 2007; Dhaka et al. 2007). CS neurons also possess background K^+ channels that are closed by cooling, contributing to depolarization and firing (Reid and Flonta 2001; Viana et al. 2002), and it has been shown that these channels participate in cold perception (Noel et al. 2009). In contrast, the ionic mechanisms underlying the rhythmic firing activity exhibited by peripheral cold receptors are still unknown. Experimental evidence suggests the involvement of slow, TTX-resistant persistent sodium currents (Brock et al. 1998; Herzog et al. 2001) and low-threshold calcium channels (Schafer et al. 1982, 1991) in the underlying oscillations that generate rhythmic firing. Mathematical simulations (Braun et al. 1998; Longtin

Address for reprint requests and other correspondence: P. Orio, Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso, Gran Bretaña 1111, 2360102 Valparaiso, Chile (e-mail: patricio.orio@uv.cl).

OPEN ACCESS Freely available online

PLoS one

Simple, Fast and Accurate Implementation of the Diffusion Approximation Algorithm for Stochastic Ion Channels with Multiple States

Patricio Orio[1]*, Daniel Soudry[2,3]

1 Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile, **2** Department of Electrical Engineering, Technion, Haifa, Israel, **3** Laboratory for Network Biology Research, Technion, Haifa, Israel

Abstract

Background: The phenomena that emerge from the interaction of the stochastic opening and closing of ion channels (channel noise) with the non-linear neural dynamics are essential to our understanding of the operation of the nervous system. The effects that channel noise can have on neural dynamics are generally studied using numerical simulations of stochastic models. Algorithms based on discrete Markov Chains (MC) seem to be the most reliable and trustworthy, but even optimized algorithms come with a non-negligible computational cost. Diffusion Approximation (DA) methods use Stochastic Differential Equations (SDE) to approximate the behavior of a number of MCs, considerably speeding up simulation times. However, model comparisons have suggested that DA methods did not lead to the same results as in MC modeling in terms of channel noise statistics and effects on excitability. Recently, it was shown that the difference arose because MCs were modeled with coupled gating particles, while the DA was modeled using uncoupled gating particles. Implementations of DA with coupled particles, in the context of a specific kinetic scheme, yielded similar results to MC. However, it remained unclear how to generalize these implementations to different kinetic schemes, or whether they were faster than MC algorithms. Additionally, a steady state approximation was used for the stochastic terms, which, as we show here, can introduce significant inaccuracies.

Main Contributions: We derived the SDE explicitly for any given ion channel kinetic scheme. The resulting generic equations were surprisingly simple and interpretable – allowing an easy, transparent and efficient DA implementation, avoiding unnecessary approximations. The algorithm was tested in a voltage clamp simulation and in two different current clamp simulations, yielding the same results as MC modeling. Also, the simulation efficiency of this DA method demonstrated considerable superiority over MC methods, except when short time steps or low channel numbers were used.

Citation: Orio P, Soudry D (2012) Simple, Fast and Accurate Implementation of the Diffusion Approximation Algorithm for Stochastic Ion Channels with Multiple States. PLoS ONE 7(5): e36670. doi:10.1371/journal.pone.0036670

Editor: Michele Giugliano, University of Antwerp, Belgium

Received October 28, 2011; **Accepted** April 11, 2012; **Published** May 22, 2012

Funding: This work was supported by Fondecyt Grant 11090308 to PO (Conicyt, Chile). The Centro Interdisciplinario de Neurociencia de Valparaíso is funded by Grant P09-022-F of the Millennium Science Institute (Ministerio de Economía, Chile). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: patricio.orio@uv.cl

Introduction

Noise and variability are present throughout the nervous system, from sensory systems to the motor output and perhaps more importantly in the higher brain areas [1]. Far from being considered as a nuisance, noise is now argued to be one of the key elements that shape the way the central nervous system (CNS) codes sensory inputs, builds internal representations and makes decisions [2]. Phenomena like stochastic resonance [3,4,5,6] enhance several aspects of sensory coding and signal detection [7,8]. Also, noise can be beneficial in various computational tasks [9,10,11,12].

One of the main sources of noise and variability is the stochastic opening and closing of ion channels, commonly called *channel noise* [13,14]. The effects of channel noise on neuronal excitability are to a large extent studied with the use of mathematical models, either by constructing and analyzing models with stochastic channels [15,16,17,18] or by introducing a noisy conductances in dynamic clamp experiments [19,20]. It is of interest, then, to develop and analyze numerical models that faithfully reproduce the stochastic nature of ion channels. It is also of interest to develop fast algorithms that can be used in large scale simulations of neural networks or in real time simulation for dynamic clamp experiments.

Ion channels are commonly modeled using the framework established by Hodgkin and Huxley [21], see also [22]. In this framework, ion channels contain one or more *gating particles* that can be either in a resting or active state. The transition rates between states are voltage-dependent, and now we know that this is because these particles contain a charged domain (the *voltage sensor*) that senses the membrane electrical potential [23]. In the pure Hodgkin and Huxley (HH) framework, the probability of a channel being open is equal to the probability of all its gating particles being active. Usually the particles are assumed to be independent and thus the probability of the open channel is the product of the probabilities of the active particles. In the limit of

THE ANATOMICAL RECORD 295:980–990 (2012)

Connexin 36 is Expressed in Beta and Connexins 26 and 32 in Acinar Cells at the End of the Secondary Transition of Mouse Pancreatic Development and Increase During Fetal and Perinatal Life

ELIA MARTHA PÉREZ-ARMENDARIZ,[1]* LOURDES CRUZ-MIGUEL,[1] CRISTINA CORONEL-CRUZ,[1] MARCELINO ESPARZA-AGUILAR,[1] ENRIQUE PINZON-ESTRADA,[2] ELIZABETH RANCAÑO-CAMACHO,[1] GERARDO ZACARIAS-CLIMACO,[1] PAOLA FERNÁNDEZ OLIVARES,[4] ANA MARIA ESPINOSA,[3] INGEBORG BECKER,[1] JUAN C. SÁEZ,[4,5] JAIME BERUMEN,[1,3] AND GREGORIO PÉREZ-PALACIOS[1]

[1]Departamento de Medicina Experimental, Facultad de Medicina, Universidad Nacional Autónoma de México, México, D.F., 04510, México

[2]Bioterio de la Facultad de Medicina, Universidad Nacional Autónoma de México, México, D.F., 04510, México

[3]Unidad de Medicina Genómica, Hospital General de México, México, D. F., 06726, México

[4]Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago, Chile

[5]Instituto Milenio, Centro Interdisciplinario de Neurociencias de Valparaíso, Valparaíso, Chile

ABSTRACT

To identify when during fetal development connexins (Cxs) 26 (Cx26) 32 (Cx32), and 36 (Cx36) begin to be expressed, as well as to characterize their spatial distribution, real time polymerase chain reaction and immunolabeling studies were performed. Total RNA from mouse pancreases at 13 and 18 days postcoitum (dpc) and 3 days postpartum (dpp) was analyzed. In addition, pancreatic sections of mouse at 13, 14, 15, 16, 18 dpc and 3 dpp and of rat at term were double labeled with either anti-insulin or anti-α-amylase and anti-Cx26 or -Cx32 or -Cx36 antibodies and studied with confocal microscopy. From day 13 dpc, Cxs 26, 32, and 36 transcripts were identified and their levels increased with age. At 13–14 dpc, Cxs 26 and 32 were localized in few acinar cells, whereas Cx36 was distributed in small beta cell clumps. From day 14 dpc onwards, the number of labeled cells and relative immunofluorescent reactivity of all three Cxs at junctional membranes of the respective cell types increased. Cxs 26 and 32 colocalized in fetal acinar cells. In rat pancreas at term, a similar connexin distribution was found. Relative Cxs levels evaluated by immunoblotting also increased (two-fold) in pancreas homogenates from day 18 dpc to 3 dpp. The early cell specific, wide distribution, and age dependent expression of Cxs 26, 32, and 36 during fetal pancreas ontogeny suggests their possible involvement in pancreas differentiation and prenatal maturation. Anat Rec, 295:980–990, 2012. © 2012 Wiley Periodicals, Inc.

Grant sponsor: Pfizer Scientific Institute in Mexico; Grant sponsor: CONACYT; Grant number: 51243Q; Grant sponsor: DGAPA (Mexico); Grant number: IN219008 and IN231011; Grant sponsor: Anillo (Chile); Grant number: ACT71.

*Correspondence to: E. Martha Pérez-Armendariz, MD, PhD, Laboratorio de sinapsis eléctricas, Torre de Investigación, 5to piso, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universitaria, 3000, Copilco Universidad, 04510, México D.F., Mexico. Fax: 5255) 57610249. E-mail: mperezarmendariz@aol.com

Received 13 October 2010; Accepted 6 March 2012.

DOI 10.1002/ar.22473

Published online 16 April 2012 in Wiley Online Library (wileyonlinelibrary.com).

Chapter 11

Mutagenesis and Temperature-Sensitive Little Machines

María Pertusa, Hans Moldenhauer, Sebastián Brauchi, Ramón Latorre, Rodolfo Madrid and Patricio Orio

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/50333

1. Introduction

In mammals a class of ion channels able to sense a wide range of temperatures (0-60 °C) has evolved. These molecular thermodynamic machines called thermo Transient Receptor Potential (thermoTRP) are spread through the different TRP channel subfamilies having members inside the TRPM (melastatin) subfamily, where TRPM2, TRPM3, TRPM4 and TRPM5 are heat-activated, whereas TRPM8 is activated by cold. The TRPV (vanilloid) subfamily contains four thermoTRP channels (TRPV1, TRPV2, TRPV3 and TRPV4), which are all activated by heat; and TRPA1 (ankyrin) channel which is activated by noxious cold (reviewed in [28, 107], Figure 1). More recently, a member of TRPC (canonical) subfamily, TRPC5, was identified as a cold receptor in the temperature range 37-25 °C [1].

Located in cutaneous nerve endings of thermoreceptors and nociceptors, and because extreme temperatures produce discomfort and pain, thermoTRP channels are involved in nociception and can be activated by a long list of other noxious stimuli such as low pH and irritant chemicals [2].

What characterizes these channels is their exquisite temperature sensitivity. Thermodynamic analyses reveal that thermoTRP channels undergo large enthalpy changes (ΔH) that account for their high temperature sensitivity [3-8]. For example, the enthalpy change between close and open in TRPV1 and TRPM8 involves ΔHs of ~100 kcal/mol and ~-60 kcal/mol, respectively [3, 5]. It is obvious that in order to make the closed-open reaction reversible these enthalpy changes must be accompanied by large entropy (ΔS) changes. These activation enthalpies are 3-5 times the enthalpy change for voltage- or ligand-dependent channel gating (ΔH ~20 kcal/mol; [108]). Actually, Yao et al. [7] pointed out that in the case of TRPV1, the ΔH involved in the closed-open transition is equivalent to an electrical energy moving 71 unit charges across 60 mV!

View Online / Journal Homepage / Table of Contents for this issue
Dynamic Article Links

Molecular BioSystems

Cite this: *Mol. BioSyst.*, 2012, **8**, 685–698

www.rsc.org/molecularbiosystems

REVIEW

Modulation of gap junction channels and hemichannels by growth factors

Kurt A. Schalper,[a] Manuel A. Riquelme,[b] María C. Brañes,[b] Agustín D. Martínez,[c] José Luis Vega,[bd] Viviana M. Berthoud,[e] Michael V. L. Bennett[f] and Juan C. Sáez*[bc]

Received 14th July 2011, Accepted 4th November 2011
DOI: 10.1039/c1mb05294b

Gap junction hemichannels and cell–cell channels have roles in coordinating numerous cellular processes, due to their permeability to extra and intracellular signaling molecules. Another mechanism of cellular coordination is provided by a vast array of growth factors that interact with relatively selective cell membrane receptors. These receptors can affect cellular transduction pathways, including alteration of intracellular concentration of free Ca^{2+} and free radicals and activation of protein kinases or phosphatases. Connexin and pannexin based channels constitute recently described targets of growth factor signal transduction pathways, but little is known regarding the effects of growth factor signaling on pannexin based channels. The effects of growth factors on these two channel types seem to depend on the cell type, cell stage and connexin and pannexin isoform expressed. The functional state of hemichannels and gap junction channels are affected in opposite directions by FGF-1 *via* protein kinase-dependent mechanisms. These changes are largely explained by channels insertion in or withdrawal from the cell membrane, but changes in open probability might also occur due to changes in phosphorylation and redox state of channel subunits. The functional consequence of variation in cell–cell communication *via* these membrane channels is implicated in disease as well as normal cellular responses.

1. Introduction

Cells within an organism communicate with each other in a number of ways. They may release hormones, neurotransmitters and other molecules that act on distant cells (as in the endocrine system) or nearby (paracrine actions, where there may also be autocrine actions on the secreting cells). In addition, cell–cell communication at chemical synpases is mediated by secreted molecules. These types of intercellular communication require the presence of specific receptors in responding cells. Usually, activation of metabotropic or ionotropic receptors is initiated by ligand binding. The former type leads to the generation of second messengers and possibly a cascade of events generating cell-specific responses, and the latter type allows permeation of ions that may have signaling functions in addition to carrying charge.

Intercellular communication can also occur without the release of substances to the extracellular space. Direct communication between contacting cells can be mediated by specialized plasma membrane structures termed gap junctions,[1] which contain intercellular channels that directly connect the cytoplasms of adjacent cells. Each gap junction channel is composed of two hemichannels also called connexons. Intercellular communication through gap junctions allows for cell groups to share ions, metabolites and second messengers. Thus, gap junctions permit a coordinated response to a wide range of stimuli, even when some cells in a coupled population lack receptors for a particular extracellular signal. In addition, gap junction proteins can form undocked hemichannels in non-junctional cell membrane, enabling communication between cytoplasm and extracellular milieu. In this case, open hemichannels become routes for autocrine/paracrine interactions through the diffusional transport of small signaling molecules.

For more detailed information regarding structure and functions of gap junction channels and hemichannels, readers are referred to more comprehensive reviews published elsewhere.[2–7]

Although hemichannels are formed of the same subunits as gap junction channels and can have similar electrical properties and permeability, mounting evidence indicates that the two

[a] *Servicio de Anatomía Patológica, Clínica Alemana de Santiago, Facultad de Medicina, Clínica Alemana - Universidad del Desarrollo, Santiago, Chile*
[b] *Departamento de Fisiología, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile. E-mail: jsaez@bio.puc.cl; Fax: +56 26862862; Tel: +56 22225515*
[c] *Instituto Milenio, Centro Interdisciplinario de Neurociencias de Valparaíso, Universidad de Valparaíso, Valparaíso, Chile*
[d] *Laboratorio de Fisiología Experimental (EPhyL), Universidad de Antofagasta, Antofagasta, Chile*
[e] *Department of Pediatrics, Section of Hematology/Oncology, University of Chicago, Chicago, IL, USA*
[f] *Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, USA*

The FASEB Journal • Research Communication

Release of gliotransmitters through astroglial connexin 43 hemichannels is necessary for fear memory consolidation in the basolateral amygdala

Jimmy Stehberg,[*,‡,1] Rodrigo Moraga-Amaro,[*] Christian Salazar,[*] Alvaro Becerra,[†] Cesar Echeverría,[†] Juan A. Orellana,[§] Geert Bultynck,[¶] Raf Ponsaerts,[¶] Luc Leybaert,[#] Felipe Simon,[†,§§] Juan C. Sáez,[‖,] and Mauricio A. Retamal[††]**

[*]Laboratorio de Neurobiologia and [†]Laboratorio de Fisiopatologia Integrativa, Departamento de Ciencias Biologicas, Facultad de Ciencias Biologicas and Facultad de Medicina, and [‡]Centro de Investigaciones Biomédicas, Universidad Andres Bello, Santiago, Chile; [§]Departamento de Neurología, Facultad de Medicina, and [‖]Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago, Chile; [¶]Laboratory of Molecular and Cellular Signaling, Department of Cellular and Molecular Medicine, Katholieke Universiteit Leuven, Leuven, Belgium; [#]Department of Basic Medical Sciences, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium; [**]Instituto Milenio, Centro Interdiciplinario de Neurociencias de Valparaíso, Valparaíso, Chile; [††]Departamento de Fisiología, Facultad de Medicina, Clínica Alemana–Universidad del Desarrollo, Santiago, Chile; and [§§]Millennium Institute on Immunology and Immunotherapy, Santiago, Chile

ABSTRACT **Recent *in vitro* evidence indicates that astrocytes can modulate synaptic plasticity by releasing neuroactive substances (gliotransmitters). However, whether gliotransmitter release from astrocytes is necessary for higher brain function *in vivo*, particularly for memory, as well as the contribution of connexin (Cx) hemichannels to gliotransmitter release, remain elusive. Here, we microinfused into the rat basolateral amygdala (BLA) TAT-Cx43L2, a peptide that selectively inhibits Cx43-hemichannel opening while maintaining synaptic transmission or interastrocyte gap junctional communication. *In vivo* blockade of Cx43 hemichannels during memory consolidation induced amnesia for auditory fear conditioning, as assessed 24 h after training, without affecting short-term memory, locomotion, or shock reactivity. The amnesic effect was transitory, specific for memory consolidation, and was confirmed after microinfusion of Gap27, another Cx43-hemichannel blocker. Learning capacity was recovered after coinfusion of TAT-Cx43L2 and a mixture of putative gliotransmitters (glutamate, glutamine, lactate, D-serine, glycine, and ATP). We propose that gliotransmitter release from astrocytes through Cx43 hemichannels is necessary for fear memory consolidation at the BLA. Thus, the present study is the first to demonstrate a physiological role for astroglial Cx43 hemichannels in brain function, making these channels a novel pharmacological target for the treatment of psychiatric disorders, including post-traumatic stress disorder.—Stehberg, J., Moraga-Amaro, R., Salazar, C., Becerra, A., Echeverría, C., Orellana, J. A., Bultynck, G., Ponsaerts, R., Leybaert, L., Simon, F., Sáez, J. C., Retamal, M. A. Release of gliotransmitters through astroglial connexin 43 hemichannels is necessary for fear memory consolidation in the basolateral amygdala. *FASEB J.* 26, 3649–3657 (2012). www.fasebj.org**

Key Words: astrocyte · amnesia · gliotransmitters · learning

DESPITE THE POPULAR BELIEF that neurons are the main constituents of the human brain, >90% of brain cells are not actually neurons, but star-shaped glial cells known as astrocytes. Their role—until recently—was believed to be neuron sustenance, neurotransmitter recycling, and maintenance of the blood-brain barrier. In recent years, it has become increasingly evident that their role in brain function may be more protagonistic than previously thought.

