Annex 3
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Hydrogen peroxide and hypochlorous acid influx through the major *S.* Typhimurium porin OmpD is affected by substitution of key residues of the channel

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ABSTRACT

OmpD is the major *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) porin and mediates hydrogen peroxide (H_2O_2) influx. The results described herein extend this finding to hypochlorous acid (HOCl), another reactive oxygen species that is also part of the oxidative burst generated by the phagosome. *S.* Typhimurium cells lacking OmpD show decreased HOCl influx, and OmpD-reconstituted proteoliposomes show an increase in the uptake of the toxic compound. To understand this physiologically relevant process, we investigated the role of key OmpD residues in H_2O_2 and NaOCl transport. Using a theoretical approach, residue K16 was defined as a major contributor to the channel electrostatic properties, and E111 was shown to directly participate in the size-exclusion limit of the channel. Together, we provide theoretical, genetic, and biochemical evidence that OmpD mediates H_2O_2 and NaOCl uptake, and that key residues of the channel are implicated in this process.

Introduction

Porins are water-filled channels that span the outer membrane (OM)[2] of Gram-negative bacteria and that mediate the uptake of hydrophilic solutes [1,2], nutrients, and toxic compounds from the external environment into the periplasm, and vice versa [3,4]. The *Escherichia coli* OmpF porin has served as a model to characterize several properties of porins including ionic preference [1], the effect of ionic strength, antibiotic uptake, antibiotic resistance [5], and the contribution of protein residues to the diffusion potential, among others. The availability of the three-dimensional structure of OmpF and other porins at atomic resolution has allowed determining a relation between the sequence, channel structure, and functional properties of porins [5]. In example, OmpF monomers, which form trimers in the outer membrane, exhibit a β-barrel domain constricted by an extracellular loop (L-3) that folds into the channel vestibule [6,7]. In the inner barrel wall, the positively charged residues K16, R42, R82, and R132 are located on the opposite side of the negatively charged residues D113 and E117 at L-3 [8]. The distribution of these residues defines the geometrical and electrostatic properties of the constriction zone, termed "eyelet", and delimits the size, charge, and properties of the molecules to be channeled. Mutation of the eyelet residues in *E. coli* OmpF and OmpC [9,10] and other homologous porins affects antibiotic diffusion and susceptibility to the compounds [11–17].

OmpD is the most abundant OM porin of *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) [18] and mediates the uptake of hydrogen peroxide (H_2O_2) [4,11], a reactive oxygen species (ROS) produced in the oxidative burst by macrophages [19]. Interestingly, the expression of *nmpC* (encoding the OmpD porin) is down-regulated when *S.* Typhimurium is exposed to H_2O_2, hypochlorous acid (NaOCl) [11], and when residing inside macrophages [4,11,20]. This suggests that the regulation of OmpD is an adaptive

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[2] *Abbreviations* used: OM, outer membrane; ROS, reactive oxygen species; LB, Luria Bertani; DHR123, dihydrorhodamine123; POPC, 1-Palmitoyl-2-oleoylphosphatidylcholine.

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Conclusions

Together, and for the first time, we provide both genetic and biochemical evidence that OmpD mediates H_2O_2 and NaOCl uptake and that key residues of the channel are implicated in this process. Furthermore, our theoretical results suggest that diffusion of both toxic compounds through OmpD depends on the overlap of specific determinants in the pore radius and electrostatic potential. New theoretical and functional assays are currently underway to demonstrate the proposed role of the amino acid side-chain reactivity on the diffusion of the toxicants through the porin channel. It is expected that in conjunction with the experimental and theoretical observations reported herein, the new assays will provide further insights into the physiological aspects and the role of OmpD in the response to ROS by the bacterium.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.abb.2015.01.005.

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A Transient Receptor Potential Ion Channel in *Chlamydomonas* Shares Key Features with Sensory Transduction-Associated TRP Channels in Mammals

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Sensory modalities are essential for navigating through an ever-changing environment. From insects to mammals, transient receptor potential (TRP) channels are known mediators for cellular sensing. *Chlamydomonas reinhardtii* is a motile single-celled freshwater green alga that is guided by photosensory, mechanosensory, and chemosensory cues. In this type of alga, sensory input is first detected by membrane receptors located in the cell body and then transduced to the beating cilia by membrane depolarization. Although TRP channels seem to be absent in plants, *C. reinhardtii* possesses genomic sequences encoding TRP proteins. Here, we describe the cloning and characterization of a *C. reinhardtii* version of a TRP channel sharing key features present in mammalian TRP channels associated with sensory transduction. In silico sequence-structure analysis unveiled the modular design of TRP channels, and electrophysiological experiments conducted on Human Embryonic Kidney-293T cells expressing the Cr-TRP1 clone showed that many of the core functional features of metazoan TRP channels are present in Cr-TRP1, suggesting that basic TRP channel gating characteristics evolved early in the history of eukaryotes.

INTRODUCTION

The transient receptor potential (TRP) channel family of cation channels is diverse in terms of structure, ion selectivity, activation mechanisms, and tissue distribution (Clapham, 2009). In mammals, the TRP family comprises 28 loosely related ion channel proteins that are classified into six subfamilies (Latorre et al., 2009). Mammalian TRP channel proteins are polymodal cation channels that participate in sensory physiology at different levels. These include thermosensation, mechanosensation, nociception (sensation of noxious stimuli), touch, taste, olfaction, and vision (Clapham, 2009; Latorre et al., 2009). Under physiological conditions, TRP channel opening allows for the fast entrance of sodium and calcium ions into the cell (Owsianik et al., 2006). Although originally found in *Drosophila melanogaster* (Cosens and Manning, 1969; Montell and Rubin, 1989), at present, TRP channels are mostly studied in mammalian cells. TRPY, from yeast vacuole, is the only TRP channel from a unicellular organism that has been cloned and described to date (Martinac et al., 2008). After the release of the *Chlamydomonas reinhardtii* genome sequence, more than 60 putative ion channels, including TRP channels, have been reported as probable gene products (Merchant et al., 2007). Commonly found in soil and freshwater, *C. reinhardtii* is a single-celled chlorophyte alga about 10 μm in diameter with two beating flagella that enable swimming with a breast-stroke-type motion (Harris, 2001). Navigating at ~50 μm/s (Harris, 2001), these algae must rapidly integrate multiple external cues to adjust their orientation relative to the source of the signal. Notably, *C. reinhardtii* possess a fine-tuned navigation system based on calcium conductances (Hegemann, 2008). The presence of putative TRP channel coding sequences in the *C. reinhardtii* genome makes them good candidates for both the generation of the input signal and/or the regulation of sensory input propagation. Two recent independent studies report behavioral changes after knocking down TRP channel transcripts in *Chlamydomonas*. Apparently expressed at the flagella, silencing the expression of TRPP2 reduces the phosphorylation of cyclic GMP-dependent protein kinase and affects algae mating behavior (Huang et al., 2007). On the other hand, TRPV-related TRP11 (also expressed at the flagella) has been associated with the mechanosensory response (Fujiu et al., 2011). Unfortunately, in both cases, neither channel heterologous expression nor algal electrical activity was described, hampering the correct interpretation of the behavioral data. Here, we present a novel functional TRP channel from *C. reinhardtii* with a predicted molecular architecture that combines features from different TRP channel subfamilies. Of equal importance, this TRP channel displays several functional properties present in TRP channels from multicellular organisms, such as outward rectification, weak voltage dependence, phosphatidylinositol 4,5-bisphosphate (PIP2) sensitization, pharmacological block by *N*-(4-*tert*-butyl-phenyl)-4-(3-chloropyridin-2-yl) tetrahydropyrazine-1(2H)-carboxamide (BCTC), and activation by temperature (Latorre et al., 2009).