Hundreds of astrocytes can be connected simultaneously to allow collective metabolic and electric coupling, as well as calcium-wave signaling (1). Such interastrocyte communication is attained by sharing cytoplasmic content through special channels called gap junction channels (2), each formed by 2 hemichannels contributed by each adjacent cell (3). Each hemi-

Abbreviations: ATP, adenosine triphosphate; BLA, basolateral amygdala; CL, cytoplasmic loop; CS, conditioned stimulus; Cx, connexin; Cx43, connexin 43; DCFS, divalent cation-free solution; Etd, ethidium bromide; GFAP, glial fibrillary acidic protein; KO, knockout; LTM, long-term memory; LY, Lucifer yellow; MAP, microtubule-associated protein; STM, short-term memory; TAT-Cx43L2, TAT-associated connexin 43, L2 region mimetic peptide; US, unconditioned stimulus; VAAC, volume-activated anion channel

[1] Correspondence: Laboratorio de Neurobiologia, Departamento de Ciencias Biologicas, Facultad de Ciencias Biologicas and Facultad de Medicina, Universidad Andres Bello, Santiago, Chile. E-mail: jstehberg@unab.cl

doi: 10.1096/fj.11-198416

0892-6638/12/0026-3649 © FASEB

ZEBRAFISH
Volume 9, Number 2, 2012

DOI: 10.1089/zeb.2012.0734

Food and Conspecific Chemical Cues Modify Visual Behavior of Zebrafish, *Danio rerio*

Jessica F. Stephenson,[1,2] Julian C. Partridge,[1] and Kathleen E. Whitlock[2]

Abstract

Animals use the different qualities of olfactory and visual sensory information to make decisions. Ethological and electrophysiological evidence suggests that there is cross-modal priming between these sensory systems in fish. We present the first experimental study showing that ecologically relevant chemical mixtures alter visual behavior, using adult male and female zebrafish, *Danio rerio*. Neutral-density filters were used to attenuate the light reaching the tank to an initial light intensity of 2.3×10^{16} photons/s/m^2. Fish were exposed to food cue and to alarm cue. The light intensity was then increased by the removal of one layer of filter (nominal absorbance 0.3) every minute until, after 10 minutes, the light level was 15.5×10^{16} photons/s/m^2. Adult male and female zebrafish responded to a moving visual stimulus at lower light levels if they had been first exposed to food cue, or to conspecific alarm cue. These results suggest the need for more integrative studies of sensory biology.

Introduction

Aquatic environments are ideally suited for the transmission of olfactory information,[1,2] and therefore fish provide some of the best examples of this cross-modal interaction. Female swordtails (*Xiphophorus pygmaeus*),[3,4] sticklebacks (*Gasterosteus aculeatus*),[5] and Mexican pupfish (*Cyprinodon* spp.)[6] vary in their preference for heterospecific or conspecific mates, depending on which sensory modality, vision or olfaction, is stimulated. Similarly, darters appear to use olfactory cues to locate their invertebrate prey, but require the visual stimulus of movement to feed.[7,8] Alarm cue is released from damaged fish skin and provides reliable information about predation risk in the immediate environment,[9] but the response of the fish to it depends on a combination of chemical and visual cues. Hartman and Abrahams[10] found that fathead minnows, *Pimephales promelas*, were more likely to respond to an olfactory alarm signal in the absence of visual information about the risk of predation. If olfactory cues were able to 'prime' the visual sensory system,[11] individuals would be less likely to miss visual information, and more likely to act appropriately on receipt of visual cues.

Since the discovery that olfactory stimulation causes an electrophysiological response in fish retinae, it has been acknowledged among many physiologists that the olfactory and visual sensory systems are functionally linked.[12–14] The effect of an olfactory stimulus on visual sensitivity has to date only been tested using amino acids. Maaswinkel and Li,[15] using a similar method to that employed here, tested whether a range of isolated amino acids as olfactory stimuli enhanced visual sensitivity during behavioral trials. Their results suggest that although olfactory input does increase visual sensitivity, this effect depends on the amino acid used. As well as having different effects on vision, single amino acids can elicit a variety of behaviors in zebrafish.[16,17] How complex mixtures involving multiple amino acids and other classes of olfactory stimuli affect either vision or visual behavior is unknown, even though such mixtures inform most aspects of fish behavior.[2]

Zebrafish, *Danio rerio*, is an important model species for analysis of visual behavior[18–21] and olfactory behaviors,[22,23] as well as the possible link between them.[15] Zebrafish visual behavior is being used to demonstrate the effects of drugs (e.g., alcohol[24] and cocaine[25]). It is therefore important to characterize the factors that affect this behavior. In order to understand better the potential interactions between visual and olfactory sensory systems, we analyzed behavioral responses of adult zebrafish to food cue and conspecific alarm cue.[26] We show that the complex, ecologically relevant chemical mixtures of food cue and alarm cue affect visually-mediated behaviors, although the neuroanatomical basis for this interaction remains to be elucidated.

[1]School of Biological Sciences, University of Bristol, Bristol, United Kingdom.
[2]Centro Interdisciplinario de Neurociencia, Facultad de Ciencia, Universidad de Valparaíso, Valparaíso, Chile.

Cellular Requirements for LARK in the *Drosophila* Circadian System

Vasudha Sundram,*[,1] Fanny S. Ng,*[,1] Mary A. Roberts,* Carola Millán,[†,‡] John Ewer,[†] and F. Rob Jackson*[,2]

*Department of Neuroscience, Center for Neuroscience Research, Tufts University School of Medicine, Boston, MA, USA, [†]Interdisciplinary Neuroscience Center of Valparaiso, University of Valparaiso, Valparaíso, Chile, and [‡]Department of Science, Facultad de Artes Liberales y Facultad de Ingeniería, Universidad Adolfo Ibañez, Viña del Mar, Chile

Abstract RNA-binding proteins mediate posttranscriptional functions in the circadian systems of multiple species. A conserved RNA recognition motif (RRM) protein encoded by the *lark* gene is postulated to serve circadian output and molecular oscillator functions in *Drosophila* and mammals, respectively. In no species, however, has LARK been eliminated, *in vivo*, to determine the consequences for circadian timing. The present study utilized RNA interference (RNAi) techniques in *Drosophila* to decrease LARK levels in clock neurons and other cell types in order to evaluate the circadian functions of the protein. Knockdown of LARK in *timeless* (TIM)– or pigment dispersing factor (PDF)–containing clock cells caused a significant number of flies to exhibit arrhythmic locomotor activity, demonstrating a requirement for the protein in pacemaker cells. There was no obvious effect on PER protein cycling in *lark* interference (RNAi) flies, but a knockdown within the PDF neurons was associated with increased PDF immunoreactivity at the dorsal termini of the small ventral lateral neuronal (s-LNv) projections, suggesting an effect on neuropeptide release. The expression of *lark* RNAi in multiple neurosecretory cell populations demonstrated that LARK is required within pacemaker and nonpacemaker cells for the manifestation of normal locomotor activity rhythms. Interestingly, decreased LARK function in the prothoracic gland (PG), a peripheral organ containing a clock required for the circadian control of eclosion, was associated with weak population eclosion rhythms or arrhythmicity.

Key words clock output, posttranscriptional, RNA binding, locomotor activity, eclosion

In both prokaryotic and eukaryotic species, circadian rhythms in biochemistry, physiology, and behavior are governed by endogenous cellular clocks. Feedback loops that drive rhythmic changes in gene transcription are important components of the clocks governing circadian behavior (Hardin, 2005; Kadener et al., 2008). However, there is evidence in several species that certain types of circadian oscillators can function in the complete absence of rhythmic clock gene transcription (Lakin-Thomas, 2006;

1. These authors contributed equally to this work.
2. To whom all correspondence should be addressed: F. Rob Jackson, Department of Neuroscience, Center for Neuroscience Research, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA; e-mail: rob.jackson@tufts.edu.

JOURNAL OF BIOLOGICAL RHYTHMS, Vol. 27 No. 3, June 2012 183-195
DOI: 10.1177/0748730412440667

Journal of Physiology - Paris 106 (2012) 120–127

Contents lists available at SciVerse ScienceDirect

Journal of Physiology - Paris

journal homepage: www.elsevier.com/locate/jphysparis

Gibbs distribution analysis of temporal correlations structure in retina ganglion cells

J.C. Vasquez [a,*], O. Marre [b], A.G. Palacios [c], M.J. Berry II [b], B. Cessac [a]

[a] NeuroMathComp Team (INRIA, ENS Paris, UNSA LJAD), 2004 Route des Lucioles, 06902 Sophia-Antipolis, France
[b] Department of Molecular Biology and Princeton Neuroscience Institute, Princeton University, USA
[c] Centro Interdisciplinario de Neurociencia de Valparaiso, Universidad de Valparaiso, Chile

ARTICLE INFO

Article history:
Available online 17 November 2011

Keywords:
Spike-train analysis
Higher-order correlation
Statistical physics
Gibbs distributions
Maximum entropy

ABSTRACT

We present a method to estimate Gibbs distributions with *spatio-temporal* constraints on spike trains statistics. We apply this method to spike trains recorded from ganglion cells of the salamander retina, in response to natural movies. Our analysis, restricted to a few neurons, performs more accurately than pairwise synchronization models (Ising) or the 1-time step Markov models (Marre et al., 2009) to describe the statistics of spatio-temporal spike patterns and emphasizes the role of higher order spatio-temporal interactions.

1. Introduction

Modern advances in neurophysiology techniques, such as two-photons imaging of calcium signals or micro-electrode arrays electro-physiology, have made it possible to observe simultaneously the activity of assemblies of neurons (Stevenson and Kording, 2011). Such experimental recordings provide a great opportunity to unravel the underlying interactions of neural assemblies. The analysis of multi-cells spike-patterns constitutes an alternative to descriptive statistics (e.g. cross-correlograms or joint peri-stimulus time histograms) which become hard to interpret for large groups of cells (Brown et al., 2004; Kass et al., 2005). Earlier multi-cells approaches (e.g. Abeles and Gerstein, 1988), focus on synchronization patterns. Using algorithms detecting the most frequent instantaneous patterns in a data set, and calculating their expected probability, these approaches aim at testing whether those patterns were produced by chance (Grün et al., 2002). This methodology relies however on a largely controversial assumption, namely Poisson-statistics (Pouzat and Chaffiol, 2009; Schneidman et al., 2006).

A second type of approach has become popular in neuroscience after works of Schneidman et al. (2006) and Shlens et al. (2006). They used a maximum entropy approach model spike trains statistics as the Gibbs distribution of the Ising model. The parameters of this distribution are determined from the mean firing rate of each neuron and their pairwise synchronizations. These works have shown that for a small group of cells (10–40 retinal ganglion cells) the Ising model describes most (~80–90%) of the statistics of the *instantaneous* patterns, and performs much better than a non-homogeneous Poisson model.

However, several papers have pointed out the importance of temporal patterns of activity at the network level (Abeles et al., 1993; Lindsey et al., 1997; Villa et al., 1999; Segev et al., 2004a). Recently, Tang et al. (2008) and Ohiorhenuan et al. (2010), have shown the insufficiency of the Ising model to predict the temporal statistics of the neural multi-cells activity. Therefore, some authors, Marre et al. (2009), Amari (2010), and Roudi and Hertz (2010), have attempted to define time-dependent Gibbs distributions on the basis of a Markovian approach (1-step time pairwise correlations). The application of such extended model in Marre et al. (2009) increased the accuracy of the statistical characterization of data with the estimated distributions.

In this paper we propose an extension of the maximal entropy approach to general spatio-temporal correlations, based on the transfer-matrix method in statistical physics (Georgii, 1988; Section 2). We describe a numerical method to perform the estimation of the Gibbs distribution parameters from empirical data (Section 3). We apply this method to the analysis of spike trains recorded from ganglion cells using multi-electrodes devices in the salamander retina (Section 4). We analyze retinal spike trains taking into account spatial patterns of two and three neurons with triplets and quadruplets terms, and temporal terms up to four time steps. Our analysis emphasizes the role of higher order spatio-temporal interactions. Section 5 contains the discussion and conclusions.

* Corresponding author. Tel.: +31 243652141; fax: +33 492387845.
E-mail addresses: jc.vasquez@sciences.ru.nl, jcvasquezbetancur@gmail.com (J.C. Vasquez).

0928-4257/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jphysparis.2011.11.001

JOURNAL OF CHEMICAL INFORMATION AND MODELING

Article

pubs.acs.org/jcim

Molecular Basis of Drug Resistance in A/H1N1 Virus

Ariela Vergara-Jaque,[†,∥] Horacio Poblete,[†,∥] Eric H. Lee,[‡] Klaus Schulten,[‡] Fernando González-Nilo,[§,¶] and Christophe Chipot*[,‡,⊥]

[†]Center for Bioinformatics and Molecular Simulation, Universidad de Talca, Chile

[‡]Beckman Institute, University of Illinois at Urbana−Champaign, Urbana, United States

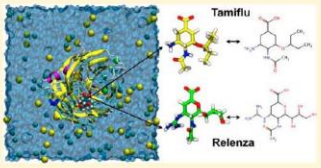
[§]Universidad Andres Bello, Center for Bioinformatics and Integrative Biology, Santiago, Chile

[⊥]Equipe de Dynamique des Assemblages Membranaires, UMR Centre National de la Recherche Scientifique/UHP 7565, Nancy Université BP 239, Nancy, France

[¶]Centro Interdisciplinario de Neurociencias de Valparaíso, Universidad de Valparaíso, Valparaíso, Chile

Supporting Information

ABSTRACT: New mutants of human influenza virus (A/H1N1) exhibit resistance to antiviral drugs. The mechanism whereby they develop insensitivity to these medications is, however, not yet completely understood. A crystallographic structure of A/H1N1 neuraminidase has been published recently. Using molecular dynamic simulations, it is now possible to characterize at the atomic level the mechanism that underlies the loss of binding affinity of the drugs. In this study, free-energy perturbation was used to evaluate the relative binding free energies of Tamiflu and Relenza with H274Y, N294S, and Y252H neuraminidase mutants. Our results demonstrate a remarkable correlation between theoretical and experimental data, which quantitatively confirms that the mutants are resistant to Tamiflu but are still strongly inhibited by Relenza. The simulations further reveal the key interactions that govern the affinity of the two drugs for each mutant. This information is envisioned to prove useful for the design of novel neuraminidase inhibitors and for the characterization of new potential mutants.



INTRODUCTION

Influenza viral infections affect all populations of the world and represent a leading cause of mortality in elderly and immune-compromised populations. The 2009 A/H1N1 pandemic clearly illustrated how drug resistant mutants can impact a population before a vaccine is available.[1] Understanding the mechanism of resistance of these mutants is likely essential to the development of potent and effective antiviral drugs and, therefore, of paramount importance for human health.

The influenza virus infects the epithelial cells of the respiratory tract through two glycoproteins, namely hemagglutinin and neuraminidase, located in the virus capsid. Specifically, neuraminidase interacts with sialic acid contained in this cell type, allowing attachment of the virus and the subsequent release of virion progeny.[2] Because the active site of neuraminidases is highly conserved, it has been employed as a target for the design of structure-based drugs. Two neuraminidase inhibitors are currently utilized to combat viral dissemination—namely, oseltamivir (Tamiflu), which binds the active site by mimicking sialic acid and zanamivir (Relenza), which is a sialic-acid derivative, wherein the 4-hydroxyl group is replaced by a guanidinium group.[3] The effectiveness of these antiviral drugs is, however, limited due to a high-mutation rate of the influenza virus.

During the 2009 pandemic of the influenza virus (A/H1N1), important mutations close to the active site were observed (H274Y, N294S, and Y252H), causing a dramatic loss of binding affinity of Tamiflu and diminishing the efficacy of the drug.[4] Similar mutations had already been reported for H5N1 neuraminidase,[5] which could be explained by the marked sequence identity of the active site of both neuraminidases. In a recent study by Collins et al.,[6] binding and inhibitory parameters were determined for Tamiflu and Relenza interacting with the wild type and three mutants of H5N1 neuraminidase: (1) the H274Y mutant observed in patients treated with Tamiflu,[7] showing a drug resistance 256-fold higher compared with the wild-type virus; (2) the N294S mutant isolated from patients treated with Tamiflu in viruses containing N1 or N2 neuraminidase,[8] exhibiting a drug resistance 81-fold higher than for the wild type; and finally (3) the Y252H mutant isolated from infected patients, has not been associated with clinical resistance, although experimentally showed to be inhibited by Tamiflu, but not Relenza.[9] The rapid emergence of drug resistance for both H5N1 avian flu and A/H1N1 influenza virus, therefore, constitutes the primary motivation for understanding its mechanism of action and should lead to the development of potent antiviral drugs that circumvent their resistance strategies.

Previous theoretical studies[10−13] have revealed the specific interactions for both wild-type and mutants H5N1 neuraminidase bound to Tamiflu and Relenza. However, the recent elucidation of the crystal structure of A/H1N1 neuraminidase[14]

Published: September 17, 2012

dx.doi.org/10.1021/ci300343w | J. Chem. Inf. Model. 2012, 52, 2650−2656

BRAIN RESEARCH 1430 (2012) 112–125

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Review

Nitric oxide signaling in the retina: What have we learned in two decades?