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TRPC6_MOUSE, NP_038866.2; TRPC7_HUMAN, NP_065122.1; TRPC7_MOUSE, NP_036165.1; TRPM1_HUMAN, NP_002411.3; TRPM1_MOUSE, NP_001034193.2; TRPM1_RAT, NP_001032823.1; TRPM2_HUMAN, NP_003298.1; TRPM2_MOUSE, NP_612174.2; TRPM3_HUMAN, NP_066003.3; TRPM4_HUMAN, NP_060106.2; TRPM4_MOUSE, NP_780339.2; TRPM4_RAT, NP_001129701.1; TRPM5_HUMAN, NP_055370.1; TRPM5_MOUSE, NP_064673.2; TRPM6_HUMAN, NP_060132.3; TRPM6_MOUSE, NP_700466.1; TRPM7_HUMAN, NP_060142.3; TRPM7_MOUSE, NP_067425.2; TRPM7_RAT, NP_446157.2; TRPM8_HUMAN, NP_076985.4; TRPM8_MOUSE, NP_599013.1; TRPM8_RAT, NP_599198.2; TRPML1_HUMAN, NP_065394.1; TRPML1_MOUSE, NP_444407.1; TRPML2_HUMAN, NP_694991.2; TRPML2_MOUSE, NP_080932.2; TRPML3_HUMAN, NP_060768.8; TRPML3_MOUSE, NP_598921.1; TRPN_DROME, NP_001245891.1; TRPP1_HUMAN, NP_000288.1; TRPP1_MOUSE, NP_032887.3; TRPP2_HUMAN, NP_057196.2; TRPP3_HUMAN, NP_055201.2; TRPP3_MOUSE, NP_058623.2; TRPV1_HUMAN, NP_061197.4; TRPV1_MOUSE, NP_001001445.1; TRPV1_RAT, NP_114188.1; TRPV2_HUMAN, NP_057197.2; TRPV2_MOUSE, NP_035836.2; TRPV2_RAT, NP_058903.2; TRPV3_HUMAN, NP_659505.1; TRPV3_MOUSE, NP_659567.2; TRPV4_HUMAN, NP_067638.3; TRPV4_MOUSE, NP_071300.2; TRPV4_RAT, NP_076460.1; TRPV5_HUMAN, NP_062815.2; TRPV5_MOUSE, NP_001007573.1; TRPV5_RAT, NP_446239.2; TRPV6_HUMAN, NP_061116.4; TRPV6_MOUSE, NP_071858.2; TRPV6_RAT, NP_446138.1; Cr-TRP1, JX173491.1, ABG54260.1, Phytozome Cre10.g452950.t1.3; Cr-TRP2, Phytozome g15856.t1; Cr-TRP11, AB596979.1, Phytozome Cre07.g341350.t1.2; Cr-TRP13, Phytozome Cre03.g175050.t1.3; Cr-TRP16 Phytozome Cre06.g278226.t1.1; Cr-TRPP2, Phytozome Cre17.g715300.t1.2; Cr-TRP5, Phytozome Cre09.g398400.t1.2; TRP15, Phytozome Cre10.g422750.t1.3; Cr-TRP6, Phytozome Cre07.g334300.t1.3; Cr-TRP21, Phytozome Cre10.g434600.t1.2; Cr-TRP22, Phytozome Cre02.g112200.t1.2; Cr-TRP23, Phytozome Cre09.g390578.t1.1; Vc-TRP1, Phytozome Vocar20010710m; Vc-TRP2, Phytozome Vocar20007683m; Vc-TRP3, XP_002947703.1, Phytozome Vocar20008340m; Vc-TRP4, XP_002954486.1, Phytozome Vocar20010765m; Cs-TRP1, XP_005642695.1, Phytozome 45570; Cs-TRP2, XP_005643399.1, Phytozome 68054; Mp_comp-TRP1, XP_003064374.1, Phytozome 49212; Mp_roc-TRP2, XP_002506421.1, Phytozome 64572; Dd-TRP1, XP_635110.1; Dd-TRP2, XP_644933.1; Dp-TRP1, XP_003289960.1; Dp-TRP2, XP_003289259.1; Dp-TRP3, XP_003283790.1; Li-TRP1, XP_001470439.1; Lm-TRP1, XP_001684103.1; Lmex-TRP1, XP_003876399.1; Lmex-TRP2, XP_003872242.1; Pt-TRP1, XP_001452370.1; Pt-TRP2, XP_001444531.1; Pt-TRP3, XP_001429751.1; TcCL-TRP1, XP_804976.1; TcCL-TRP2, XP_804854.1; Tc-TRP1, ADWP02012431.

Supplemental Data

Supplemental Figure 1. Multiple Sequence Alignment Depicting the Ankyrin Domains Present in Cr-TRP1 and Other TRPN, TRPC, TRPV, and TRPA Family Representatives.

Supplemental Figure 2. TRPM Homology Regions (MHR) in Cr-TRP1.

Supplemental Figure 3. Cr-TRP1 Amino Acid Sequence.

Supplemental Figure 4. The Effect of Cr-TRP1 Blockers.

Supplemental Figure 5. Alignment at the TM3-TM4 Region.

Supplemental Figure 6. Inward and Outward Whole-Cell Currents Are Affected after 10 min Incubation with 10 nM Wortmannin.

Supplemental Data Set 1. Fasta Format Multiple Sequence Alignment of the 68 Functionally Characterized TRPs Plus the 33 Sequences Identified in Algae and Unicellular Organisms Used to Construct Phylogeny Shown in Figure 2.

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AUTHOR CONTRIBUTIONS

S.B. and D.E.A. designed the research. L.A.-D., D.C., M.A.-A., F.B.-M., R.M., and S.B. performed research. I.V.-C. and C.K.C. contributed new analytic/computational tools. D.E.A., S.B., and C.K.C. analyzed data. S.B., D.E.A., and C.K.C. wrote the article.

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Tryptophan Scanning Reveals Dense Packing of Connexin Transmembrane Domains in Gap Junction Channels Composed of Connexin32*

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Background: Transmembrane domain interactions in gap junction channels are poorly understood.
Results: Tryptophan substitution experiments involving all four TM domains of Cx32 revealed tight packing.
Conclusion: After modeling, tight packing was found to occur in the midregion. Pore-facing residues were highly sensitive to substitution, whereas lipid-facing residues were variably tolerant.
Significance: Connexin-based channels are more densely packed than their innexin-based counterparts.

Tryptophan was substituted for residues in all four transmembrane domains of connexin32. Function was assayed using dual cell two-electrode voltage clamp after expression in *Xenopus* oocytes. Tryptophan substitution was poorly tolerated in all domains, with the greatest impact in TM1 and TM4. For instance, in TM1, 15 substitutions were made, six abolished coupling and five others significantly reduced function. Only TM2 and TM3 included a distinct helical face that lacked sensitivity to tryptophan substitution. Results were visualized on a comparative model of Cx32 hemichannel. In this model, a region midway through the membrane appears highly sensitive to tryptophan substitution and includes residues Arg-32, Ile-33, Met-34, and Val-35. In the modeled channel, pore-facing regions of TM1 and TM2 were highly sensitive to tryptophan substitution, whereas the lipid-facing regions of TM3 and TM4 were variably tolerant. Residues facing a putative intracellular water pocket (the IC pocket) were also highly sensitive to tryptophan substitution. Although future studies will be required to separate trafficking-defective mutants from those that alter channel function, a subset of interactions important for voltage gating was identified. Interactions important for voltage gating occurred mainly in the mid-region of the channel and focused on TM1. To determine whether results could be extrapolated to other connexins, TM1 of Cx43 was scanned revealing similar but not identical sensitivity to TM1 of Cx32.