Alex H. Vielma[a,b], *Mauricio A. Retamal*[c], *Oliver Schmachtenberg*[a,*]

[a]*Centro Interdisciplinario de Neurociencia de Valparaíso (CINV), Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile*
[b]*Programa de Doctorado en Neurociencia, Facultad de Ciencias, Universidad de Valparaíso, Chile*
[c]*Laboratorio de Fisiología, Clínica Alemana — Universidad del Desarrollo, Santiago, Chile*

ARTICLE INFO

Article history:
Accepted 27 October 2011
Available online 4 November 2011

Keywords:
Vision
Photoreceptor
Amacrine cell
NOAC
Soluble guanylyl cyclase
Light adaptation

ABSTRACT

Two decades after its first detection in the retina, nitric oxide (NO) continues to puzzle visual neuroscientists. While its liberation by photoreceptors remains controversial, recent evidence supports three subtypes of amacrine cells as main sources of NO in the inner retina. NO synthesis was shown to depend on light stimulation, and mounting evidence suggests that NO is a regulator of visual adaptation at different signal processing levels. NO modulates light responses in all retinal neuron classes, and specific ion conductances are activated by NO in rods, cones, bipolar and ganglion cells. Light-dependent gap junction coupling in the inner and outer plexiform layers is also affected by NO. The vast majority of these effects were shown to be mediated by activation of the NO receptor soluble guanylate cyclase and resultant cGMP elevation. This review analyzes the current state of knowledge on physiological NO signaling in the retina.

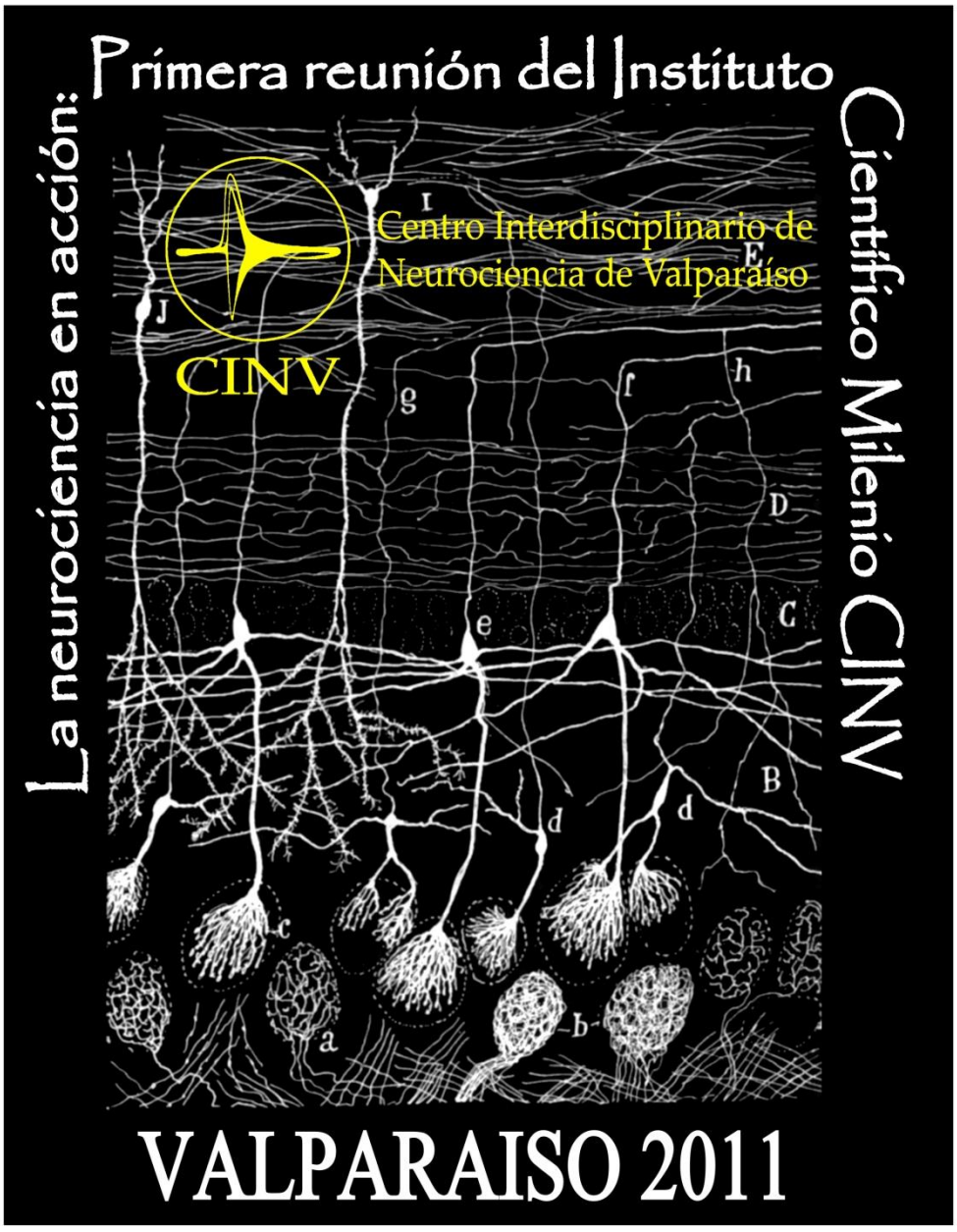
Contents

* *Corresponding author at:* CINV, Universidad de Valparaíso, Avda. Gran Bretaña 1111, Casilla 5030, Correo 4, 2360102 Valparaíso, Chile. Fax: +56 32 2508027.
E-mail addresses: schmachtenberg.uv@gmail.com, Oliver.Schmachtenberg@uv.cl (O. Schmachtenberg).

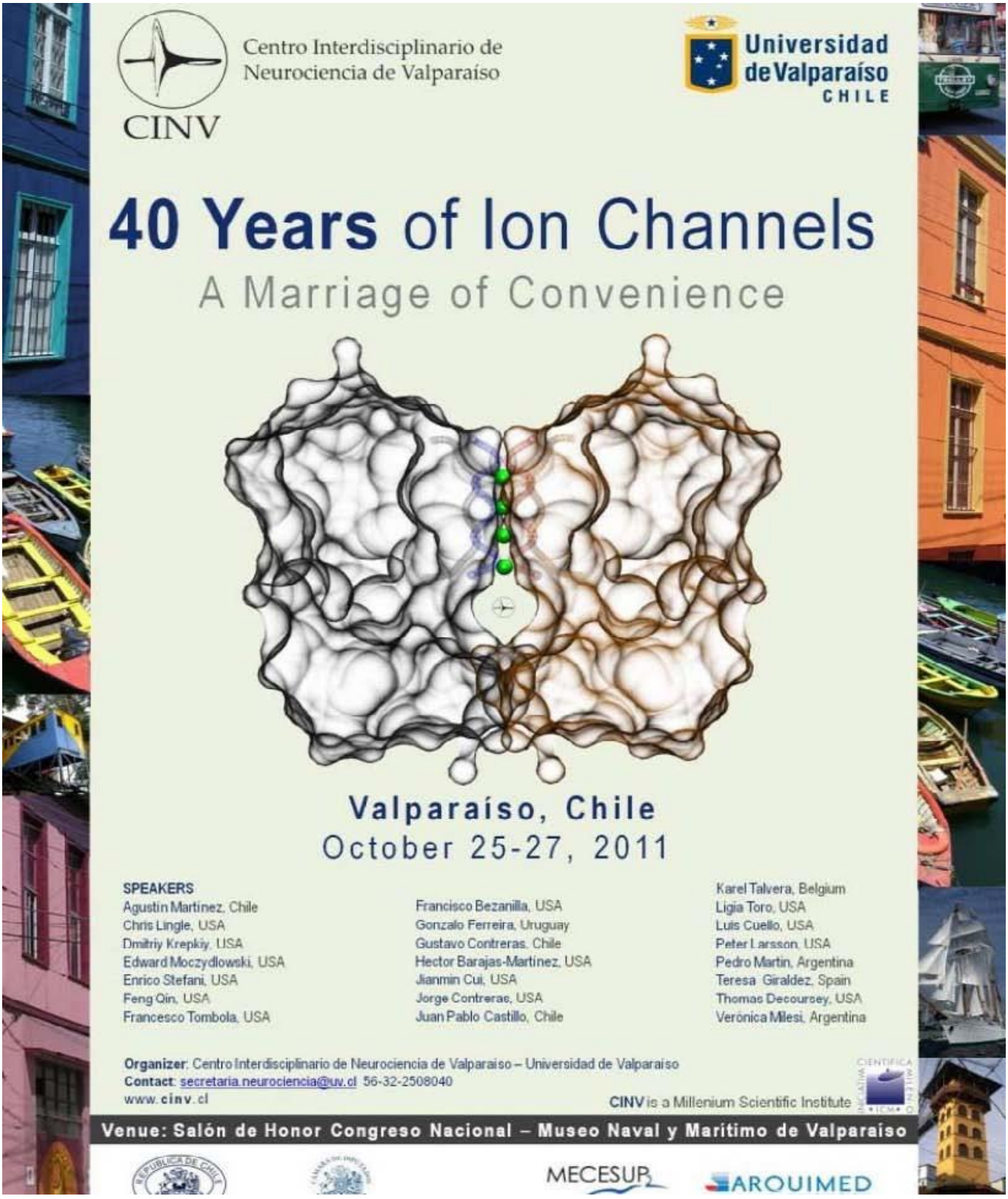
Abbreviations: 8-Br-cGMP, 8-bromo cyclic guanosine monophosphate; CNGC, cyclic nucleotide-gated channel; C-PTIO, carboxy-PTIO (NO scavenger); Cx, connexin; DAF-2DA, 4,5-diaminofluorescein diacetate (fluorescent NO indicator); DAPI, 4′,6-diamidino-2-phenylindole; DEA/NO, diethylamine NONOate (NO donor); ERG, electroretinogram; GCL, ganglion cell layer; IBMX, isobutyl methylxanthine (phosphodiesterase inhibitor); INL, inner nuclear layer; IR, immunoreactivity; IS, (photoreceptor) inner segments; l-NAME, L-NG-nitroarginine methyl ester (NOS inhibitor); l-NMMA, N5-[imino(methylamino)methyl]-L-ornithine, monoacetate (NOS inhibitor); NADPH:, nicotinamide adenine dinucleotide phosphate; NOAC, NO producing amacrine cell; NOC-12, 1-hydroxy-2-oxo-3-(N-ethyl-2-aminoethyl)-3-ethyl-1-triazene (NO donor); NOS, nitric oxide synthase; ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin1-one (sGC inhibitor); OPL, outer plexiform layer; OPs, oscillatory potentials; OS, (photoreceptor) outer segments; PIS, photoreceptor inner segments; PKG, protein kinase G (cGMP-dependent protein kinase); SEM, standard error of mean; sGC, soluble guanylate cyclase; SIN-1, 3-morpholino-sydnonimine (NO donor); SNAP, S-nitroso-N-Acetyl-D,L-penicillamine (NO donor); SNO-Cys, S-nitroso-cysteine; SNP, sodium nitroprusside (NO donor)

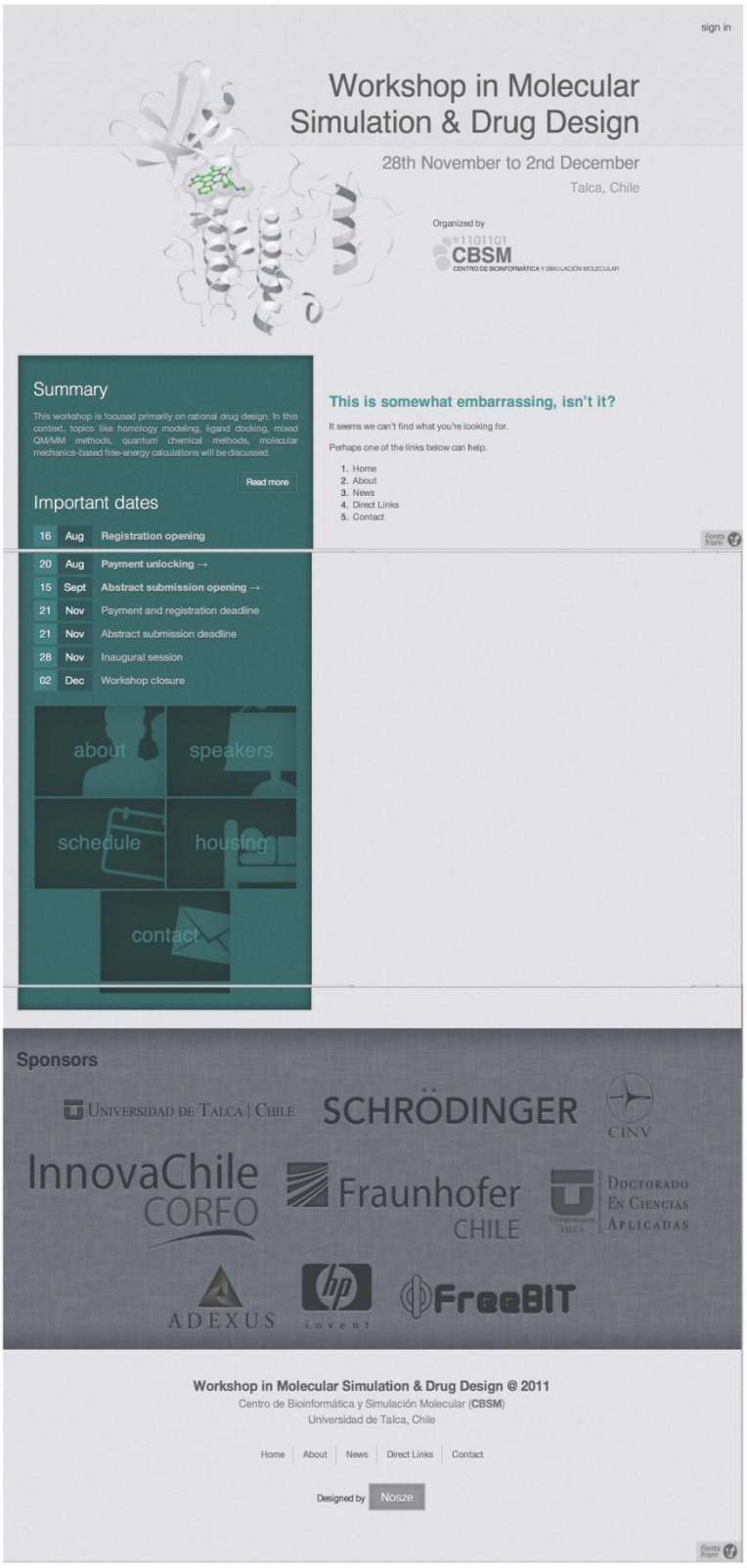
0006-8993/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2011.10.045

Annex 4
Organization of Scientific Events



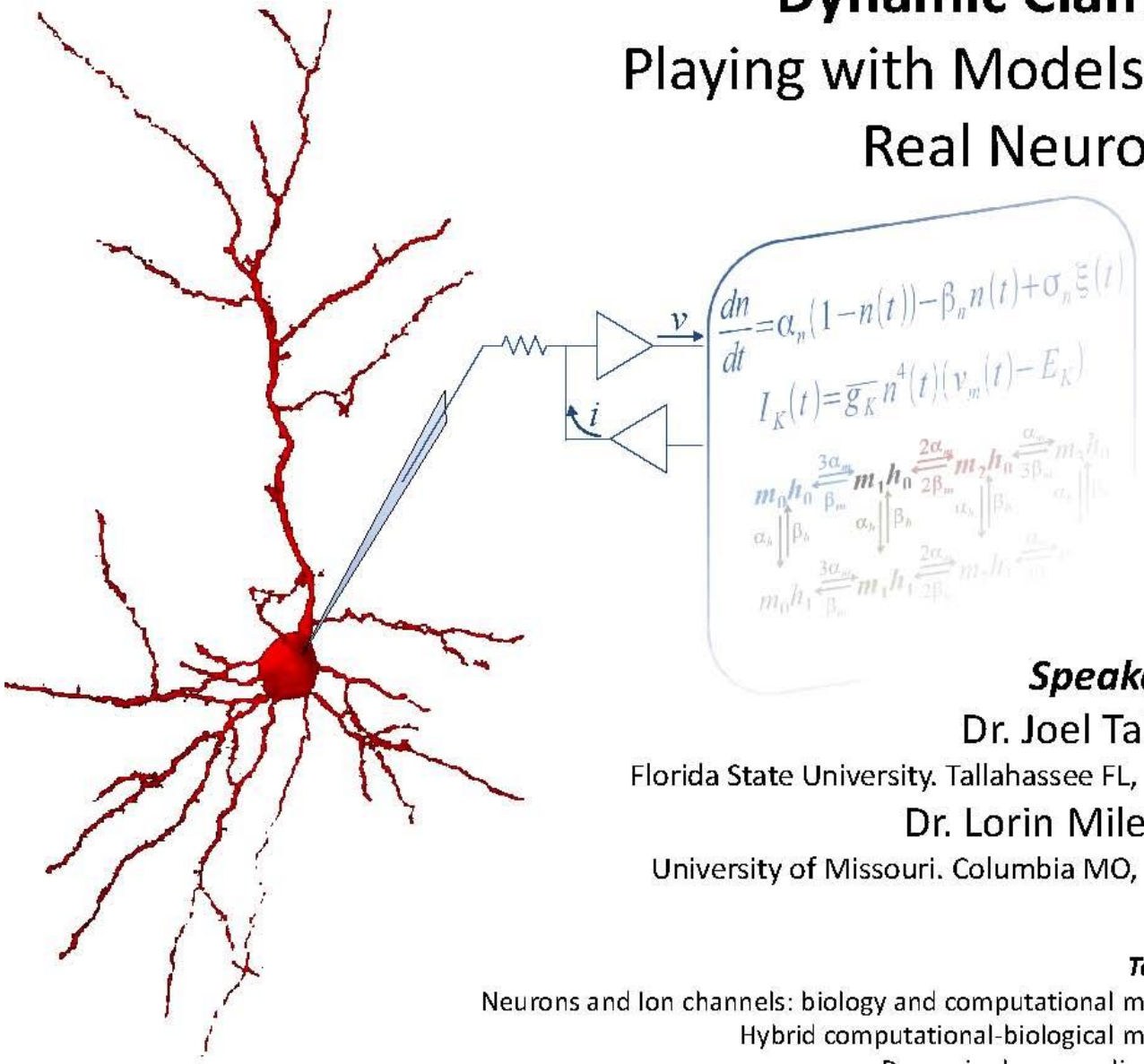
Viernes 23 de Septiembre, 9:00 AM.
Instituto de Sistemas Complejos Valparaíso &
Auditorio Museo Naval.
Paseo 21 de Mayo, Cº Artillería, Playa Ancha, Valparaíso.
Contacto: CINV= 32-2508040


Centro Interdisciplinario de Neurociencia de Valparaíso
CINV
Universidad de Valparaíso CHILE
40 Years of Ion Channels
A Marriage of Convenience
Valparaíso, Chile
October 25-27, 2011
SPEAKERS
Agustin Martinez, Chile
Chris Lingle, USA
Dmitriy Krepkiy, USA
Edward Moczydlowski, USA
Enrico Stefani, USA
Feng Qin, USA
Francesco Tombola, USA
Francisco Bezanilla, USA
Gonzalo Ferreira, Uruguay
Gustavo Contreras, Chile
Hector Barajas-Martinez, USA
Jianmin Cui, USA
Jorge Contreras, USA
Juan Pablo Castillo, Chile
Karel Talvera, Belgium
Ligia Toro, USA
Luis Cuello, USA
Peter Larsson, USA
Pedro Martin, Argentina
Teresa Giraldez, Spain
Thomas Decoursey, USA
Verónica Milesi, Argentina
Organizer: Centro Interdisciplinario de Neurociencia de Valparaíso – Universidad de Valparaíso
Contact: secretaria.neurociencia@uv.cl 56-32-2508040
www.cinv.cl
CINV is a Millenium Scientific Institute
Venue: Salón de Honor Congreso Nacional – Museo Naval y Marítimo de Valparaíso
MECESUP
AROUIMED


sign in
Workshop in Molecular Simulation & Drug Design
28th November to 2nd December
Talca, Chile
Organized by
CBSM
CENTRO DE BIOINFORMÁTICA Y SIMULACIÓN MOLECULAR
Summary
This workshop is focused primarily on rational drug design. In this context, topics like homology modeling, ligand docking, mixed QM/MM methods, quantum chemical methods, molecular mechanics-based free-energy calculations will be discussed.
Read more
Important dates
16 Aug Registration opening
20 Aug Payment unlocking →
15 Sept Abstract submission opening →
21 Nov Payment and registration deadline
21 Nov Abstract submission deadline
28 Nov Inaugural session
02 Dec Workshop closure
about
speakers
schedule
housing
contact
This is somewhat embarrassing, isn't it?
It seems we can't find what you're looking for.
Perhaps one of the links below can help.
1. Home
2. About
3. News
4. Direct Links
5. Contact
Sponsors
Universidad de Talca | Chile
SCHRÖDINGER
CINV
InnovaChile CORFO
Fraunhofer CHILE
Doctorado En Ciencias Aplicadas
ADEXUS
invent
FreeBIT
Workshop in Molecular Simulation & Drug Design @ 2011
Centro de Bioinformática y Simulación Molecular (CBSM)
Universidad de Talca, Chile
Home
About
News
Direct Links
Contact
Designed by
Nosze

Post graduate course

Dynamic Clamp:
Playing with Models in Real Neurons



Speakers:

Dr. Joel Tabak

Florida State University. Tallahassee FL, USA.

Dr. Lorin Milescu

University of Missouri. Columbia MO, USA.

Topics:

Neurons and Ion channels: biology and computational models
Hybrid computational-biological models
Dynamic clamp applications
Introduction to QuB
Student tutorials on the computer

December 14-16, 2011

Instituto de Sistemas Complejos de Valparaíso

Organizers:

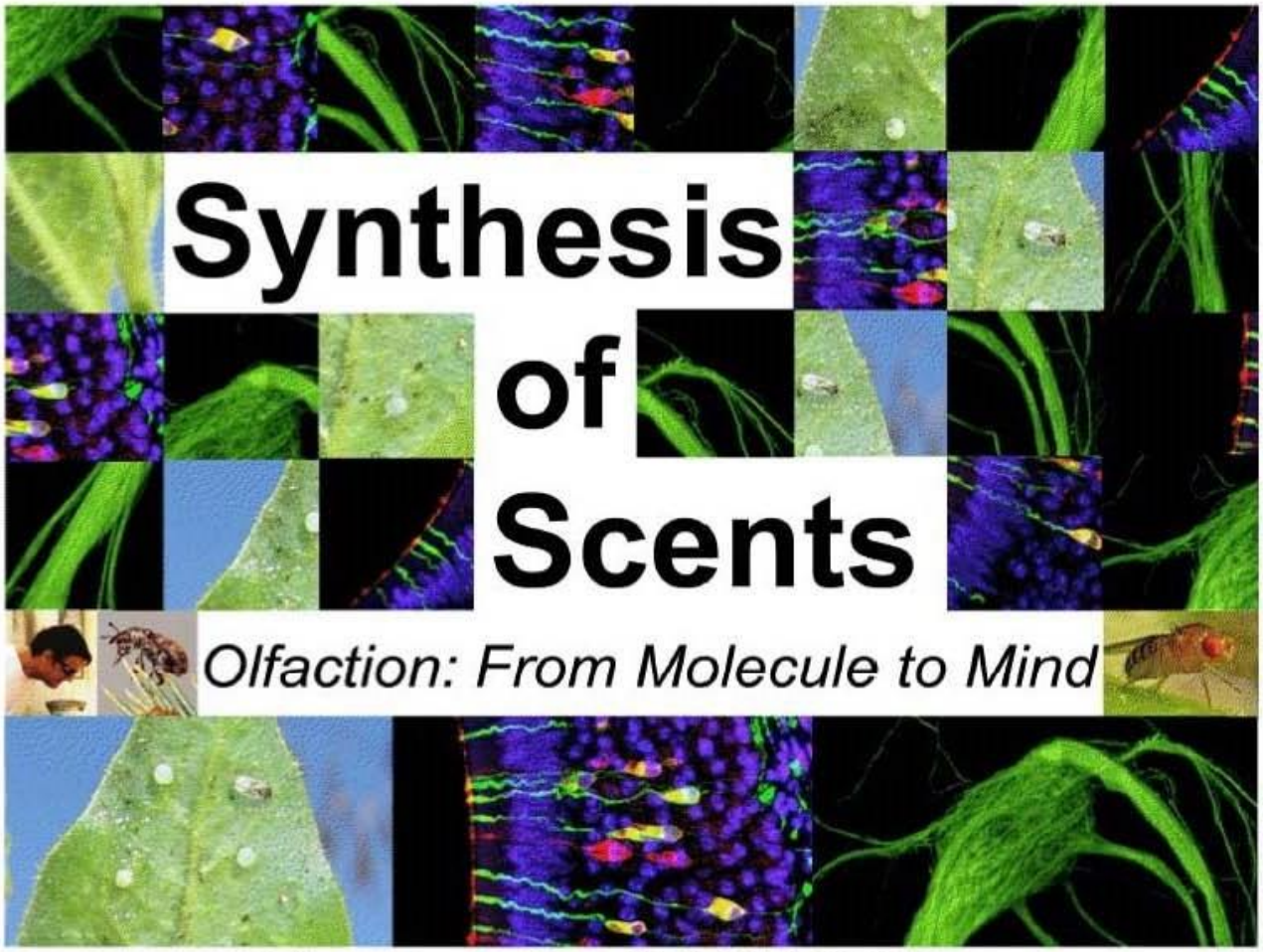
Dr. Patricio Orio

Centro Interdisciplinario de Neurociencia de Valparaíso CINV
Universidad de Valparaíso.

Dr. Patricio Rojas

Universidad de Santiago de Chile.

Support Fondecyt – MECESUP – Iniciativa Científica Milenio – DICYT-USACH.



Monday, April 16, 2012

9:30-17:30

Tom Baker, PhD, (USA)
Joerg Bohlmann, PhD, (Canada)
Bill Hansson, PhD, (Germany)
Brian Key, PhD, (Australia)
Peter Mombaerts, PhD, (Germany)
Ivan Rodriguez, PhD, (Switzerland)
Andreas Schaefer, PhD, (Germany)
Dieter Wicher, PhD, (Germany)

Introduction by Kathleen Whitlock, PhD, (Chile)

Universidad de Valparaíso CHILE

Centro Interdisciplinario de Neurociencia de Valparaíso
CINV

PC de V Parque Cultural de Valparaíso

INICIATIVA CIENTIFICA MILENIO ICM

Organizer: Centro Interdisciplinario de Neurociencia (CINV) - Universidad de Valparaíso
Contact: secretaria.neurociencia@uv.cl - (56) 32-250.80.40
www.cinv.cl

Centro Interdisciplinario de Neurociencia de Valparaiso

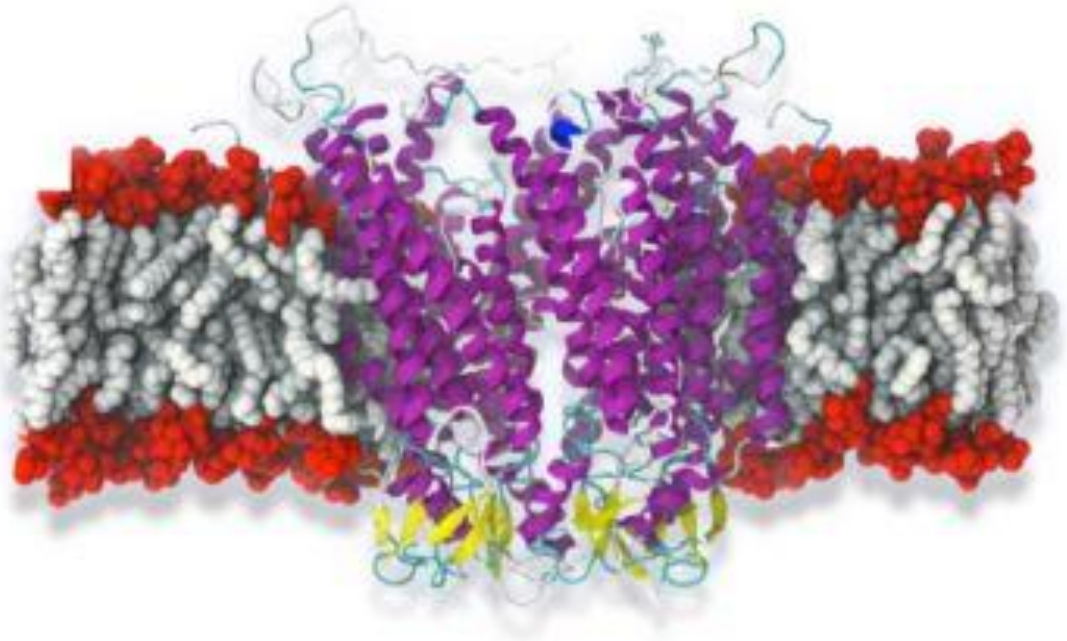
CINV

29-30 October 2012
CINV - Universidad de Valparaiso



International Workshop

Structure and Function of Connexin and Pannexin Channels



Participants:

Juan Andrés Orellana (Chile)
Viviana Berthoud (USA)
Adrián Palacios (Chile)
Luis Cea (Chile)
Silvia Perfuela (Canada)
Xavier Figueroa (Chile)
Tomás Pérez-Acle (Chile)
Christian Giaume (France)
Mauricio Retamal (Chile)
Ross Johnson (USA)
Juan Carlos Sáez (Chile)
Agustín Martínez (Chile)
Oliver Schmachtenberg (Chile)

Organizers:

Universidad de Valparaiso CHILE

Support:

CONICYT

Small Brains, Big Ideas-PLUS

Neurobiological Insights from Invertebrates

International Symposium, **November 6th, 2012**

Valparaíso, Chile

Museo Naval, 9:00 AM- 6:00 PM

Seeding ideas for the future of science

Overview, John Ewer, Universidad de Valparaíso, Chile

Participants

Mark Alkema, UMASS Med, USA. Behavior in *C.elegans*
Vivian Budnik, UMASS Med, USA. Synaptic formation in *Drosophila*
Michael Francis, UMASS Med, USA. Synapse function in *C.elegans*
Carolina Rezával, University of Oxford, UK. Behavior in *Drosophila*
Steven Reppert, UMASS Med, USA. Navigation in Butterflies
Brian H. Smith, Arizona University, USA. Learning in Bees
Scott Waddell, University of Oxford, UK. Learning in *Drosophila*

Registration and more information

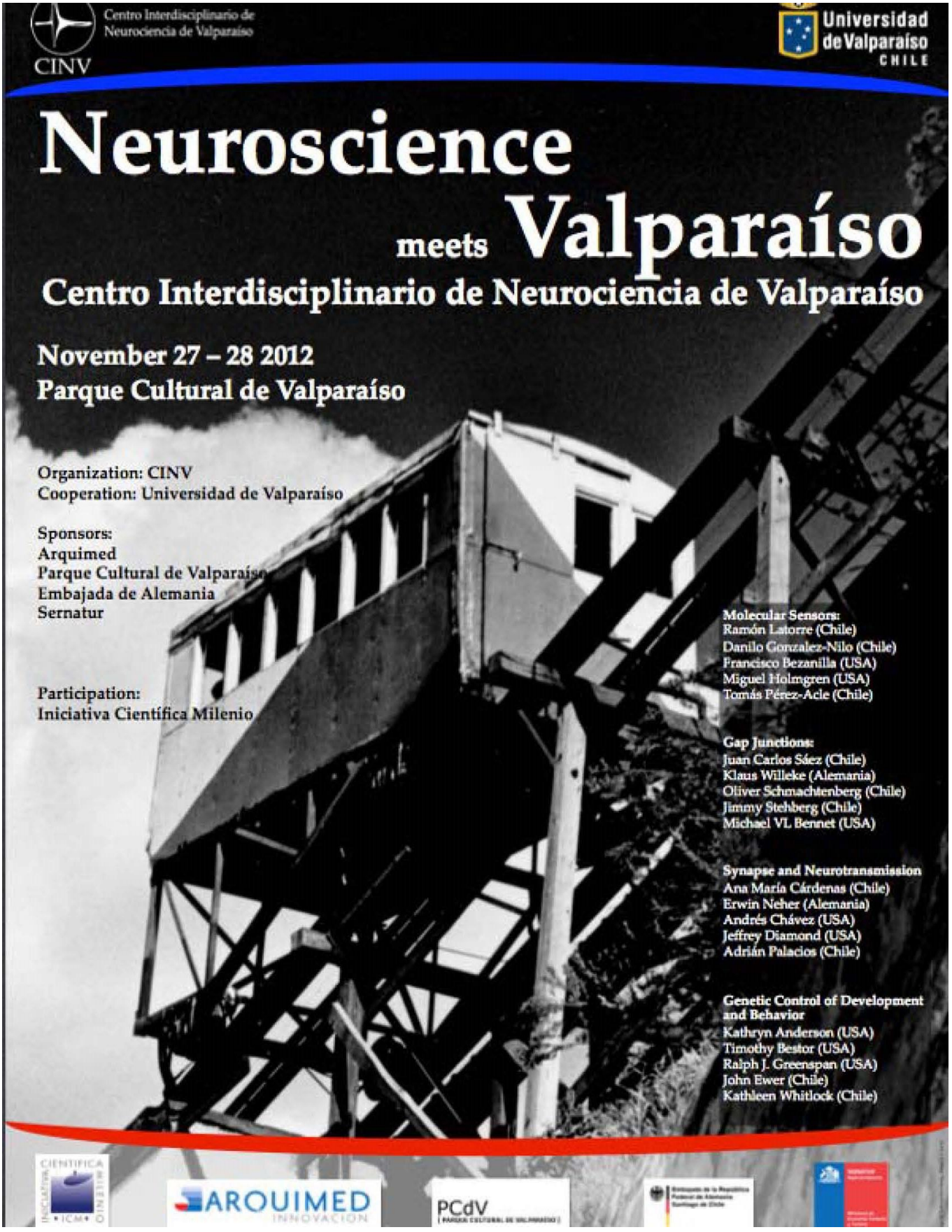
www.smallbrains.org

CINV

FACULTAD de MEDICINA

Organizers:
Jimena Sierralta Ph. D.
John Ewer Ph. D.
Yuly Fuentes-Medel, Ph.D.
Contact us at **sbbiPLUS@gmail.com**

wellcome trust


Centro Interdisciplinario de Neurociencia de Valparaíso
CINV
Universidad de Valparaíso CHILE
Neuroscience
meets Valparaíso
Centro Interdisciplinario de Neurociencia de Valparaíso
November 27 – 28 2012
Parque Cultural de Valparaíso
Organization: CINV
Cooperation: Universidad de Valparaíso
Sponsors:
Arquimed
Parque Cultural de Valparaíso
Embajada de Alemania
Sernatur
Participation:
Iniciativa Científica Milenio
Molecular Sensors:
Ramón Latorre (Chile)
Danilo Gonzalez-Nilo (Chile)
Francisco Bezanilla (USA)
Miguel Holmgren (USA)
Tomás Pérez-Acle (Chile)
Gap Junctions:
Juan Carlos Sáez (Chile)
Klaus Willeke (Alemania)
Oliver Schmachtenberg (Chile)
Jimmy Stehberg (Chile)
Michael VL Bennet (USA)
Synapse and Neurotransmission
Ana María Cárdenas (Chile)
Erwin Neher (Alemania)
Andrés Chávez (USA)
Jeffrey Diamond (USA)
Adrián Palacios (Chile)
Genetic Control of Development and Behavior
Kathryn Anderson (USA)
Timothy Bestor (USA)
Ralph J. Greenspan (USA)
John Ewer (Chile)
Kathleen Whitlock (Chile)
ARQUIMED INNOVACION
PCdV

Annex 7.3. Articles and Interviews

Title: "The relationship between smell and emotion remains unanswered"

Media: "Ya" Magazine, from newspaper "El Mercurio" (National)
Date: September 27, 2011

KATHLEEN WITHLOCK, NEUROBIÓLOGA NORTEAMERICANA EN CHILE:

"LA RELACIÓN ENTRE OLFATO Y EMOCIÓN SIGUE SIN RESPUESTA"

Acádémica de la Universidad de Valparaíso, Kathleen Withlock decidió realizar su carrera en Chile y no en Estados Unidos, porque necesita "un sentimiento de aventura". Observa peces para descubrir cómo el sistema olfatorio regenera las neuronas. Dice que el olfato es el único sentido que sigue siendo un enigma para los científicos, pero sí está comprobado que es el sentido "más sentimental".