Gap junctions mediate direct intercellular communication between animal cells. A typical gap junction includes hundreds or thousands of gap junction channels localized to a region of cell contact. The channels permit the passage of ions, nutrients, and cellular metabolites up to about 1 kDa in size (1). The connexin protein family constitutes gap junction channels in mammalian tissues, and 21 different connexin proteins have been identified in humans (2). Connexins are named according to their molecular mass; for example, connexin32 (Cx32) has an estimated molecular mass of 32 kDa. Connexins are also classified in groups based on sequence and evolutionary origins (3). For example, Cx32 is a β-connexin, and it shows higher sequence identity with β-connexins (*e.g.* Cx26, Cx30, and Cx31) than α-connexins (*e.g.* Cx40, Cx43, and Cx50).

Connexins are expressed in specific and overlapping patterns, and Cx32 is expressed in liver, Schwann cells, and oligodendrocytes (2). Mutations in the human Cx32 gene (GJB1) are associated with a peripheral neuropathy known as Charcot-Marie-Tooth disease type X (CMTX)[4] (4) with over 400 mutations identified in patients (5). In Schwann cells, Cx32 appears to form a critical pathway for the flow of cellular metabolites between layers of the myelin sheath (6), and mutations ranging from complete loss of function to fairly conservative missense mutations induce similar severity of disease (7). CMTX mutations have been characterized in a variety of experimental systems and have been shown to alter trafficking, voltage gating, and permeability (5). Because neuropathy is usually the only clinical symptom associated with CMTX mutations, it is suspected that other connexins compensate for the loss of Cx32 in tissue such as liver (5).

Connexins have four transmembrane domains (Fig. 1*A*, *TM1–TM4*) and cytoplasmic N and C termini (8) as indicated in Fig. 1. An intercellular gap junction channel is formed when

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[4] The abbreviations used are: CMTX, Charcot-Marie-Tooth disease type X; TM, transmembrane; MD, molecular dynamics; μS, microsiemens; h, human; r, rat.

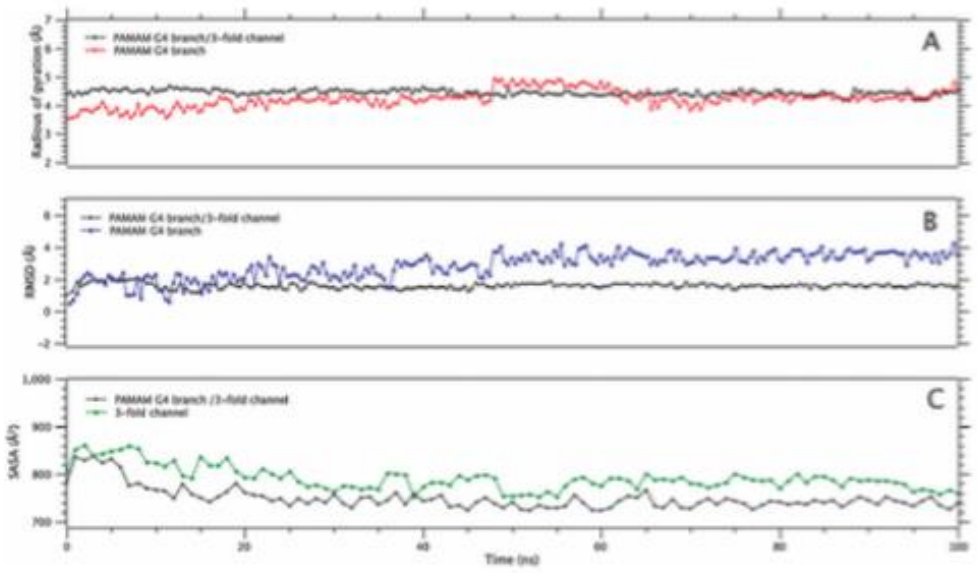

Fig. 10 (A) Radius of gyration, (B) root-mean-square deviation (RMSD) of a branch of PAMAM G4 interacting and not interacting with the 3-fold channel of L-Ftn and (C) surface accessible solvent area (SASA) of a free 3-fold channel and a 3-fold channel being blocked by PAMAM G4.

to the penetrating branch get rapidly stabilized and reach a constant behavior as the simulation time progressed.

The surface accessible solvent area (SASA) was also plotted for an interacting and non-interacting 3-fold channel of L-Ftn with dendrimer branches (Fig. 10C). Before $t = 2.8$ ns, while the dendrimer is still enough separated from the protein, SASA exhibit very similar values for both pores. However, in the case of the interacting pore, this value progressively decays until $t = 46$ ns, when reaches a mean value of 703 Å^2. As shown at Fig. 5B, at this time there was a decrease in the distance of the terminal amine of ATR89 to the COM of the pore, reaching the lowest average value of distance during the molecular dynamic simulation and therefore producing a diminution in the SASA values.

The profiles displayed in Fig. 10 demonstrate that electrostatic interactions together with hydrogen-bond contacts effectively hold up the dendrimer to the 3-fold channel of L-Ftn. The stability of PAMAM/protein complex during the time of simulation confirms that PAMAM G4 is an effective blocker of 3-fold channels in L-Ftn.

This study characterized at atomic level dendrimer–Ftn complexes. All results demonstrated that PAMAM G4 effectively interacts and blocks the 3-fold channels of L-Ftn, reducing the iron storage capacity of this protein. Therefore, PAMAM G4 dendrimers could affect negatively iron homeostasis.

4. Conclusions

In this work, the chemical interactions between PAMAM G4 and L-Ftn were studied. Experimental measurements demonstrated that PAMAM G4 effectively inhibits the iron storage properties of L-Ftn. The molecular dynamics analysis shows that PAMAM G4 interacts with the 3-fold channel of L-Ftn, suggesting that this interaction is responsible for the low iron storage properties of L-Ftn in the presence of PAMAM G4. Computational approaches confirmed the relevance of electrostatic interactions in the stabilization of the PAMAM G4/L-Ftn system. H-Bond type interactions preferentially between the terminal protonated amino groups of the dendrimer and acidic amino acids such as Glu and Asp contributed greatly to the permanent blockage of the channel from the first contact between both molecules, till the end of the simulation. Both superficial and inner-channel interactions were stable along the simulation, confirming that PAMAM G4 acts as an effective channel blocker of L-Ftn. This study suggests that amino terminated dendrimers can affect the iron metabolism. To efficiently address this issue, it is required to develop a new dendrimer which has the ability to bind nucleic acids and also avoid its non-specific interaction with plasma proteins, by modulating the charge of its terminal groups and introducing neutral molecules.

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PRIORITY REVIEW

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Biophysical analysis of thermosensitive TRP channels with a special focus on the cold receptor TRPM8

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Keywords: DRG, dorsal root ganglion; F, Faraday; T, temperature; TG, trigeminal ganglion; TRP, transient receptor potential; R, universal gas constant; G^0, Standard molar Gibbs free energy; H^0, Standard molar enthalpy; Q_{10}, temperature coefficient; S^0, Standard molar entropy.

Abbreviations: DRG, dorsal root ganglion; F, Faraday; G^0, Standard molar Gibbs free energy; H^0, Standard molar enthalpy Q_{10}, temperature coefficient; S^0, Standard molar entropy; TG, trigeminal ganglion; TRP, transient receptor potential; T, temperature; R, universal gas constant.