Por **MARÍA JOSÉ VIERA-GALLO.**

De todas las incógnitas de la neurociencia hay una que el microscopio de Kathleen Withlock (47) probablemente jamás resolverá: por qué, desde que era una niña que cazaba pirigüines en las afueras de Nueva York, se ha obstinado en entender lo inentendible: cómo funciona el cerebro humano.

Las solicitadas investigaciones de esta biólogo del desarrollo y genética, especialista en sistema olfatorio, nacida en Albany, han pasado por los mejores laboratorios norteamericanos, le han valido varias becas de excelencia —entre éstas, una de dos millones de dólares del National Institute of Health de su país (dinero gracias al cual se vino a Chile el 2006)—, y un puesto en el Centro Genómica de la Célula del proyecto Milenio recientemente financiado por el gobierno chileno.

Para sorpresa de su propio currículum, hoy Kathleen vive y trabaja en Playa Ancha, junto a su marido, el científico chileno John Ewar, y es profesora titular del Centro de Neurociencia de la Universidad de Valparaíso. También fundó el programa de educación "Ciencia Al Tiro" para acercar Darwin a las escuelas públicas.

Media tarde en su compañía quizás no basten para recorrer todos los pasillos de una disciplina cada día más en boga en el mundo, que empezará la construcción de su nueva sede internacional en el puerto, pero sí para observar de cerca a esos peces cebras que Kathleen se trajo en barco desde Estados Unidos junto a sus invaluables microscopios. "El es mi embrión favorito, Rudolf, ¿qué te parece?". Rudolf emana unas luces infra-rojas por los ojos, una técnica avanzada de escáner única en Chile, que, en palabras fáciles, "ilumina con fluorescente el interior de las neuronas activas de un animalito transgénico vivo".

El laboratorio y las oficinas del Centro de Neurociencia donde transita Kathleen no son cubículos hi-tech como los de las películas de ciencia ficción. Entre sus muros desbordan frascos, anotaciones de fórmulas, instrumentos, y también fetiches como un minicerebro de juguete y un móvil de peces de madera: un caos natural que recuerda que la verdadera ciencia tiene más de corazón y genialidad que de cálculo y pretensión.

"Soy muy fiona, me encantan mirar estas luces", se ríe Kathleen, alejándose al fin de su nuevo microscopio Nikon.

No será la primera ni la última risa de esta entrevistada. En un mundo científico estigmatizado de grave, Kathleen sabe que para empezar a derribar mitos basta usar un delantal de colores en vez de uno blanco.

—Cuando alguien te pregunta en qué trabajas, ¿qué dices?

—Que observo peces cebras para investigar parte del desarrollo neuroendocrino. El sistema olfatorio permite ver cómo se están diferenciando las neuronas, ya que en él hay un tipo de células troncales que regeneran, no sabemos cómo, nuevas neuronas durante el crecimiento humano. Pero lo que más me interesa es cómo los genes y los estímulos del medio ambiente que nos encontramos en la vida cotidiana interactúan durante el desarrollo temprano del sistema nervioso, creando nuevos circuitos.

"LA NEUROCIENCIA NO ES LOCA, ES FASCINANTE, SORPRENDENTE Y PROFUNDAMENTE PERSONAL. COMPRENDER EL SISTEMA NERVIOSO ES ENTENDER QUIÉNES SOMOS, CÓMO NOSOTROS, COMO LOS SERES HUMANOS «FUNCIONAMOS»".

—¿Cómo descubriste tu alma científica? ¿Cuándo para un cumpleaños pediste una lupa en vez de una muñeca?

—Me crié en el campo, al aire libre y siempre tuve muchos animales: conejos, gatos, roedores, serpientes, insectos y pirigüines, que eran mis favoritos y recogía del lago. ¡Mi mamá odiaba verlos debajo de mi cama! En realidad, yo siempre quise estudiar ciencias y arte, hasta que fui aceptada en ambas carreras en SUNY la Universidad Pública de NY, y me vi obligada a elegir.

—¿Cuáles fueron tus primeras impresiones sobre el submundo de la ciencia?

—No muy divertidas, la verdad. De hecho, el primer semestre me arranqué a los cursos de arte. No fui una alumna modelo, me gustaba ir al bar, leía cosas que estaban fuera del programa y hacía preguntas que no eran curriculares. Era muy chicha fresca. Nunca fui competitiva como mis compañeros de medicina. Quería avanzar rápido e investigar biología del desarrollo sensorial en animales marinos. El doctor Murphy, mi profesor guía, terminó aceptándome en su laboratorio porque me iba bien en genética (susurra casi con vergüenza que era la

Title: "Valparaiso begins a race to become an international center for scientific research"
Media: Newspaper "El Mercurio" (National)
Date: November 24, 2011

C 10 NACIONAL

Universidad local consentó a recibir $12 mil millones para impulsar la neurociencia:

Valparaíso inicia carrera para convertirse en centro de investigación científica internacional

Proyecto liderado por el premio Nacional Ramón Latorre busca, además, contribuir a la recuperación urbana del puerto.

$3.500

Title: "Colossal *porteño* project" (Opinion column of the Mayor of Valparaíso)
Media: Newspaper "El Mercurio de Valparaíso" (Regional)
Date: November 14, 2011

C
Columna

Jorge Castro Alcalde de Valparaíso

"Este centro será pivote de nuestro proyecto Puerto Viejo, que busca potenciar y poner en valor el barrio fundacional de la ciudad"

Colosal proyecto porteño

Valparaíso potencia hoy su industria universitaria y lo hace para insertarse en la Sociedad del Conocimiento. La Ciencia y la Tecnología han sido parte de nuestra historia. Tenemos fortalezas naturales en ese campo.

Hay hitos memorables que lo confirman. Uno de ellos acaeció el 28 de junio de 1968 cuando en el Hospital Naval el doctor Jorge Kaplan Meyer puso a Valparaíso en las portadas de todo el mundo con el primer trasplante cardíaco de Chile.

Hoy estamos a las puertas de otro logro gigantesco. De la mano de un científico descomunal y de la Universidad de Valparaíso surge en pleno Barrio Puerto el proyecto de un Instituto que será polo mundial de investigación y estudio para los científicos más avanzados del planeta.

Ese hombre extraordinario, gran impulsor de la iniciativa es el doctor Ramón Latorre. Se trata de un porteño de corazón que ha estado por dos décadas junto a nosotros en las investigaciones de biología marina en Montemar y se ha dado tiempo para explorar múltiples otros emprendimientos en el campo de la ciencia.

El doctor Latorre debe ser el científico chileno más citado a nivel internacional. Es una autoridad en neurociencia; una disciplina que está a la vanguardia de las nuevas fronteras de la investigación. La neurociencia explora las claves de lo que puede ser mañana un ámbito inimaginable de conocimientos y progreso, incluidos la inteligencia artificial, la robótica y toda una gama del saber de tercera generación que puede ser, incluso, la clave de un tercer Premio Nobel para Chile.

Y ese líder de tremenda magnitud comparte con nosotros; eligió a Valparaíso como su casa para grandes investigaciones, pasea por nuestras calles y -en su tremenda sencillez- comparte nuestra vida como un porteño más. Quiere respetuosamente levantar este centro internacional en el barrio fundacional de Valparaíso, un poco como "pidiéndoles permiso" a sus vecinos por ocupar este espacio muy cerca de La Matriz.

Hasta allí llegarán hombres de ciencia de todo el mundo para dar vida a este extraordinario centro científico. Lo encabezará este porteño que tiene a todo el mundo como su campo de operaciones; doctorado en la Universidad de Chile, y también en las de Chicago y Harvard. Es toda una eminencia que nos hace un gigantesco regalo.

Este edificio que tendrá "techo verde" será sede de congresos, simposios y estará abierto a las organizaciones vecinales. Incluye un museo y acomodaciones para que adultos y jóvenes lleguen hasta este centro en busca de motivación, admiración y diálogo con la ciencia y los investigadores de todo el mundo.

Con orgullo le hemos otorgado al doctor Latorre nuestra Medalla Bicentenario. Con el mismo orgullo hemos agradecido a la Universidad de Valparaíso este aporte que nos pone a todos en el ranking mundial. Con orgullo afirmamos que -una vez construido- este centro será pivote de nuestro proyecto "Puerto Viejo" que busca potenciar y poner en valor el barrio fundacional de la ciudad.

Title: "PUERTO DE IDEAS: The festival that introduces a new experience of culture begins in Valparaíso"
Media: Newspaper "El Mercurio" (National)
Date: November 23, 2011

Title: "Chileans participate in obtaining the first genome from an acarus"
Media: Newspaper "El Mercurio" (National)
Date: November 28, 2011

Elaborado por la Asociación Estadounidense de Psiquiatría:

Nueva edición de manual sobre trastornos mentales tiene locos a especialistas

En mayo de 2013 aparecerá la versión actualizada de esta biblia para psiquiatras, neurólogos y psicólogos. Hoy se discute qué nuevos trastornos deben ser incluidos y cuáles deben salir.

Enfermo imaginario

Un ejemplo de las cosas que le preocupan a la Asociación Americana de Psiquiatría (APA) es el déficit atencional y la sobrediagnóstico. La APA sostiene que procesos perfectamente normales, que van y vienen en una persona sana, están siendo "medicalizados" y "etiquetalizados". Esto implica el "riesgo de provocar una epidemia de falsos positivos". Y, de esta manera, que una gran cantidad de personas están en riesgo de ser diagnosticadas con algo que simplemente no tienen.

Desde 1952, año en que la Asociación Estadounidense de Psiquiatría (APA, su sigla en inglés) hizo el primer Manual de Diagnóstico y Estadísticas de desórdenes mentales o DSM (por Diagnostic and Statistical Manual of Mental Disorders) el conocimiento sobre salud mental creció considerablemente.

Ahora, la comunidad de expertos en salud mental está debatiendo en distintas partes del mundo cómo debería ser la quinta edición del DSM, que aparecerá en mayo de 2013.

No es una discusión fácil.

Especialistas del mundo entero usan el actual DSM-4 para diagnosticar a sus pacientes, basándose en las descripciones y síntomas que el manual detalla. Y, por lo mismo, a través de distintas agrupaciones, están tratando de entregar sus puntos de vista para que la nueva versión incluya sus sugerencias.

De hecho, la APA creó el sitio *www.dsm5.org* para que cualquier persona pueda leer en qué va el debate y, claro, para enviar comentarios y propuestas. Es decir, la tarea es casi titánica.

Todos quieren algo. Y la APA estaría preocupada por no excederse. En no transformar cualquier conducta en un potencial desorden y, por lo tanto, convertir a una persona sana en una que recibe un tratamiento farmacológico innecesario.

"Estamos preocupados por la redacción de los umbrales para el diagnóstico de trastorno de múltiples categorías. Sobre la introducción de enfermedades que pueden llevar a un tratamiento médico inadecuado de las poblaciones vulnerables, y sobre las propuestas específicas que parecen carecer de fundamento empírico. Además, cuestionamos los cambios propuestos a la definición de trastorno mental que les resta importancia a aspectos socioculturales, mientras que le da más énfasis a la teoría biológica", dice una carta abierta que publicó la APA sobre esta discusión.

Sobre aspectos socioculturales, el psiquiatra chileno Lister Rossel piensa que habría que solicitar que los países del tercer mundo tengan más peso en la elaboración de estas guías. "Los conceptos de salud mental son distintos en el primer mundo que en el tercer mundo. La autonomía en el primer mundo, por ejemplo, es muy sana. Pero en el tercer mundo, no tanto: es muy importante incorporar la interrelación de la familia y de las redes; hay una fuerte integración de criterios entre lo que es antropológico y lo que es psiquiátrico".

Un ejemplo: en Filipinas, los cuidados post-natales son muy distintos que en Hong Kong. "La diferencia está en las redes familiares y sociales que hay entre filipinos y que en Hong Kong casi no se dan", explica Rossel.

"El mundo sajón valora mucho la autonomía, no depender de nadie. Que tenga trabajo ojalá como emprendedor. En cambio en otros países, el tema de la colaboración es considerado lo máximo. Yo considero de un tango no saludable alguien que no participa del colectivo".

Descuentos en tiendas *online*:

Hoy Chile tendrá su propio Cyber Monday

Después de las ofertas del Black Friday, le siguen sucesivos descuentos en línea en el Cyber Monday. La Cámara de Comercio de Santiago tomó la costumbre estadounidense y hoy hará su versión criolla. A la iniciativa se sumaron siete de las más grandes tiendas de comercio electrónico de Chile.

Falabella.com, el mall de Ripley, también ofrecerá descuentos durante la jornada.

Los tres reyes magos del pesebre recortable.

Sitio con actividades:

Iglesia invita a preparar la Navidad en la web

Un especial sobre la Navidad está disponible en Iglesia.cl, el portal de la Conferencia Episcopal de Chile. La iniciativa busca ayudar a prepararse en familia durante el adviento e incluye tarjetas para imprimir, juegos para los niños, un pesebre para recortar e imágenes para colorear. También presenta el verdadero sentido del árbol de Navidad y otros adornos.

De Naciones Unidas:

Parte cita en Durban sobre cambio climático

El personal de la ONU podrá ir sin chaqueta ni corbata durante la Cumbre del Cambio Climático que comienza hoy en la ciudad sudafricana de Durban para ahorrar energía debido a las altas temperaturas que se registran, y los casi 20.000 asistentes están invitados a seguir el ejemplo. La cita se inicia con el reto de renovar el Protocolo de Kioto, el único acuerdo vinculante para la reducción de emisiones, que expira en 2012, y la necesidad de limitar el calentamiento global a menos de 2 grados con respecto a la era preindustrial.

OPINIÓN

Trío de nobeles a Santiago

No sé si me dé el ánimo para entrevistar a otro Premio Nóbel; cada vez que lo he hecho —ejemplo— me ha significado una preparación entretenida pero agotadora. Además, como periodista, después de un entrevistado con me podigrí no podía llegar donde el editor del diario y decirle que no había entrevistado. Así es que Nóbel entrevistado = tensión chamboncada, siempre.

Esta semana, desde el miércoles, llegan un Nóbel de Física 1984, Carlo Rubbia, a quien ya he entrevistado dos veces, una con la periodista Lorena Cuzmár; Yuan Tseh Lee, Nóbel de Química 1986, y Rajendra K. Pachauri, el del Cambio Climático, Nóbel de la Paz 2007, al que también ya entrevisté con la Lorena.

Visitan al "Congreso del Futuro", que organizan los dos ramas del Congreso chileno. Quiere decir que el Parlamento le interesa la ciencia. Me colard, capaz que entrevisto.

Carlo Rubbia es especialista en energía nuclear. Un tipo divertido, cordial, con visión política. Italiano. Le pregunté, por ejemplo, si se tiñe el pelo, porque da gran curiosidad su aspecto a los 75 años. Y él, que es una simpatía, no se daría por ofendido y capaz que confiese su verdad capilar. Además, podría desahogarse por el desastre de Fukushima, o de si se arrepiente de haber estudiado física nuclear.

En cambio, Yuan Tseh Lee, de 75, se ve un señor demasiado solemne y taiwanés. Yo he estado en Taiwán y me impresionó lo alegres y dicharacheros que son los habitantes, cargar este estímulo tengo sentido del humor. Pero no me arriesgaría. Con los asiáticos uno nunca sabe; lo único en que hay que entregarles las tarjetas de visita con las dos manos y recibir igual las de ellos... y leerlas concienzudamente. Le podría preguntar al premio Nóbel sobre el informe mundial sobre el cambio climático. Se pregunta si sobrevivió el planeta Tierra.

Bueno, todos sabemos que no sobrevivió, la cosa es cuántos millones de años le quedan.

La difícil tarea de mediar entre estos dos nobeles le corresponderá a nuestro ágil presidente de la Academia de Ciencias, el doctor Juan Asenjo, que diseña la producción industrial de elementos bioquímicos útiles para el ser humano, como las bacterias especializadas. Por eso, recibió el Premio Nacional de Ciencias Aplicadas 2004. Aquí tendrá que entrujar a dos nobeles a la vez, ambos de Asia... no lo envidio. Lo ayudarán esos antojos todavía secretos que le gusta usar.

De la impresionante lista de protagonistas de estos días, quien más me tinca es un chileno de 32 años, doctorado en física, experto en gráficos. Estudia en el MIT. "Astro multitalento y laboratorista de ciencia" (Oberlin Arts & Science Lab). Se llama César Hidalgo. Me tinca porque sin imaginar un "aroma", el cuadro visual de lo que uno estudia, no hay caso de seguir investigando. Apuesto a que Hidalgo abre esos mundos.