Mammals maintain homeostatic control of their body temperature. Therefore, these organisms are expected to have adaptations that confer the ability to detect and react to both self and ambient temperature. Temperature-activated ion channels have been discovered to be the primary molecular determinants of thermosensation. The most representative group of these determinants constitutes members of the transient receptor potential superfamily, TRP, which are activated by either low or high temperatures covering the whole range of physiologically relevant temperatures. This review makes a critical assessment of existing analytical methods of temperature-activated TRP channel mechanisms using the cold-activated TRPM8 channel as a paradigm.

Introduction

Living organisms are forced to exist in an environment in which temperature is constantly changing. Since virtually all known chemical reactions display temperature dependency, biological processes are unavoidably affected by this thermodynamic intensive parameter. Hence, it is advantageous for organisms to possess adaptive mechanisms or structures that confer them a special ability for sensing ambient and potentially dangerous temperatures. If we move up the evolutionary ladder, the more complex structures and mechanisms for sensing temperature are found in mammals. The neuronal pathways that participate in thermosensation of external stimuli in mammals have been well described.[1] Thermal stimuli excite sensory nerve endings of primary afferent neurons that project from trigeminal ganglia (TG) in the head and from the dorsal root ganglion (DRG) of the spinal cord to the rest of the body. Sensory nerve fibers convert thermal stimuli into action potentials that carry information to integrative centers in the spinal cord and brain. Identification and characterization of the molecular determinants of thermal sensitivity in neuronal endings has been an important undertaking in physiology for the past decades.

Propagated action potentials are initiated by a membrane depolarization at the nerve ending. Although several mechanisms have been proposed to explain the membrane depolarization evoked by cold in different tissues,[2–6] there is a large body of evidence suggesting that cold and warming can promote Ca^{2+} influx into DRG neurons, which would imply that Ca^{2+} channels are involved in thermosensation.[7,8] In fact, in the last decades several non-selective cation channels belonging to the transient receptor potential (TRP) superfamily have been identified as the molecular determinants in thermosensitive neurons.[9–11] In addition to thermosensitive TRP channels, there are other families of proteins involved in thermosensation, such as ANO1 and ANO2 (2 Ca^{2+}-activated Cl^- channels),[12] the endoplasmic reticulum Ca^{2+} sensor of store-operated Ca^{2+} entry STIM1[13] as well as several K^+ and Na^+ channels, whose thermosensitivity can modulate the excitability of neurons.[1] In addition changes in the intracellular concentration of the TRP channel modulator phosphatidylinositol 4,5-bisphosphate (PIP2) contribute to the temperature detection *in vivo*.[14] In this review, we

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The equation for the open probability at any temperature and any membrane potential is:

$$P_O = \frac{1}{1 + \frac{1}{ML}\left(\frac{1 + J + K + JKE}{1 + JDF + KCG + JDFKCGE}\right)^4 + \frac{1}{L}\left(\frac{1 + JD + KC + JDKCE}{1 + JDF + KCG + JDFKCGE}\right)^4} \quad (43)$$

This model produces a conductance vs voltage curves as are display if **Figure 4H-I** plotted using the parameters reported by Raddatz et al.[56] The activation of both sensors additively affects the standard molar free energy of the C_0-C_1 and C_1-O transitions and can reproduce the steady-state behavior of TRPM8 in a wide range of conditions. Even though the fit to the experimental steady state does not improve compared with mode of **Figure 4D**, this model is able to account for the complex channel kinetics.

Conclusions

The classical approaches used to describe the thermosensitivity of ion channels consist of 2 parameters: the thermal coefficient Q_{10} and the thermal threshold. We have shown that both parameters must be used with extreme caution because obtained values are strongly dependent on the precise experimental conditions.

Within the thermosensitive proteins in mammals, the TRP channels represent a very extended and important family. We have focused on a well-studied thermosensitive TRP member, namely the cold-activated TRPM8 channel, which has been extensively studied in terms of mathematical modeling of temperature and voltage coupling to the channel gating. Evidence has been presented in support of the inadequacy of the classical 2-state or linear sequential models, in which voltage and temperature sensors are strictly coupled to the pore domain, as they fail to describe the complex behavior of the gating of this channel. In contrast, allosteric models have more accurately described TRPM8 gating, possibly posing a general rule for all polymodal TRP channel receptors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Research Article

Hydrophobic interaction between contiguous residues in the S6 transmembrane segment acts as a stimuli integration node in the BK channel

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Large-conductance Ca^{2+}- and voltage-activated K^+ channel (BK) open probability is enhanced by depolarization, increasing Ca^{2+} concentration, or both. These stimuli activate modular voltage and Ca^{2+} sensors that are allosterically coupled to channel gating. Here, we report a point mutation of a phenylalanine (F380A) in the S6 transmembrane helix that, in the absence of internal Ca^{2+}, profoundly hinders channel opening while showing only minor effects on the voltage sensor active–resting equilibrium. Interpretation of these results using an allosteric model suggests that the F380A mutation greatly increases the free energy difference between open and closed states and uncouples Ca^{2+} binding from voltage sensor activation and voltage sensor activation from channel opening. However, the presence of a bulky and more hydrophobic amino acid in the F380 position (F380W) increases the intrinsic open–closed equilibrium, weakening the coupling between both sensors with the pore domain. Based on these functional experiments and molecular dynamics simulations, we propose that F380 interacts with another S6 hydrophobic residue (L377) in contiguous subunits. This pair forms a hydrophobic ring important in determining the open–closed equilibrium and, like an integration node, participates in the communication between sensors and betwe Scale document up Moreover, because of its effects on open probabilities, the F380A mutant can be used for detailed voltage sensor experiments in the presence of permeant cations.

INTRODUCTION

Large-conductance Ca^{2+}- and voltage-activated K^+ channels (BK, Slo1) increase their open probability in the presence of membrane depolarization and/or during an increase in the intracellular Ca^{2+} concentration (Marty, 1981; Pallotta et al., 1981; Latorre et al., 1982). The BK channel is one of the most broadly expressed channels in mammals and plays important roles in both excitable and nonexcitable cells. For example, in vascular smooth muscle cells it regulates the contractile tone, in neurons it colocalizes with voltage-dependent calcium channels and is involved in the control of neurosecretion, and in hair cells it is involved in frequency tuning (Lancaster and Nicoll, 1987; Brayden and Nelson, 1992; Gola and Crest, 1993; Edgerton and Reinhart, 2003; Gessner et al., 2005; Miranda-Rottmann et al., 2010). BK channels are tetramers in which the pore-forming α subunit is coded by a single gene (*Slo1*; *KCNMA1*) ubiquitously expressed across mammalian tissues (Toro et al., 1998). However, BK channels display a variety of phenotypes in different cells and tissues as a result of alternative splicing, metabolic regulation and modulation by β (Orio et al., 2002) and γ (Yan and Aldrich, 2012) subunits. This diversity of phenotypes is fundamental for their adequate function in each tissue.

The voltage-sensing domain (VSD) and pore-gating domain (PGD) of BK channels share similarity with voltage-dependent K^+ channels (Kv). However, an evolutionary relationship between them seems distant (Yu and Catterall, 2004). Evidence suggests that pore domains in both channels are different. For instance, the inner pore in BK channels is larger (Brelidze et al., 2003; Li and Aldrich, 2004; Zhou et al., 2011) than in Kv channels. Also, the ion permeation gate of BK channels resides at the selectivity filter (Piskorowski and Aldrich, 2006), whereas that of Kv channels is controlled by an intracellular gate (Liu et al., 1997). Moreover, Zhou et al. (2011) found that BK S6 transmembrane helix residues are rotated relative to Shaker, which is a Kv channel.

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Abbreviations used in this paper: HA, Horrigan and Aldrich; IbTx, iberiotoxin; MD, molecular dynamics.

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340 times slower in the F380A mutant. In contrast, large hydrophobic amino acids such as tyrosine or tryptophan should strengthen the interaction between the L377 and 380 residue, increasing the intrinsic equilibrium constant L_0. As predicted, this mutation decreased the free energy difference between open and closed state, whereas the result of the in silico calculation of the energy between W380 and L377 was −16.5 kcal/mol when the F380 and L377 interaction energy is taken as reference (Fig. 7 F). Although it appears to be a clear negative correlation between the strength of the interaction and the free energy difference between open and closes states, the effects on the allosteric constants are not so clear and suggest that the hydrophobic ring is involved in the coupling between sensors and pore opening in a complex way. The coupling between voltage sensor and opening is decrease in both mutants, whereas E and C are decreased only in one of the mutants (F380A and F380W, respectively).

In the lack of a crystal structure of BK channel, we can only speculate about the possible interaction between the sensors and the structure formed by residues L377 and F380. Previous modeling (Carvacho et al., 2008) and the present molecular model (Fig. 7) show that the smallest diameter of the internal vestibule is attained at the level of F380. We hypothesized that the hydrophobic ring can be the fulcrum of a lever where the forces produced by the sensors converge. For example, the distances between the four S6 helixes can increase or decrease in response to calcium binding, and these movements could produce a stretching or relaxing of the structure formed by the set of the four S6 helixes, whereas this structure is really pivoting at the level of the hydrophobic ring and the diameter to this lever does not change substantially. In the case of voltage sensor, if the S4–S5 linker plays a similar role in BK channels as it does in Kv channels where this linker interacts with the S6, it is possible that the hydrophobic ring plays the same role as it does when the channel is activated by Ca^{2+}.

The gating phenotype of the F380A in the high Ca^{2+} mutant resembles that of the Shaker ILT mutant (Ledwell and Aldrich, 1999), where the voltage activation curve is shifted to the right along the voltage axis, whereas most of the gating charge moves at voltages at which channels are closed. In the ILT Shaker mutant, channel opening is associated with the charge movement linked to the last rate-limiting transition in the ILT activation pathway. Thus, the F380A mutant isolated the BK opening voltage-dependent transition from earlier gating transitions and may provide a valuable experiment tool for teasing out the molecular basis of structural transitions in the activation pathway of BK channels.

Additionally, our results also support the idea that the inner vestibule in BK channels plays an important role in determining unitary conductance (Lippiat et al., 2000). Our fluctuation analysis experiments show that the F380A and F380W mutations decrease the channel conductance by ~60% and 50%, respectively.

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Mechanism of potassium ion uptake by the Na^+/K^+-ATPase

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The Na^+/K^+-ATPase restores sodium (Na^+) and potassium (K^+) electrochemical gradients dissipated by action potentials and ion-coupled transport processes. As ions are transported, they become transiently trapped between intracellular and extracellular gates. Once the external gate opens, three Na^+ ions are released, followed by the binding and occlusion of two K^+ ions. While the mechanisms of Na^+ release have been well characterized by the study of transient Na^+ currents, smaller and faster transient currents mediated by external K^+ have been more difficult to study. Here we show that external K^+ ions travelling to their binding sites sense only a small fraction of the electric field as they rapidly and simultaneously become occluded. Consistent with these results, molecular dynamics simulations of a pump model show a wide water-filled access channel connecting the binding site to the external solution. These results suggest a mechanism of K^+ gating different from that of Na^+ occlusion.

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Author contributions

M.H., F.B., R.L. and B.R. conceived the project. J.P.C., D.B., R.L., F.B. and M.H. designed and performed the experiments. J.P.C., R.L., F.B. and M.H. analysed the experimental data. H.R., A.D. and B.R. designed and performed the molecular modelling. All authors contributed to writing of the manuscript.

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Molecular mechanism underlying β1 regulation in voltage- and calcium-activated potassium (BK) channels

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Being activated by depolarizing voltages and increases in cytoplasmic Ca^{2+}, voltage- and calcium-activated potassium (BK) channels and their modulatory β-subunits are able to dampen or stop excitatory stimuli in a wide range of cellular types, including both neuronal and nonneuronal tissues. Minimal alterations in BK channel function may contribute to the pathophysiology of several diseases, including hypertension, asthma, cancer, epilepsy, and diabetes. Several gating processes, allosterically coupled to each other, control BK channel activity and are potential targets for regulation by auxiliary β-subunits that are expressed together with the α (BK)-subunit in almost every tissue type where they are found. By measuring gating currents in BK channels coexpressed with chimeras between β1 and β3 or β2 auxiliary subunits, we were able to identify that the cytoplasmic regions of β1 are responsible for the modulation of the voltage sensors. In addition, we narrowed down the structural determinants to the N terminus of β1, which contains two lysine residues (i.e., K3 and K4), which upon substitution virtually abolished the effects of β1 on charge movement. The mechanism by which K3 and K4 stabilize the voltage sensor is not electrostatic but specific, and the α (BK)-residues involved remain to be identified. This is the first report, to our knowledge, where the regulatory effects of the β1-subunit have been clearly assigned to a particular segment, with two pivotal amino acids being responsible for this modulation.

BK channels | gating currents | voltage sensor | BK beta-subunits

High-conductance voltage- and calcium-activated potassium (BK) channels are homotetrameric proteins of α-subunits encoded by the *slo1* gene (1). These channels are expressed in virtually all mammalian tissues, where they detect and integrate membrane voltage and calcium concentration changes dampening the responsiveness of cells when confronted with excitatory stimuli. They are abundant in the CNS and nonneuronal tissues, such as smooth muscle or hair cells. This wide distribution is associated with an outstandingly large functional diversity, in which BK channel activity appears optimally adapted to the particular physiological demands of each cell type (2). On the other hand, small alterations in BK channel function may contribute to the pathophysiology of hypertension, asthma, cancer, epilepsy, diabetes, and other conditions in humans (3–8). Alternative splicing, post-translational modifications, and regulation by auxiliary proteins have been proposed to contribute to this functional diversity (1, 2, 9–16).

The BK channel α-subunit is formed by a single polypeptide of about 1,200 amino acids that contains all of the key structural elements for ion permeation, gating, and modulation by ions and other proteins. Tetramers of α-subunits form functional BK channels. Each subunit has seven hydrophobic transmembrane segments (S0–S6), where the voltage-sensor domain (VSD) and pore domain (PD) reside (2). The N terminus faces the extracellular side of the membrane, whereas the C terminus is intracellular. The latter contains four hydrophobic α-helices (S7–S10) and the main Ca^{2+} binding sites (2). VSDs formed by segments S1–S4 harbor a series of charged residues across the membrane that contributes to voltage sensing (2). Upon membrane depolarization, each VSD undergoes a rearrangement (17) that prompts the opening of a highly K^+-selective pore formed by the four PDs that come together at the symmetry center of the tetramer.

Although BK channel expression is ubiquitous, in most physiological scenarios their functioning is provided by their coassembly with auxiliary proteins, such as β-subunits. This coassembly brings channel activity into the proper cell/tissue context (11, 13). Four different β-subunits have been cloned (β1–β4) (18–24), all of which have been observed to modify BK channel function. Albeit to a different extent, all β-subunits modify the Ca^{2+} sensitivity, voltage dependence, and gating properties of BK channels, hence modifying plasma membrane excitability balance. Regarding auxiliary β-subunits, β1- and β2-subunits increase apparent Ca^{2+} sensitivity and decelerate macroscopic current kinetics (14, 20, 21, 25–30); β2 and β3 induce fast inactivation as well as an instantaneous outward rectification (20, 21, 24, 31, 32); and β4 slows down activation and deactivation kinetics (12, 23) and modifies Ca^{2+} sensitivity (12, 33, 34).