En la sesión final, el viernes en la tarde, el matemático Eric Goles, Premio Nacional de Ciencias, animador del programa "Enlaces", de TVN, moderará un foro con el francés Ivar Ekeland y con el estadounidense Jerome Glenn. El primero, matemático de la economía; el segundo, futurista. Participan

Varias arañitas rojas sobre una hoja de gardenia. En el recuadro, la *Tetranychus urticae* vista de cerca.

De la Universidad de Valparaíso:

Chilenos participan en el primer genoma obtenido desde un ácaro

Analizaron a la arañita roja, que afecta a más de 1.100 cultivos de todo el mundo, incluyendo Chile.

Nuevas pistas sobre la evolución de los artrópodos y su relación con las plantas ofrece el genoma de la arañita roja o bimaculada (*Tetranychus urticae*)

El estudio, que fue realizado por un consorcio de investigadores internacional, encabezado por el canadiense Miodrag Grbic, de la Universidad de Western, Ontario, se publicó en la edición del jueves de la revista científica *Nature*.

El trabajo incluyó a los científicos del Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso, Ivber Ewer y Rodrigo

agrícola porque ataca al tomate, la frutilla, la manzana, la uva y a especies ornamentales", explica.

Destaca que el animal en el primero que se secuencia de un grupo que incluye muchas plagas agrícolas y otras que hacen daño a la salud animal y humana, como las garrapatas.

Trabajo de verificación

Formaron parte de un consorcio grande que incluye más de 50 personas. Los científicos chilenos participaron en lo que se llama la notación del genoma.

nos encargamos de verificar algunas familias de genes que tienen que ver con el desarrollo, la estructura del esqueleto del animal y parte sustancia «duro», destaca el científico.

Asimismo que en estos días es difícil que un artículo sobre la secuenciación de un genoma termine en una buena revista, porque se hacen muchas. "La gracia que tiene éste, aparte de ser una gran plaga, es que como puede desarrollar resistencia a muchos cosas y comer tantas plantas diferentes, no es común".

El científico destacó que la información obtenida también le servirá

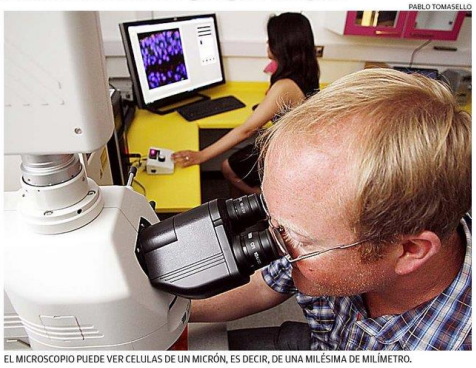
Title: "UV inaugurates a microscope that can see neurons in 3D"
Media: Newspaper "El Mercurio de Valparaíso" (Regional)
Date: January 11, 2012

UV inaugura microscopio que puede ver las neuronas en 3D

CIENCIA. *Más de $ 100 millones costó el instrumental que permitirá estudiar con más detalle el sistema nervioso. Es el tercero que opera en el país.*

Christian González G.
ciudades@mercuriovalpo.cl

PABLO TOMASELLO



EL MICROSCOPIO PUEDE VER CELULAS DE UN MICRÓN, ES DECIR, DE UNA MILÉSIMA DE MILÍMETRO.

Con el objetivo de mejorar la capacidad tecnológica para la formación de estudiantes de posgrado, ofrecer nuevas oportunidades de investigación con equipamiento de alto nivel y proveer cobertura de microscopía confocal de barrido láser, se inauguró en la Universidad de Valparaíso el Laboratorio de Microscopía Avanzada de Fluorescencia (Lamaf), junto con nuevas oficinas del Centro Interdisciplinario de Neurociencia de Valparaíso (CINV).

Este ostentoso elemento costó 105 millones de pesos, y fue financiado por fondos del mismo establecimiento de educación superior en conjunto con el proyecto Fiac-Mecesup 2, siendo pionero actualmente en nuestra región, registrándose sólo dos de estos objetos en el país (ambos en Santiago).

"El microscopio opera con fluorescencia, esto quiere decir que marca la célula y la ve con el láser. Con eso obtenemos una mejor resolución e imágenes en tres dimensiones y así podemos mejorar las conclusiones relevantes para las enfermedades. Su finalidad es la de obtener investigación de punta en el sistema nervioso básicamente, además de entender y curar algunas enfermedades", señaló Oliver Schmachtenberg, director del proyecto.

FONDOS

Las obras realizadas en el inmueble del pasaje Harrington se realizaron gracias a una inversión de 35 millones de pesos, los que fueron aportados por el CINV y la misma universidad, siendo el responsable de esta iniciativa, el director del centro, doctor Ramón Latorre, quien se mostró muy agradecido a la universidad por la finalización de los trabajos y la entrega de los mismos.

$105 millones costó el microscopio que será utilizado para fines científicos.

$230 millones fue el monto de inversión en el Laboratorio de Microscopía de la UV.

ALEGRÍA

La habilitación de las nuevas oficinas del CINV produjeron plena alegría entre quienes asistieron a la actividad desarrollada en la casona del pasaje Harrington, a un costado de la facultad de ciencias de la U. de Valparaíso, ya que en éstas se llevarán a cabo una serie de trabajos que generarán mayores espacios para investigadores y personal de apoyo del mismo centro.

"Para la universidad es muy gratificante que podamos contribuir a revitalizar un barrio como es el cercano al pasaje Harrington, y luego hacerlo para el desarrollo de la ciencia de alto nivel como la que se desarrolla en el CINV. Para nosotros se trata de la realización de fines públicos, producir ciencia y conocimiento es aportar al desarrollo humano", agregó el rector de la establecimiento, Aldo Valle.

Para el alcalde de Valparaíso "esto es altamente provechoso, ya que tiene que ver con la apuesta de una universidad para dotar un centro con equipos que permitan que los mejores puedan estar haciendo ciencia. También está la apuesta de la universidad por la recuperación, ya que donde va a estar cobijado el centro tiene una historia muy importante. El Pasaje Harrington es un conjunto de casas muy típicas de la arquitectura porteña", declaró el jefe comunal de Valparaíso, Jorge Castro.

PABLO TOMASELLO

LOS NIÑOS "CAPEAN" EL CALOR EN LAS FUENTES DE AGUA EN VIÑA.

Ola de calor sube los termómetros sobre los 35° en algunas zonas

VIÑA DEL MAR. *Altas temperaturas de la región seguirían durante todo enero.*

Este verano ha sido muy caluroso y un ejemplo es que en el sector costero la temperatura alcanzaría hoy los 25° C, según las proyecciones de la Dirección Metereológica. Peor será para Los Andes el jueves, cuando la máxima alcance los 35° a la sombra.

Según el jefe del centro meteorológico de Valparaíso, Roberto Díaz, esto se debe a que hay una ola de aire cálido proveniente del norte del país, que provoca un incremento de la sensación térmica.

"Este es un fenómeno cíclico, que varía dependiendo de los sistemas frontales. Podrían pasar los días y meterse una cuña de aire frío, que haría cambiar el clima a nublado", explicó el meteorólogo.

"La temperaturas altas provocan una evaporación del aire y ahí viene la vaguada costera, entonces cambiaría todo el panorama", precisó. Pero para tranquilidad de quienes hoy disfrutan de sus vacaciones en la playa, el clima y la temperatura no descenderían mientras le factor viento esté presente, "Durante el mes de enero las altas temperaturas se mantendrían mientras haya viento, que no permite que se forme la vaguada costera", aseguró Díaz.

La gente en las playas trata de capear el calor de la mejor forma. Los rociadores de agua, y los tradicionales quitasoles son los favoritos de quienes acuden a los balnearios para disfrutar de los días soleados. Los más felices son los comerciantes que tienen sus negocios en los balnearios.

Cecilia Martínez arrienda quitasoles a los bañistas y asegura que han aumentado este año: "la gente trata de protegerse más, incluso hay personas que cuando ya no hay arriendo de quitasoles se van porque el calor y el sol están muy fuertes. Ya no se puede estar expuesto al sol sin una protección".

Turistas provenientes de Bueno Aires, aseguran que si llegan a las dos de la tarde a la playa, a las cinco tienen que irse porque no dan más con el calor. E insisten en la importancia del protector solar.

DGA hace positivo balance de acuerdo para el uso de las aguas del río Aconcagua

QUILLOTA. *Según el organismo, ha permitido abastecer a los regantes de la zona.*

Las distintas secciones del río Aconcagua han cumplido el convenio que permite el uso de las aguas, lo que se suma a un aumento de las labores de control de parte de la DGA y de la Dirección de Obras Hidráulicas (DOH), de acuerdo a un informe emanado de estos organismos.

El control se inició el primer fin de semana de enero y contempla más personal para la vigilancia del cierre de los canales en los distintos puntos de la cuenca del Aconcagua.

Esta iniciativa especial tuvo un impacto positivo en el cumplimiento del acuerdo, percibiéndose un mayor respeto por los horarios de cierre de las compuertas que el registrado en las semanas previas.

"Ya hubiésemos querido estos niveles de agua antes del movimiento. Si el logro era tener agua, está cumplido"

Álex Salazar
Presidente canal Hijuelas

El fin de semana el caudal promedio registrado fue de 19,5 m3/s , cifra menor a la semana pasada donde se promedió un caudal de 27,4 m3/s, sin embargo el agua llegó casi íntegro desde la cabecera de la cuenca hasta la tercera sección, verificándose así el cumplimiento del acuerdo por parte de la primera y segunda sección del río.

El promedio del domingo fue superior al caudal registrado en la estación Chacabuquito, ubicada en el inicio del río.

MOP ES GARANTE

En total, 43 monitores se desplegaron en los tramos del río Aconcagua para vigilar que las condiciones del acuerdo sean respetadas por los regantes, a fin de permitir el acceso al agua. Este equipo es el encargado de denunciar irregularidades y levantar posibles contingencias.

El Ministerio de Obras Públicas actúa como garante de la resolución de los conflictos que han mantenido los regantes del río Aconcagua, que incluso motivó una manifestación y la solicitud de intervención por parte de los productores de la provincia de Quillota.

Álex Salazar, presidente del canal Hijuelas, quien a fines de diciembre firmó un acuerdo con el MOP para velar por el cumplimiento del convenio, aseveró que pese a que hay buenos resultados se mantienen en estado de alerta.

"ALERTA CONSTANTE"

"Cuando tenemos a más de 30 personas con facultades de cerrar compuertas, es prácticamente una intervención pequeña, que es lo que estábamos pidiendo, intervenir los canales que no cumplen. Hay canales que han cumplido, pero estamos en alerta constante para vigilar que el agua llegue a los regantes de la zona y poder regar sus cultivos", Alex Salazar.

El dirigente explicó que los dos últimos fines de semana, el canal Hijuelas ha tenido un buen caudal para el riego.

"Ya hubiésemos querido estos niveles de agua antes del movimiento. Si el logro del movimiento era tener agua, está cumplido", dijo Salazar.

Para complementar el acuerdo de los regantes, el 20 de enero deben comenzar a operar los pozos profundos de Curimón y Panquehue.

"La idea es poder iniciar el aporte de los pozos, cuando comienza a bajar el caudal producto de los deshielos, entonces poder trabajar con el agua que venga en el río, mas la compensación de los pozos", dijo el seremi de Obras Públicas Pedro Sariego. Esta agua será conducida a través de un canalón directamente a la tercera sección.

RODRIGO BAEZA

EL FIN DE SEMANA AUMENTA EL CAUDAL DEL RÍO ACONCAGUA.

Title: "Three national price winners launch the *Explora* year"
Media: Newspaper "Diario Austral" (Regional)
Date: March 29, 2012

6 | Actualidad

DIARIO AUSTRAL

Tres premios nacionales dieron inicio al año de Explora

TRABAJO. *En el programa de Conicyt dedicarán todo el 2012 a la difusión de la neurociencia, a través de video conferencias, charlas y ferias*

Gerardo Pavez Vargas

Ayer se dio inicio oficial al año de actividades del programa Explora de la Comisión Nacional de Investigación Científica y Tecnológica, Conicyt, en Los Ríos, en el año de la neurociencia.

La ceremonia de inicio se realizó en el Aula Magna del campus Teja de la Universidad Austral de Chile. Luchi y el primer gran hito importante de la jornada fue el Premio Nacional de Ciencias Naturales 2002, Ramón Latorre, quien dictó la conferencia "El arte del descubrimiento". La actividad contó además con la presencia del intendente Juan Andrés Varas, el director nacional del programa Explora, José Santiago Arellano, el rector de la Universidad Austral de Chile, Oscar Galindo, el seremi de Educación Carlos Cruz y la coordinadora regional de Explora en Los Ríos, Lillian Villanueva, junto a directores, profesores y estudiantes de establecimientos educacionales de la toda la región. Incluso, los alumnos de otras partes de Chile pudieron ser parte de la actividad a través de internet.

INVITADOS DESTACADOS

Además de Ramón Latorre, otros dos científicos destacados con el Premio Nacional de Ciencias fueron participes de la jornada de ayer, se trató de Claudio Hunneus, Premio Nacional de Ciencias Exactas 1995 y director del Centro de Estudios Científicos, Cecs y Pedro Labarca, Premio Nacional de Ciencias Naturales 2004 e investigador de dicho centro. Su situación fue destacada por José Santiago Arellano, director nacional de Explora, quien expresó que "este es un privilegio y un entusiasmo para el Estado de cada uno de los estudiantes de la región". Quien también se refirió al inicio del año Explora, fue el intendente Varas quien manifestó que "estamos avanzando en disminuir la desigualdad, por medio de la educación, que es una de las armas que nos conducirá al desarrollo". Por último, el seremi Carlos Cruz expresó que "nuestro país está en la búsqueda del avance científico y es necesario que los jóvenes participen de aquello".

CON UNA CONFERENCIA COMENZÓ OFICIALMENTE EL AÑO DE ACTIVIDADES DE EXPLORA EN LOS RÍOS.

"El arte del descubrimiento"

En la conferencia de inicio del año de Explora, Ramón Latorre relató a los jóvenes asistentes a la charla las circunstancias en que grandes científicos de la historia realizaron sus descubrimientos. En ese sentido indicó que "el azar favorece a una mente preparada", poniendo como ejemplo a Newton con la historia de la manzana con que descubrió la fuerza de la gravedad.

LOS PRESUPUESTOS MUNICIPALES SE DISCUTIRÁN EN SESIONES.

Profesores molestos por evaluaciones

DESPIDOS. *Amancay que se opondrán a desvinculaciones por desempeño.*

El presidente comunal Valdivia del Colegio de Profesores, Ángel Toro, señaló que el gremio rechaza colectivamente las medidas que se tomarán con los profesores con mal desempeño en la Evaluación Docente, los cuales serán desvinculados de sus puestos de trabajo en el sistema municipal.

Toro expresó que "aún no tenemos claro el número de profesores a nivel regional que serán desvinculados de sus trabajos por esta evaluación, debido a que aún estén llegando los expedientes, sólo sabemos que se trata de 106 a nivel nacional, pero en vamos a asegurar que los profesores puedan sus trabajos por una evaluación como esta que presenta tantos vicios".

El dirigente agregó que "han habido situaciones anormales en el tema de las evaluaciones, ya que no siempre se cumple el rigor de la evaluación docente, lo que produce muchos vicios". En ese sentido, el seremi de Educación Carlos Cruz indicó que "los directivos locales que se realicen se han dado a la baja de las matrículas antes establecimientos educacionales, lo que hará reestructurar los cursos, pero hasta fines de marzo no se conocerá una cifra exacta".

Escuela Ann Sullivan recibió su nuevo bus

ENTREGA. *Gracias a inversión de 32 millones de pesos del FNDR.*

Title: "The stubbornness of Latorre"
Media: Magazine "Que Pasa" (National)
Date: January 2012

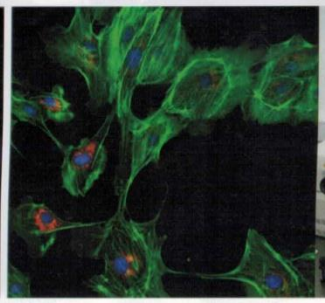
LA PORFÍA DE

LATORRE

El Premio Nacional de Ciencias Ramón Latorre reparte su tiempo entre viajes anuales para dictar clases en la U. de Chicago, conferencias y, más que nada, su labor como director del Centro Interdisciplinario de Neurociencia de Valparaíso. Ahí está puesta su verdadera obsesión. Su sueño es instalar el nuevo CINV en el Edificio Severín, hoy una más de las ruinas del barrio Puerto.

[Por Paulo Ramírez // Foto: Esteban Zúñiga]

Title: "Valparaiso at the forefront of neuroscience"
Media: Magazine "Tell" (Regional)
Date: April 2012



El Laboratorio de Microscopía Avanzada de Fluorescencia (LAMAF) es un conjunto de equipos que permite analizar en detalle las características de las neuronas.

Valparaíso, a la vanguardia de la neurociencia

En una tradicional casona de Playa Ancha, un grupo de científicos del Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso investiga cómo funcionan el sistema nervioso y las neuronas que nos permiten relacionarnos con nuestro medio ambiente.

Durante los últimos veinte años, John Ewer ha dedicado buena parte de sus esfuerzos a estudiar la estructura genética de *Drosophila*, la mosca del vinagre. Lo hizo en la Universidad de Brandeis, en Boston, donde se doctoró en Neurogenética en 1991, y más tarde como profesor asociado en la prestigiosa Universidad de Cornell. ¿Por qué ese interés en el ADN de un insecto? Porque allí, dice, podría estar la clave para entender algunas de las lógicas de funcionamiento del sistema nervioso de todos los animales, incluyendo el de los seres humanos.

"La *Drosophila* –o mosca del vinagre, precisa– tiene bastante más en común con los seres humanos de lo que se podría creer; por ejemplo, investigaciones que en 1995 culminaron en un Premio Nobel en Medicina, mostraron que la forma en que se desarrolla el embrión de la mosca tiene piezas similares al de casi todos los animales. Por eso los llamamos sistemas modelo; en general, los seres vivos tienen numerosos componentes comunes y también lógicas comunes de funcionamiento. Se podría decir que todos tenemos algo de mosca, somos variantes de un diseño similar", explica Ewer.

Hace algunos años, sin embargo, este chileno con pinta y acento de gringo abandonó su envidiado puesto en la costa este de Norteamérica y volvió a nuestro país. En una casona de Playa Ancha, junto a una decena de investigadores permanentes, un puñado de científicos asociados y decenas de estudiantes de posgrado, da vida al Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso (CINV-UV), que nació en 1999, al alero de la Facultad de Ciencias de dicha casa de estudio, y hoy se ha convertido en un núcleo científico de primer nivel que, en sus propias palabras, poco tiene que envidiar a los mejores del planeta.

"El concepto de neurociencia es reciente y se refiere al conjunto de disciplinas interesadas en el estudio del sistema nervioso, que tratan de responder a preguntas tales como el origen del lenguaje, nuestra habilidad por la matemática, por qué vemos los colores o sentimos el frío, cómo aprendemos y memorizamos; en breve, el origen de la conciencia", añade Adrián Palacios, doctor en Neurociencia de la Universidad de Pierre y Marie Curie (Paris VI), ex investigador de la Universidad de Yale y uno de los creadores de este instituto. "Se trata de entender cómo a partir de un ensamble de millones de células que forman el cerebro se genera nuestra conciencia, nuestra manera de sentir, de aprender o de olvidar".

CIENCIA BÁSICA

En el CINV-UV conviven especialistas de distintas áreas: biofísicos, neurobiólogos, genetistas y bioinformáticos. Un denominador común es que todos hacen ciencia básica, es decir, no aspiran a que sus avances o descubrimientos se transformen en productos o aplicaciones, sino que sirvan para responder preguntas que abran nuevos caminos en el desarrollo de la ciencia. Algo que muchas veces es difícil de comprender en un país donde casi todos los parámetros de éxito están ligados a resultados inmediatos y, mejor aún, rentables.

"Entender el funcionamiento de un órgano es prerrequisito para entender a un órgano enfermo y, desde esa perspectiva, todas las preguntas relativas al sistema nervioso, o endocrino, son válidas. Desde el punto de vista práctico, las curas para las distintas enfermedades se han descubierto gracias a que alguien, antes, investigó el organismo y nos permitió saber cómo operaba. Por otro lado, la conexión entre el conocimiento básico de un sistema biológico y su aplicación es una cosa que yo califico como mágica. No hay ningún país en el mundo que haga buena innovación si no tiene buena ciencia básica. Las dos cosas están enhebradas, salen cuando tienen que salir. Uno no puede forzar las cosas, no puede decir 'vamos a curar el cáncer', porque se han gastado millones y millones de dólares para ello y los primeros avances, justamente, han venido de la ciencia básica".

Quien así habla es Ramón Latorre. Doctor en Ciencias de la Universidad de Chile —uno de los primeros que hubo en el país—, Premio Nacional de Ciencias en 2002, miembro de la Academia de Ciencias de Estados Unidos, investigador de la Universidad de

Title: "Lack of advanced human capital hinders national development"
edia: Magazine "Universidad de Talca" (Regional)
Date: April 2012


UNIVERSIDAD DE TALCA

WWW.UTALCA.CL | SEMANA DEL 16 AL 22 ABRIL DE 2012

PREMIO NACIONAL DE CIENCIAS, RAMÓN LATORRE, EN INAUGURACIÓN DE AÑO 2012:

Escaso capital humano avanzado dificultará desarrollo del país

Chile
Japón
EEUU

Pese a "orfandad" estatal que enfrentan las Universidades Públicas, nuestra Corporación continúa avanzando a paso firme en su rol de favorecer la ciudadanía, con más carreras de pregrado, numero de matrícula, nuevos campus y más investigaciones. "Enfrentamos el año con la convicción que la luz que nos guía el camino de la excelencia, nos sigue alumbrando y no se extingue, que hay un país esperando lo mejor de sus universidades públicas; que no tenemos derecho a defraudarlo", dijo el rector Álvaro Rojas (Págs. 4 y 5).

Title: "A natural animal model for Alzheimer's disease"
Media: Magazine "Pour la Science" (International)
Date: August 15, 2012

POUR LA SCIENCE .fr
Le magazine de référence de l'actualité scientifique

Rechercher

Actualités | Multimédia | Agenda | Offres d'emploi | En kiosque | Archives | Éditions numériques | S'abonner | Librairie

MATHS | PHYSIQUE - CHIMIE | ASTRONOMIE | TERRE - ENVIRONNEMENT | BIOLOGIE - SANTÉ | NEUROSCIENCES | PALÉONTOLOGIE | SCIENCE ET SOCIÉTÉ | TECHNOLOGIE

Abonnez-vous à Pour la Science
25 % de remise minimum !
> Abonnement simple
> Abonnement couplé
Déjà abonné

ACTUALITÉS | 15/08/2012 | 1 réaction(s) | < Précédent - Suivant >

NEUROSCIENCES

Un modèle animal naturel pour la maladie d'Alzheimer

Des neurobiologistes chiliens et français ont montré que le dégu du Chili – un petit rongeur – souffre souvent, en vieillissant, d'une pathologie semblable à la maladie d'Alzheimer. Il pourrait représenter un excellent modèle d'étude pour les chercheurs.

Bénédicte Salthun-Lassalle

La maladie d'Alzheimer est la forme de démence la plus fréquente. En 2010, en France, elle concernait 850 000 personnes âgées de plus de 75 ans, soit 1,2 pour cent de la population. Elle est donc un enjeu majeur de santé publique : une grande partie des équipes de recherche en neurosciences, en France et ailleurs, étudient cette pathologie afin de la dépister le plus tôt possible, ralentir son évolution et trouver un traitement. Adrian Palacios, du Centre interdisciplinaire de neurosciences à l'Université de Valparaíso au Chili, et ses collègues chiliens, américains et français ont montré qu'un petit rongeur, le dégu du Chili *(Octodon degus)*, pourrait bien aider les chercheurs : quatre animaux sur cinq développent naturellement, avec l'âge, une maladie d'Alzheimer et constitueraient donc de bons modèles d'étude.

Afin de comprendre et de combattre une maladie neurodégénérative telle que la maladie d'Alzheimer, les équipes de recherche ont plusieurs outils à leur disposition, notamment : des cellules et des neurones en culture, dans des « boîtes » de pétri, pour tester des molécules, déterminer les dysfonctionnements cellulaires et comment les cellules interagissent ; des modèles animaux, souvent créés par manipulation génétique, qui reproduisent les signes cliniques et

Le dégu du Chili serait un bon modèle d'étude de la maladie d'Alzheimer, car il développe avec l'âge une forme semblable de la pathologie, sans intervention humaine.

© Centre interdisciplinaire de neurosciences, Université de Valparaíso, Chili

Pour en savoir plus

A. Ardiles *et al.*, Postsynaptic dysfunction is associated with spatial and object recognition memory loss in a natural model of Alzheimer's disease, *PNAS*, en ligne le 6 août 2012.

L'auteur

Bénédicte Salthun-Lassalle est journaliste à *Pour la Science* et *Cerveau & Psycho*.

Restez connectés

Actuellement en kiosque

N°425
Mars 2013

›› Sommaire
›› Télécharger le pdf
›› Acheter ce numéro
›› Abonnements
›› Feuilleter

En couverture
Les fractales lisses
Un nouvel objet mathématique
Il est possible de transformer une portion de plan en une surface torique sans modifier les longueurs. Le résultat, visualisé pour la première fois, inaugure une nouvelle famille d'objets géométriques.

Mathématiques
Des cartes bien mélangées

Neurosciences
Human brain project : simuler le cerveau humain

Astrophysique
Les secrets des météorites primitives

Psychologie
Rythmes scolaires : les recentrer sur l'enfant

Recevez gratuitement nos newsletters

Entrez votre e-mail | ok

Dossier Pour la Science

N°78
Janvier - mars 2013

›› Sommaire
›› Télécharger le pdf
›› Acheter ce numéro
›› Feuilleter
›› Abonnements

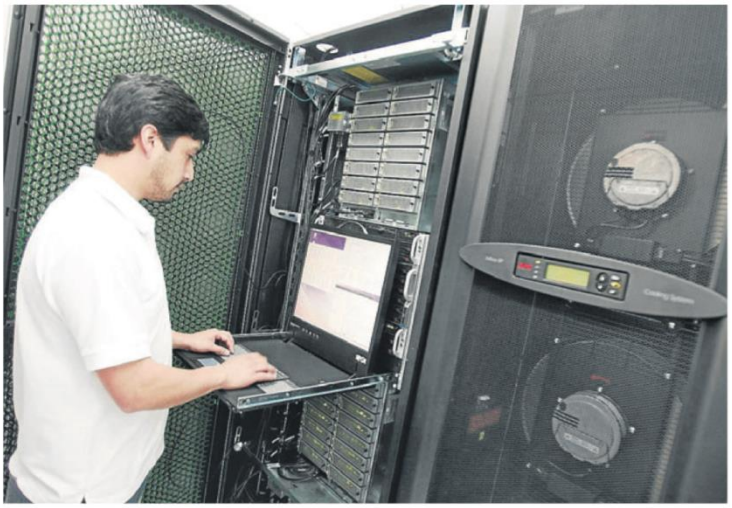
Title: "Chile's largest computer doubles the processing capacity in science"
Media: Newspaper "La Tercera" (National)
Date: September 4, 2012

Tendencias

6 m **cuadrados** mide la habitación donde el computador es alojado.

1 **día demora** realizar un cálculo que un PC tardaría 4 años y medio.

6 **meses** tardó su construcción, a cargo de la empresa Silicon Graphics.



▸▸ Desde una pequeña consola se opera el computador diseñado para la investigación. FOTO: PATRICIO FUENTES

Computador más grande de Chile duplica capacidad de cálculo para ciencia

▸ Proyecto de Unab y UV será inaugurado hoy y se usará para nanotecnología.

▸ Calcula en un día lo que un PC común haría en cuatro años y medio.

Axel Christiansen Z.

Es tan grande que se necesita una habitación de seis metros cuadrados entera para alojarlo y sólo puede manejarse de pie. Su rapidez es tal que es capaz de hacer en un día el cálculo que un computador normal realizaría en cuatro años y medio. Y usando sólo un tercio de su capacidad.

Se trata del nuevo supercomputador que hoy se inaugura en el Centro de Bioinformática y Biología Integrativa de la U. Andrés Bello, en el campus República, y que lo convierte en el más grande de Chile. Una iniciativa realizada con el Centro Interdisciplinario de Neurociencia de la U. de Valparaíso y que permite a nuestro país avanzar en investigación de medicamentos y nanotecnología.

Danilo González-Nilo, director del Centro de Bioinformática y Biología Integrativa de la Unab, estuvo encargado del proceso de creación de este computador, bautizado como Nano Biotech por el uso que se le tiene planeado dar. "Su creación tomó seis meses y fue encargado a Estados Unidos", cuenta a **La Tercera.**

La empresa Silicon Graphics, especializada en el desarrollo de este tipo de equipos, creó el computador a la medida de las necesidades del centro.

¿Cuál es la diferencia con un computador tradicional? Estos equipos no están diseñados para correr programas tradicionales, sino que para realizar cálculos intensivos, simulaciones y analizar grandes cargas de datos.

"Nuestros estudios requieren evaluar, por ejemplo, la interacción que tienen miles de átomos. Un cálculo normal requiere unos 500 mil átomos interactuando entre sí, lo que precisa equipos dedicados a ese análisis".

Allí es donde entran en juego sus especificaciones: el computador posee 1.536 núcleos -o cores- de procesamiento y 1.024 GB (3TB) de memoria RAM. Los primeros son los que se dedican a la velocidad y complejidad del cálculo, mientras que la memoria RAM permite ir acumulando más información calculada sin que ésta se vaya "atorando" y haga lento el proceso.

Para hacerse una idea, un computador de escritorio promedio posee hoy de dos a cuatro núcleos de procesamiento y cuatro GB de RAM.

"Con este computador al menos se duplicará la capacidad de cálculo de la ciencia en Chile", dice González-Nilo, quien ya había trabajado armando el supercomputador de la Universidad de Talca, que posee 300 núcleos, al que en 2010 se le sumó el de la Universidad de Chile, con cerca de 800.

Investigaciones

El uso de este computador será básicamente para el estudio de proteínas y nanotecnología. Uno de sus primeros proyectos será entender cómo actúa el calor y el dolor a nivel molecular con el fin de producir en el futuro inhibidores del dolor, por ejemplo, para pacientes que sufren osteoporosis.

También se usará en la aplicación de nanomedicina; es decir, la creación de fármacos que actúan a nivel molecular y que son particularmente efectivos en el tratamiento del cáncer, por ejemplo.

Los planes para la máquina ya están delineados: el próximo año se lanzará un concurso para que los interesados puedan aplicar y hacer sus cálculos en el computador. "Nuestra idea no es dejar obsoletos a los actuales equipos, sino que complementar y aumentar este tipo de investigaciones en Chile", dice.●

COMPARACION

El más rápido del mundo
Con 1.572.864 núcleos de procesamiento, el Sequoia, ubicado en EE.UU., es el supercomputador más veloz del mundo. Se usa para analizar datos científicos y simulaciones de armas nucleares.

Al servicio del petróleo
Los supercomputadores también son útiles en la industria. El Grifo04, de Petrobras, en Brasil, es el más poderoso de Sudamérica (17.408 núcleos). En investigación científica el cetro es del Tup, Brasil, (31.104 núcleos).

El antiguo récord
El año 2010, la Universidad de Chile estrenó el IBM iDataPlex, un supercomputador de 600 núcleos y 1.500 GB de memoria RAM. El equipo ha sido usado en cálculos climáticos y también astronómicos.

Personas con altos niveles de azúcar en la sangre se les achica el cerebro

Un nuevo estudio realizado por científicos de la U. Nacional Australiana, en Canberra, demostró que las personas que tienen altos niveles de azúcar en la sangre -sin ser diabéticos aún- tienen un mayor riesgo de encogimiento cerebral: un fenómeno que ocurre producto del envejecimiento y algunas demencias.

"Numerosos estudios han demostrado una relación entre la diabetes tipo 2 y la contracción del cerebro y la demencia, pero no sabíamos mucho acerca de si las personas con un nivel normal de azúcar en la sangre, aunque en su límite máximo, tienen los mismos efectos", dijo el autor del estudio, Nicolás Cherbuin.

Para comprobarlo, siguieron a 249 pacientes entre los 60 y 64 años que tenían un rango de azúcar en la sangre considerado normal, de acuerdo a la definición de la OMS. Es decir, de hasta 110 mg/dl. A todos ellos se les hizo un escáner cerebral al inicio del estudio y otro cuatro años después. Tras comparar las imágenes con la cantidad de azúcar presente en la sangre de los participantes en ayunas, observaron que aquellos que estaban más cerca del límite eran más propensos a tener una pérdida en el volumen cerebral entre un 6% y un 10% en las áreas del hipocampo y la amígdala implicadas en la memoria y habilidades cognitivas, en comparación con aquellos que tenían los niveles de azúcar más bajo.

"Estos hallazgos sugieren que, incluso para las personas que no tienen diabetes, los niveles de azúcar en la sangre podrían tener un impacto en la salud del cerebro", dijo Cherbuin.

Aunque no está totalmente descrito el mecanismo que hay tras el fenómeno, se sabe que el azúcar aumenta los niveles de cortisol, la hormona del estrés, la que tras permanecer por largo tiempo en alta cantidad, provoca muerte neuronal.●

"Incluso para quienes no tienen diabetes, altos niveles de azúcar impactan negativamente el cerebro".

Dr. Nicolás Cherbuin
U. Nacional Australiana

Title: "Chilean rodent becomes a model for studying Alzheimer and other diseases"
Media: Newspaper "El Mercurio" (National)
Date: September 12, 2012

Habita exclusivamente en la zona centro-norte del país:

Roedor chileno se convierte en modelo para estudiar el alzheimer y otras enfermedades

En laboratorios de diversos países, el afable degu (*Octodon degus*) se está utilizando para estudiar diabetes, cataratas, tumores y conductas como el apego y la crianza.

PAULA LEIGHTON N.

A la clásica rata de laboratorio le salió competencia 'made in Chile'. El degu (*Octodon degus*), un pequeño y sociable roedor nativo de la zona centro-norte del país, ha ido ganando terreno como modelo de estudio de enfermedades y conductas sociales humanas (ver recuadro).

En agosto, la prestigiosa PNAS, de la Academia de Ciencias de EE.UU., tuvo a este pariente de la chinchilla entre sus páginas. Investigadores validaron su uso para estudiar las etapas iniciales de la enfermedad de alzheimer, patología que los degus desarrollan en forma natural, a diferencia de las ratas y ratones de laboratorio tradicionales, a los que se les debe inducir genéticamente para estudiarlos.

Un artículo sobre el estudio se convirtió en uno de los más comentados del mes en Alzheimer Research Forum, el principal foro en línea de expertos en la materia. Y en las próximas semanas, la revista Cold Spring Harbor Protocols —dedicada a la divulgación de métodos de investigación científica— publicará dos artículos destacando al degu como un animal cuya similitud biológica con el humano lo convierte en una especie ideal para estudiar capacidades mentales superiores, como aprendizaje y memoria, e investigar numerosas enfermedades y posibles terapias para tratarlas.

Adrián Palacios (izq.) y Álvaro Ardiles estudian el alzheimer en degus. Descubrieron que características naturales de estos roedores pueden ayudar a la detección precoz de esta patología neurodegenerativa.

"El degu se está usando en laboratorio porque desarrolla en forma natural patologías como diabetes, cataratas, alzheimer y algunos tipos de tumores. Asimismo, a diferencia de la rata, tiene su actividad máxima de día, por lo que su ritmo circadiano es bastante similar al humano", explica Adrián Palacios, profesor del Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso.