It should be kept in mind that β-subunits are potential targets for different molecules that modulate channel function, such as alcohol (35), estrogens (15), hormones (36), and fatty acids (37, 38). Additionally, scorpion toxin affinity in BK channels would tend to increase when β1 is coexpressed with the α-subunit (22).

To identify the molecular elements that give β1 the ability to modulate the voltage sensor of BK channels, we constructed

Significance

β-Subunits (β1–β4) play a critical role in defining the properties of the voltage- and calcium-activated potassium (BK) channel, which in turn determines the physiological role that this channel can perform in different tissues. In particular, the β1-subunit causes an increase in the apparent BK Ca^{2+} sensitivity due to a stabilization of the voltage sensor in the active configuration. We investigated the molecular details of such voltage-sensor stabilization by mutagenesis and gating current measurements. Mixing regions of β1 and β3 made it possible to identify the N terminus, in particular the third and fourth lysine residues, as the structural element necessary to recover the full effect of β1 on the voltage sensor.

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

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$V_{0.5} = <V_{0.5}>$, allowing us to construct an average Q–V curve that preserved the shape of the individual Q–V curves.

The amount of free energy required to activate the sensors was calculated as $\Delta G = zFV_{0.5}$, with the z and $V_{0.5}$ values determined from Q–V curves as was previously done (61). All $\Delta\Delta G$ values were calculated as $\Delta\Delta G = Fl z_i V_{0.5\ i} - z_0 V_{0.5}$, where z_0 and $V_{0.5}$ correspond to the values for BK + β1, which are taken as reference, and z_i and $V_{0.5\ i}$ are the values for the other BK + β combinations (chimeras and mutants).

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Review

Voltage-gated proton (H_v1) channels, a singular voltage sensing domain

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ABSTRACT

The main role of voltage-gated proton channels (H_v1) is to extrude protons from the intracellular milieu when, mediated by different cellular processes, the H^+ concentration increases. H_v1 are exquisitely selective for protons and their structure is homologous to the voltage sensing domain (VSD) of other voltage-gated ion channels like sodium, potassium, and calcium channels. In clear contrast to the classical voltage-dependent channels, H_v1 lacks a pore domain and thus permeation necessarily occurs through the voltage sensing domain. H_v1 channels are activated by depolarizing voltages, and increases in internal proton concentration. It has been proposed that local conformational changes of the transmembrane segment S4, driven by depolarization, trigger the molecular rearrangements that open H_v1. However, it is still unclear how the electromechanical coupling is achieved between the VSD and the potential pore, allowing the proton flux from the intracellular to the extracellular side. Here we provide a revised view of voltage activation in H_v1 channels, offering a comparative scenario with other voltage sensing channels domains.

1. Introduction

1.1. Voltage-gated ion channels

In all living organisms, ion channels and transporters are responsible for maintaining the electrical homeostasis that is essential for a wide variety of physiological processes from neurotransmitter release to fecundation. Ion channels comprise a superfamily of proteins allowing the passage of ions through their pores at near diffusion limited rates (10^6–10^8 ions/s), and exhibiting exquisite specificity. Among them, voltage-gated ion channels, present in excitable and non-excitable cells, regulate ion conductance in response to changes in the voltage across the membrane. This family of voltage-gated ion channels shares a common structural and functional domain called the voltage sensor domain (VSD), which is able to detect fluctuations in the voltage across the membrane. Voltage sensing relies on a series of positively charged residues distributed along the fourth transmembrane segment of the domain. The electrical energy generated by the displacement of these residues during voltage activation is then transduced to the pore domain (PD), leading to channel opening. The PD contains a selectivity filter that determines which ions can permeate through the channel [1].

H_v1 is a unique voltage-gated ion channel due to its lacking of the classical PD. For this characteristic, it was dubbed VSOP (Voltage Sensor Only Protein) [2,3]. Since its cloning, the molecular determinants associated with proton permeation are thought to be harbored within the VSD (Fig. 1) [4].

In this review we discuss critically what makes the H_v1 channel a singular voltage sensing domain which harbors a conductive pathway.

1.2. Voltage-gated proton channels

The functional manifestation of proton channel conductance was first reported in neurons of the garden snail *Helix aspersa* [5]. Almost 10 years passed before these channels were recorded in mammalian and human cells [6–9]. Genes encoding a proton channel were first identified simultaneously in human, mouse and *Ciona intestinalis* [2,3], revealing a surprising similarity with the voltage-sensor domain (VSD) of most members of the voltage-gated ion channel family. H_v1 channels are very conspicuous members of the voltage-gated ion channel superfamily, due to their markedly different architecture (Fig. 1A). They are homodimers, containing a conduction pathway in each subunit. This allows it to function without the typical S5–S6 pore-forming seg-

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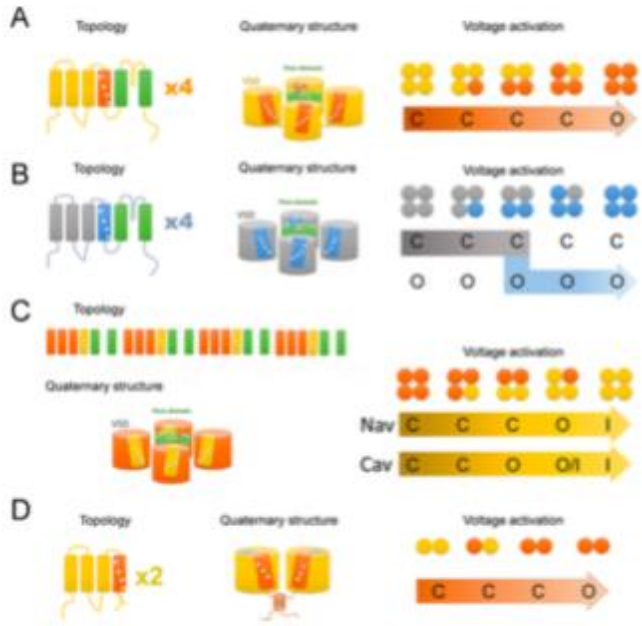

Fig. 4. Differences between VSDs among different voltage-gated ion channels and their roles in voltage activation. (A) The K_v channel presents a tetrameric structure, wherein each subunit has a VSD. Most K_v channels require that the entirety of the VSD remains in the active position in order to open the channel. This type of activation is related to a sequential kinetic mechanism as is shown in the right part of the figure. (B) Voltage-dependent K^+ channels with allosteric kinetic mechanism. In this case the architecture is the same as is shown in A, but these allosteric channels (such as KCNQ or BK channels) do not necessarily need to activate all of the VSDs to open the channel. These channels present VSD modulation by Ca^{2+} and/or auxiliary subunits that may change the number of VSDs needed to open the channel. (C) Na_v and Ca_v channels share the same architecture as Kv channels but in this case it is only one polypeptide containing four domains, each one with a VSD. In this case, two or three VSDs in the active position are required to open the channel for Na_v and Ca_v, respectively. Despite the high homology among domains, some of them are responsible for opening the channel while others are related to the inactivation process. (D) The extreme case of the voltage-gated H^+ channel, where the topology and the quaternary structure are completely different from any other voltage-gated ion channel. However, the kinetic mechanism remains similar to the voltage activation of K_v channels.

between both subunits and would take place before the coordinated opening of each monomer conduction pathway. The H_v1 channel VSD is unique among voltage gated ion channels in allowing proton permeation, and shows an exquisite selectivity and pH dependency.

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ARTÍCULO DE INVESTIGACIÓN / ORIGINAL RESEARCH PAPER

REGISTRO DE ACTIVIDAD ELÉCTRICA EN LA RETINA DE UNA RATA ALBINA EMPLEANDO UNA MATRIZ DE MICROELECTRODOS

Recording of Electrical Activity in the Retina of an Albino Rat Employing a Microelectrode Array

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RESUMEN

Las matrices de microelectrodos (MEA) son dispositivos que permiten la detección de potenciales de acción o espigas en poblaciones de células excitables, ofreciendo varias aplicaciones en el campo de las neurociencias y la biología. Este trabajo muestra un protocolo para el registro de espigas en una población de células ganglionares retinales empleando una matriz de microelectrodos. La retina de una rata albina fue extraída y preparada para ser estimulada *in vitro* con luz led blanca, con el fin de registrar sus espigas evocadas ante estos estímulos. Cada microelectrodo puede registrar espigas de más de una célula ganglionar, razón por la cual se determinó a qué célula pertenece cada espiga aplicando un procedimiento conocido como "clasificación de espigas". El trabajo permitió obtener el registro de un periodo de estimulación y otro de no estimulación, con el fin de representar los potenciales de acción evocados con luz y los espontáneos. Los registros fueron almacenados para visualizar las espigas de las células ganglionares y poder aplicar la herramienta de clasificación de espigas. De este modo, se almacenan los instantes de tiempo en los cuales cada célula ganglionar registrada generó potenciales de acción. Este trabajo conllevó al establecimiento de un protocolo de experimentación básico enfocado al uso de matrices MEA en el laboratorio de adquisición de potenciales extracelulares de la Universidad Antonio Nariño Sede Bogotá, no sólo para caracterizar los potenciales de acción de células ganglionares retinales, sino también para otro tipo de células que puedan ser estudiadas empleando matrices de microelectrodos.

Palabras clave: células ganglionares retinales, clasificación de espigas, potenciales evocados, potenciales extracelulares.

ABSTRACT

The microelectrode arrays (MEA) are devices that allow the detection of action potentials or spikes in populations of excitable cells, offering a wide spectrum of applications in topics of Neurosciences and Biology. This work describes a protocol for recording of spikes in a population of retinal ganglion cells employing a microelectrode array. The retina of an albino rat was dissected and prepared to be stimulated *in vitro* with white led light and to record their evoked spikes. Each microelectrode can record spikes from more than a ganglion cell, for which it was necessary to determine which cell fires each spike applying a procedure known as spike sorting. The work allowed to obtain the recording of a stimulation period and another of non-stimulation, representing evoked and spontaneous action potentials. The recordings were saved, in order to visualize the action potentials of the ganglion cells detected and to apply a computational method for the spike sorting. In this way, it was saved the time stamps in which each action potential was fired by its respective cell. This work established a basic experimentation protocol focused to the use of MEA devices

rapida de la respuesta, lo cual representa un problema en experimentos de larga duración.

Por último, cabe resaltar la posibilidad de utilizar los recursos del laboratorio para desarrollar experimentos en otros ámbitos, como los que se indican en la sección de introducción del presente documento.

CONCLUSIONES

Se ha acondicionado un laboratorio basado en tecnología MEA que permitirá realizar procedimientos donde se requiera estudiar las respuestas extracelulares en poblaciones de células excitables. La funcionalidad del laboratorio ha sido demostrada mediante un procedimiento básico en neurofisiología como guía para futuros experimentos. Aunque es necesario aún complementar algunas condiciones del laboratorio, se tienen las condiciones iniciales para considerar la posibilidad de buscar otras aplicaciones que permitan explotar las capacidades del sistema con tecnología MEA instalado en este laboratorio.

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RESEARCH ARTICLE

Zebrafish adult-derived hypothalamic neurospheres generate gonadotropin-releasing hormone (GnRH) neurons

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ABSTRACT

Gonadotropin-releasing hormone (GnRH) is a hypothalamic decapeptide essential for fertility in vertebrates. Human male patients lacking GnRH and treated with hormone therapy can remain fertile after cessation of treatment suggesting that new GnRH neurons can be generated during adult life. We used zebrafish to investigate the neurogenic potential of the adult hypothalamus. Previously we have characterized the development of GnRH cells in the zebrafish linking genetic pathways to the differentiation of neuromodulatory and endocrine GnRH cells in specific regions of the brain. Here, we developed a new method to obtain neural progenitors from the adult hypothalamus *in vitro*. Using this system, we show that neurospheres derived from the adult hypothalamus can be maintained in culture and subsequently differentiate glia and neurons. Importantly, the adult derived progenitors differentiate into neurons containing GnRH and the number of cells is increased through exposure to either testosterone or GnRH, hormones used in therapeutic treatment in humans. Finally, we show *in vivo* that a neurogenic niche in the hypothalamus contains GnRH positive neurons. Thus, we demonstrated for the first time that neurospheres can be derived from the hypothalamus of the adult zebrafish and that these neural progenitors are capable of producing GnRH containing neurons.

KEY WORDS: Kallmann syndrome, GnRH receptors, Testosterone

INTRODUCTION

The hypothalamus integrates information essential for the control of homeostasis including blood pressure, appetite, social behaviors, and reproduction. Multiple nuclei, including the neuroendocrine magnocellular and parvocellular nuclei, regulate complex processes through direct synaptic contacts as well as release into tissues and portal systems (Garcia-Segura, 2009; Machluf et al., 2011). The gonadotropin-releasing hormone (GnRH) cells of the parvocellular nucleus in mammals regulate puberty and fertility through pulsatile hormonal release via projections in the median eminence into the hypophyseal-portal-vasculature system, resulting in the release of the target hormones from the adenohypophysis. Although structurally different from mammals, the brain of teleost fish contain all the hypothalamic cell types (Machluf et al., 2011) including GnRH cells localized to the parvocellular nucleus (Gomes et al., 2013).

In humans, the congenital failure in the function of the GnRH neuroendocrine system results in reproductive disorders termed hypogonadotropic hypogonadism (HH), and these patients can also show a wide variety of non-reproductive phenotypes. Within the HH phenotype there exists a congenital GnRH deficiency with associated anosmia called Kallmann syndrome, which is now known to be a heterogeneous disease (Balasubramanian et al., 2010). Subsequent analysis of human patients have shown that HH results from mutations falling into two basic categories: those that affect peptides and/or ligands (*GnRH*, *kisspeptin*, *prokineticin2*) and those that affect the patterning and early development of the brain (*fgfr1*, *fgf8*, *anosmin1*, *CHD7*). In the case of mutations in the ligand receptor pairs of *kisspeptin* and *GnRH*, the defects are restricted to the loss of the pulsatile GnRH secretion necessary for the onset and maintenance of puberty. In contrast, mutations affecting brain patterning affect not only GnRH cell development but also cause a variety of associated phenotypes: cleft lip, cleft palate, high arched palate and other midline defects (reviewed in Silveira et al., 2010).

Males suffering from infertility associated with idiopathic HH (IHH) undergoing hormone therapy using testosterone, GnRH, or both, can show a reversal of IHH accompanied by a restoration of pulsatile GnRH release. Unexpectedly, these patients can continue to show pulsatile GnRH after removal of hormone treatment (Raivio et al., 2007) suggesting that the hypothalamus can recover GnRH function in adult humans.