El investigador es coautor de los artículos en ambas revistas junto a

está utilizando el roedor chileno, al igual que en Alemania, Holanda, España, Japón e Inglaterra.

Modelo completo

En 2005, el degu logró notoriedad cuando por primera vez se publicó en Neurobiology of Aging un estudio que lo describía como el primer roedor silvestre que serviría como modelo para estudiar alzheimer. Esto, tras descubrir que al envejecer, a los dos o tres años de vida, acumulaban en el cerebro beta-amiloide y proteína tau, características de la enfermedad.

"Sobre todo cuando lo sacas de su medio natural y empieza a comer alimentos con azúcares, el degu se en-

Una especie amigable

"El modo de vida de lo
semeja al de los huma
cestrales, con hábitos
donde diferentes grup
liares coexisten en un
población, con gran in
entre individuos de dif
familias", destaca Roc
quez, investigador del
de Ecología y Biodiver
Depto. de Ciencias Ec
la U. de Chile.
La organización social
el apego, la angustia d
ción, el juego y la crian
nitaria son algunas de
ductas del degu que se
diado en Chile y en el e
Sus depredadores son
aves rapaces, como ág
peucos. "Si bien en co
naturales raramente s
el año de edad, en cau
pueden llegar a vivir é
e incluso 10", señala V

tor principal de esa in
director del Centro de E
to y Regeneración de l
Ciencias Biológicas de l
Adrián Palacios agre
ciente estudio en PNAS
como el primer modelo
ral para estudiar esta
neurodegenerativa, ir
sus etapas más precoce
"Como es posible
también puede usarse p
cómo los cambios a niv
relacionan con modific
conducta, por ejemplo
dad de reconocer objet
Además, ayudarían
macos para recuperar
ciones, una línea que
junto al laboratorio d

Title: "**Scientist from Valparaíso analyzes Chilean rodents to erradicate Alzheimer**"
Media: Newspaper "El Mercurio de Valparaíso" (Regional)
Date: September 20, 2012

Científico porteño analiza roedores chilenos para terminar con el Alzheimer

VALPARAÍSO. *El pequeño animal (Octodon degus), endémico de nuestro país, presenta durante su envejecimiento los síntomas cerebrales más comunes de la enfermedad.*

José Ossandón
josandon@mercuriovalpo.cl

Un roedor nativo de nuestro país podría ser la solución para terminar con el mal de Alzheimer. Así lo establece un reciente estudio hecho en el laboratorio del investigador Adrián Palacios, del Centro Interdisciplinario de Neurociencia (CINV) de la Universidad de Valparaíso, que fue publicado recientemente en la prestigiosa revista Proceeding of National Academy of Science.

Según el análisis elaborado en esa institución, el pequeño animal chileno (Octodon degus) presenta durante su envejecimiento los síntomas cerebrales más comunes de la enfermedad neurodegenerativa de tipo Alzheimer.

El modelo propuesto permitirá el estudio de los mecanismos neurobiológicos precisos involucrados y el ensayo de agentes protectores de este mal.

Cabe consignar que el Alzheimer es un problema de salud pública mayor y afecta a millones de personas alrededor del mundo.

EMV

EL ROEDOR PRESENTA DURANTE SU ENVEJECIMIENTO LOS SÍNTOMAS CEREBRALES MÁS COMUNES DEL ALZHEIMER.

"Quedan aún importantes etapas"

● "Si bien hemos avanzado un gran paso en establecer un buen modelo de estudio para Alzheimer, quedan aún importantes etapas para iniciar la validación de un diagnóstico precoz, como el uso de medidas terapéuticas que apacigüen los efectos de la enfermedad", aseveró el investigador del Centro Interdisciplinario de Neurociencia de la UV, Adrián Palacios.

ORIGEN

Se conoce que en cerca del 2% de los casos el origen de la enfermedad se relaciona con algún factor genético de tipo familiar; aunque, en definitiva, el 98% de las situaciones es esporádico, sin causas conocidas.

Los pacientes con Alzheimer poseen problemas de aprendizaje y memoria, y ven su vida cotidiana afectada de manera invalidante.

Durante años los estudios científicos han descubierto una serie de agentes esenciales en producir la enfermedad.

Uno de estos es la acumulación temprana de proteínas ß-amiloide soluble y tau fosforilada, generando disfunción celular y, por consecuencia, un deterioro de la capacidad cognitiva de las personas.

En etapas más avanzadas, estas proteínas forman cúmulos de fibras (placas) que se depositan de manera intra y extracelular en neuronas, ahondado en la neurodegeneración.

MODELO ANIMAL

Un problema frecuente en el estudio de las enfermedades neurodegenerativas, como el Alzheimer, es disponer de un modelo animal que reproduzca de manera natural en el tiempo los síntomas biológicos que la caracterizan.

A falta de modelos naturales, los biólogos crearon ratones transgénicos, a los que se les introdujo modificaciones genéticas que expresan proteínas como el ß-amiloide, cuya toxicidad fue previamente establecida.

Sin embargo, un problema frecuente en este tipo de modelo ratón transgénico es la sobreproducción de las proteínas de interés; la ausencia de varios otros agentes tóxicos; la escasez de pruebas conductuales cognitivas concluyentes y, por último, que el desarrollo de la neuropatología no ocurre en el contexto natural de envejecimiento paulatino que caracteriza al humano.

Recientemente la atención se ha dirigido sobre algunos modelos animales que presentan de manera natural algunos hitos de la enfermedad de Alzheimer.

Por ejemplo en el "conejillo de India" o cuye, el precursor APP de la proteína ß-amiloide es idéntico al del humano. Y el cuye presenta acumulación de ß-amiloide en su cerebro.

No obstante, no se ha descrito en el cuye la presencia de otros bioagentes importantes de la enfermedad.

CONSECUENCIAS

Hace algunos años el laboratorio del doctor Nibaldo Inostrosa, de la Universidad Católica de Chile, demostró que en el Octodon degus se encontraban presentes varios agentes del Alzheimer y que este roedor podría constituir un buen modelo de estudio.

"Desde hace ya algunos años nos hemos dedicado, en particular junto a Álvaro Ardiles, un estudiante de nuestro doctorado, a estudiar de manera detallada las consecuencias cognitivas y de plasticidad neuronal en el modelo degus. A partir de 36 meses, el degus presenta un deterioro de su memoria espacial y la de reconocimiento de objetos, algo similar a lo que ocurre en los humanos", explicó el doctor Adrián Palacios.

Sostuvo que "junto a esto, ocurre desde temprana edad acumulación de la proteína ß-amiloide soluble y tau fosforilada en el cerebro del animal. Curioso en entender las bases neurobiológicas del deterioro cognitivo, estudiamos las propiedades de plasticidad sináptica a nivel del hipocampo, una estructura cerebral cuya función es crítica para el aprendizaje y la memoria espacial".

Agregó que "encontramos que, acorde a las observaciones anteriores, la respuesta fisiológica del circuito hipocampal se veía también alterada en degus envejecidos. La disfunción, entonces, pudimos encontrarla en la falla de algunas proteínas claves a nivel de neuronas post-sinápticas del hipocampo". CS

PDI

LAS MENORES SON DE LIMACHE.

Violento atraco a menor de 15 años en la playa El Sol de Viña del Mar

● Semidesnuda y totalmente shockeada fue encontrada ayer una menor de 15 años, quien sufrió el robo de casi la totalidad de su vestimenta en la playa El Sol de Viña del Mar, donde fue atacada por tres niñas oriundas de Limache, quienes violentamente le arrebataron sus prendas. La víctima llegó de madrugada al balneario junto a una amiga, tras disfrutar de las celebraciones de Fiestas Patrias en el Sporting Club. Tras recibir un llamado de alerta al fono 134, personal de la PDI se constituyó en el balneario y junto a la afectada dieron con el paradero del grupo de niñas. Las menores, de entre 15 y 17 años, fueron trasladadas hasta el Juzgado de Garantía. CS

Sujeto disparó contra un carabinero en plena vía pública

● Con una bala incrustada en su hombro derecho quedó un sujeto identificado como G.S.L., de 36 años, quien disparó contra un furgón policial y posteriormente contra un cabo primero ayer en Quintero. Los hechos sucedieron cuando el sujeto se encontraba caminando por la avenida Francia a torso desnudo y con un revólver nueve milímetros en su poder. El hecho fue alertado a personal de la Subcomisaría de Quintero, que se constituyó en el sector e intentó detenerlo. Ante esto, G.S.L. disparó contra el furgón policial y se escapó del lugar. Al ser interceptado, disparó esta vez contra un uniformado, quien en defensa propia utilizó su arma de servicio, hiriéndolo en un hombro. CS

Title: "What we know about memory and vision?"
Media: Magazine "Explora" (Nacional)
Date: September, 2012

¿Qué sabemos de memoria y visión?

Adrián Palacios: Doctor en Neurociencia
Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso.

Los colores no existen, podría decir un físico. Existen ondas y fotones que nuestros ojos separan como colores gracias a la retina, una compleja red de neuronas asociada a bastones y conos, células fotosensibles que nos ayudan a pintar la realidad. ¿Cómo percibimos? ¿Qué rol tienen los colores en la construcción de lo que nos rodea? Son algunas dudas que ocupan la investigación del neurocientífico Adrián Palacios, quien explica en esta entrevista las principales características de la visión humana y su evolución. También habla sobre la memoria, complejo proceso que no es posible situar en un sector específico del cerebro. "Aún no sabemos dónde está, sólo sabemos que está ahí, que es parte de un circuito de neuronas".

Distinguimos más colores que el resto de los mamíferos, pero menos que los peces, reptiles y aves. ¿A qué se debe?

Los mamíferos evolucionaron a partir de reptiles primitivos, que se adaptaron a un hábitat nocturno con gran éxito. Estos pequeños roedores lo pasaban bien cazando y se desarrollaron como nunca antes lo hizo otra especie.

Sin embargo, el costo de esta adaptación a la oscuridad fue perder ciertas características de la visión de colores que poseían sus ancestros. Éste es el origen del humano y recién hace 40 millones de años comienza a reconstituir esta visión, a re-inventarla. El primate tricrómata (que posee tres tipos de sensores para el color) es relativamente nuevo y todavía los sensores del sistema visual en humanos están adaptados a la oscuridad. El 95% de las células de la retina son bastones (células sensibles al blanco y negro).

¿Qué hace diferente al ojo humano de otras especies?

La diferencia la marca una pequeña zona de la retina llamada fóvea, de gran concentración de conos y con una mejor resolución tanto espacial como cromática, que nos permite disfrutar plenamente de un mundo en color. La evolución desde un sistema de visión dicromata, presente en la mayoría de los mamíferos, al del humano requirió de un tercer tipo de sensor (en la región del verde y rojo) naciendo una gama de colores que para nosotros es extraordinaria. Pero si tuviéramos 4 ó 5 diferentes tipos de sensores el mundo sería cromáticamente muy diferente, viviríamos de otra manera, podríamos ver objetos que reflejan, por ejemplo, ultravioleta o infrarrojo, es decir algo invisible al ojo humano.

6

Title: "Scientist explains how a zombies attack to Santiago would be like"
Media: Newspaper "Las Ültimas Noticias" (National)
Date: October 18, 2012

Biólogo usó ecuaciones para medir el fenómeno

Científico explica cómo sería un ataque zombi en Santiago

ORIETTA SANTA MARÍA

La imagen de los muertos vivientes, gimiendo y caminando con torpeza en busca de alimento, es fuente de inspiración para la ciencia. El doctor en biotecnología Tomás Pérez-Acle creó un modelo matemático y simulaciones computacionales para analizar el impacto de un ataque zombi sobre diez ciudades similares a Santiago.

Tomás, director del laboratorio de biología computacional de la Fundación Ciencia para la Vida y profesor de la Universidad de Chile, creó varias ecuaciones para estudiar la dispersión de los zombies proyectando los resultados a enfermedades contagiosas reales y a situaciones catastróficas como terremotos.

El científico, fanático de la serie "The walking dead", dice que el modelo de la infección zombi se parece (en teoría, claro, porque recordemos que los muertos vivientes no existen) al de varias enfermedades contagiosas que atacan a los humanos. "Si una persona se contagia con el Síndrome de Inmunodeficiencia Adquirida (SIDA), nunca deja de estar contagiada y si un zombi muerde a alguien esta persona tampoco nunca deja de estar infectada", explica el biólogo.

> Cuatro muertos vivientes tardarían once días en contagiar a diez grandes ciudades.

La investigación del biólogo chileno fue publicada por la Universidad de Cornell.

La investigación del chileno, publicada por la Universidad de Cornell, en Estados Unidos, planteó la aparición de cuatro zombies metidos en una ciudad con similar cantidad de habitantes que Santiago.

"En once días todos los habitantes de diez ciudades estaban infectados por los zombies. Tuvimos que incluir unos soldados o exterminadores para matarlos e incorporar tratamiento médico para el 30% de la población", explicó el investigador.

El biólogo detalla que detrás de este resultado hay modelos matemáticos que incluso incluyen autos con los cuales se transporta la gente mordida de una ciudad a otra. Tomás dará hoy una charla sobre el tema en la facultad de ingeniería de la U. de Chile.

> "Si un zombi muerde a alguien, esta persona nunca deja de estar infectada"

Title: "Flies, worms and butterflies entice scientists from Chile and Latin America"
Media: Newspaper "El Mercurio" (National)
Date: November 25, 2012

EL MERCURIO
JUEVES 15 DE NOVIEMBRE DE 2012

Simposio "Pequeños cerebros, grandes ideas":

Moscas, gusanos y mariposas conquistan a científicos de Chile y América Latina

Tres chilenos se propusieron ampliar el uso de invertebrados en laboratorios de la región. Aquí explican por qué.

PAULA LEIGHTON N.

Moscas. Diminutas moscas del vinagre (*Drosophila melanogaster*) aparecieron frente a los ojos de Yuly Fuentes, bioquímica de la U. de Concepción.

—"Voy a trabajar con moscas... ¡chuta!"—, pensó.

La científica iniciaba su doctorado en el Departamento de Neurobiología de la U. de Massachusetts.

Las moscas, los gusanos, las arañas, las abejas y otros tantos invertebrados que se emplean como modelos en investigación biológica tienden a ser poco valorados por investigadores acostumbrados a trabajar con mamíferos. "Me di cuenta de que yo también tenía esa resistencia", dice Fuentes.

En poco tiempo esa impresión se revirtió, al punto que junto a los científicos Jimena Sierralta, del Instituto Milenio de Neurociencias Biomédicas de la U. de Chile, y John Ewer, del Centro Interdisciplinario de Neurociencia (CINV) de la U. de Valparaíso —también investigadores de *Drosophila*— crearon "Small Brains, Big Ideas" (Pequeños cerebros, grandes ideas), un curso que busca expandir el uso de invertebrados como modelo de estudio en América Latina y que la semana pasada concluyó su segunda versión.

Durante 10 días una treintena de científicos jóvenes y estudiantes de posgrado en ciencias biológicas participaron en charlas y talleres prácticos dictados por destacados investigadores de universidades de EE.UU., Inglaterra, Chile y Argentina.

"La idea es convertir al país en un polo donde científicos latinoamericanos de alto nivel vayan a entrenarse en el uso de invertebrados y luego monten sus laboratorios usándolos como modelos", avizora Fuentes.

Aunque nos parezcan distantes, los invertebrados son el origen de hallazgos que han permitido grandes avances en la comprensión de enfermedades que afectan a los humanos, incluyendo el cáncer, trastornos neurodegenerativos y de la inmunidad. El genoma humano no se habría podido decodificar si antes no se hubiera secuenciado el genoma del *C. Elegans*, un gusano que no mide más de 2 mm, pero que tiene prácticamente la misma cantidad de genes que el humano.

Siete premios Nobel de Medicina y Fisiología se han otorgado a investigadores que hicieron sus hallazgos en invertebrados.

Técnicas de microscopía y disección de tejidos, como los de *Drosophila* (arriba), son algunas destrezas que aprenden los jóvenes científicos.

Jimena Sierralta, John Ewer y Yuly Fuentes son el motor detrás de "Small Brains, Big Ideas". Su objetivo es masificar el uso de invertebrados en América Latina.

Rápido y barato

"No sólo son modelos muy poderosos para hacer investigación en cualquier área de la biología. También tienen la gran ventaja de que es muy barato mantenerlos, lo que es un gran atractivo para países de América Latina, porque permiten hacer buena investigación a bajo costo", subraya John Ewer.

Lo sabe Diego Rayes, del Instituto de Investigaciones Bioquímicas Bahía Blanca (Argentina), que usa *C. Elegans*. "Para mantenerlos basta un incubador, unas placas de Petri y bacterias para alimentarlos. ¡Nada, comparado con el espacio y recursos que requiere un bioterio de ratones!".

Otra ventaja, destaca, es que "como tienen ciclos de vida muy cortos, puedes analizar en poco tiempo muchas generaciones". Eso permite hacer en meses experimentos que con mamíferos tomarían años. Así, las investigaciones avanzan rápido y también los descubrimientos.

El primer simposio, en 2010, ya dio frutos. "De ese curso ya hay una chica en Colombia y otra persona en Perú que empezaron sus laboratorios estudiando *C. elegans* y *Drosophila*", dice Jimena Sierralta, quien destaca que este año sumaron expertos que enseñaron el uso de mariposas y de abejas melíferas.

Para Fuentes, los primeros pasos son promisorios: "Estos animalitos han sido subvalorados, pero hoy estamos demostrando que sí en Latinoamérica quieres hacer buena ciencia con bajos costos, ellos son el complemento perfecto".

Title: "Nobel Prize winner will deliver a speech at the Parque Cultural de Valparaíso as part of the Simposium of Neuroscience hosted by the University of Valparaíso"
Media: TV Channel "24 Horas" (National)
Date: December 27, 2012