The ability to generate new GnRH cells in the adult brain would require a quiescent progenitor population in the hypothalamus. It is widely accepted that adult neurogenesis occurs in the subventricular zone of the lateral ventricles and the subgranular zone of the hippocampus. More recent *in vivo* studies support the hypothalamus as a source of neurogenic and gliogenic precursors (Pérez-Martín et al., 2010; Sousa-Ferreira et al., 2014, 2011; Xu et al., 2005). The discovery of proliferating and neural stem cell (NSC) populations in the hypothalamus have been linked to the maintenance of body weight and energy expenditure (Bolborea and Dale, 2013). The observations that the vertebrate brain has the ability to generate new neurons have led us to examine genesis of GnRH cells in the adult zebrafish. To date no convincing studies have shown GnRH positive cells in the preoptic area (POA) nuclei of the adult zebrafish hypothalamus, though it has been suggested that these cells migrate to this region (Abraham et al., 2009) and that the hypothalamus in fact does not contain GnRH positive cell bodies. Here we show that GnRH cells can be detected by immunocytochemistry in the POA of adult zebrafish, that neurospheres can be isolated from the adult hypothalamus and differentiate into GnRH cells, that the number of GnRH cells increases in a dose dependent manner following hormone exposure (testosterone/GnRH), and that there is a potential neurogenic niche for GnRH cells in the hypothalamus of the adult

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disaggregated in proliferation medium to obtain individual cells (Fig. 3A2, disaggregation). Cells were cultured in the same medium and cytokines and heparin were added every 2 days. After 5 days the neurospheres (Fig. 3A3, neurospheres) were seeded or dissociated and seeded in NUNC LAB-TEK II CC2 slide 8 chamber coated with 0.2 mg/ml poly-L-lysine (Sigma-Aldrich) and 10 mg/ml laminin (Invitrogen) (Fig. 3A4 upper panel: four plates per culture) and maintained in differentiation medium containing by NeuroCult® NS-A Basal Medium (STEMCELL Technologies Inc, Vancouver, Canada; 05770) supplemented with NeuroCult® NS-A Differentiation Supplement (STEMCELL Technologies Inc; 05773), 100 U/ml penicillin, 100 mg/ml streptomycin, 2.5 mg/ml Fungizone (Invitrogen). The cells were grown up in the same medium for 6 h (Fig. 3A5 upper panel, undifferentiated) or 7 days (Fig. 3A6 upper panel, differentiated) and samples were collected to perform further analyses. The generation of neurospheres from adult zebrafish required rapid and careful dissection in agreement with (Pastrana et al., 2011): a known number of cells were plated, single cell plating was performed, the density was low avoiding the accidental formation of aggregates through mechanical disruption. Quantification was done at consistent times after plating and sphere size was recorded (Fig. 3B-D). All the cells were grown at 28.5°C, 5% CO_2 in an incubator chamber (Nuaire 5500E).

Hormone treatment

After one week in proliferation medium, cells were disaggregated in differentiation medium and seeded (see above) (Fig. 3A4, bottom panel). Neurospheres were supplemented every 2 days (3, 5 and 7 day) with vehicle, 10 µM testosterone (Sigma-Aldrich; T1500), or 10 nM GnRH (Sigma-Aldrich; L4897) (prepared in ethanol or PBS respectively, according to manufacturer guidelines). A dose-response curve was performed for GnRH using the following concentrations: 0.01, 0.10, 1.0, 10, 100, 1000 or 10,000 nM. After 5 days of treatment cells were used for immunocytochemistry assays (Fig. 3A5-6, bottom panel).

Immunocytochemistry of cultured cells

Cells were fixed in PFA 4% for 30 min at room temperature and immunocytochemical procedures were carried out as previously described (Cortés-Campos et al., 2011). The following primary antibodies were used: rabbit anti-sGnRH BB8 (1:1000; Kah et al., 1986), rabbit anti-GFAP (1:200; Dako, Campintene, CA, USA; Z0334), chicken anti-vimentin (1:200; Millipore; AB5733), mouse anti-HUC (1:100; Invitrogen; A-21271), mouse anti-neurofilament (1:200; Sigma-Aldrich; N2787), mouse anti-sox2 (1:1000; Millipore; AB5603), mouse anti-nestin (1:100; BD Biosciences, San Jose, CA, USA; 611659) and mouse anti-PCNA (1:100; Sigma-Aldrich; P8825). The samples were DNA counter-stained with DAPI (1:1000; Invitrogen) and the reactivity revealed using Alexa-labeled secondary antibodies (1:500; Invitrogen).

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA from neurosphere cultures was isolated using Trizol (Invitrogen) and treated with DNase I (Invitrogen). RT-PCR was performed according to the manufacturer's protocol using 1 µg RNA (Invitrogen) and oligodT(20) primer (Invitrogen). The PCR reaction was performed with 1 µl cDNA using the primers previously described to amplify GnRH receptors (Tello et al., 2008) and androgen receptor (Hossain et al., 2008). Each reaction mixture was incubated at 95°C for 5 min followed by 35 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C and a final extension of 7 min at 72°C. The expected products were: 582 bp for *GnRHR1*, 765 bp for *GnRHR2*, 421 bp for *GnRHR3*, 298 bp for *GnRHR4*, 237 bp for *AR*, 154 bp for *β-actin* and 192 bp for *gapdh*.

Microscopy

Bright field images were obtained using a Leica DMR microscope (Leica Microsystems CMS GmbH, Wetzlar, Germany) and a Leica DFC 480 camera (Leica Microsystems Ltd, Heerbrugg, Switzerland); images were processed with the Leica Application Suite 2.3.3 software (Leica Microsystems Ltd). Fluorescent images were taken using a Spinning Disc microscope Olympus BX-DSU (Olympus Corporation, Shinjuku-ku, Tokyo, Japan) and acquired with ORCA IR2 Hamamatsu camera (Hamamatsu Photonics, Higashi-ku, Hamamatsu City, Japan). Images acquired using the Olympus Cell^R software (Olympus Soft Imaging Solutions, Munchen, Germany) were processed using the deconvolution software AutoQuantX 2.2.2 (Media Cybernetics, Bethesda, MD, USA) and ImageJ® software (National Institute of Health, Bethesda, MD, USA).

Statistical analyses

The total number of cells (DAPI positive), neurons (neurofilament positive) and cells expressing GnRH (BB8 positive) were counted in control and hormone-treated experimental groups and compared by Mann-Whitney-Wilcoxon non-parametric test using GraphPad Prism Version 4.0 software (GraphPad Software, San Diego, CA, USA).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

K.E.W. and C.C.-C. developed the concepts or approach, C.C.-C., J.L., R.C., and K.E.W. performed experiments or data analysis, and prepared or edited the manuscript prior to submission.

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Supplementary material

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Plug-and-Play Genetic Access to *Drosophila* Cell Types using Exchangeable Exon Cassettes

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SUMMARY

Genetically encoded effectors are important tools for probing cellular function in living animals, but improved methods for directing their expression to specific cell types are required. Here, we introduce a simple, versatile method for achieving cell-type-specific expression of transgenes that leverages the untapped potential of "coding introns" (i.e., introns between coding exons). Our method couples the expression of a transgene to that of a native gene expressed in the cells of interest using intronically inserted "plug-and-play" cassettes (called "Trojan exons") that carry a splice acceptor site followed by the coding sequences of T2A peptide and an effector transgene. We demonstrate the efficacy of this approach in *Drosophila* using lines containing suitable MiMIC (Minos-mediated integration cassette) transposons and a palette of Trojan exons capable of expressing a range of commonly used transcription factors. We also introduce an exchangeable, MiMIC-like Trojan exon construct that can be targeted to coding introns using the Crispr/Cas system.

INTRODUCTION

Genetically based tools for perturbing cellular function are increasingly used to study the contributions of different cell types to development, physiology, and behavior. The utility of these tools depends critically on the cell-type specificity of their expression, and there is considerable demand for targeting methods with greater selectivity than is currently available. In general, selectivity of expression is achieved by using DNA regulatory elements of one or more genes normally expressed by a cell type of interest to drive the expression of a primary transgene, such as Gal4 or Cre; the primary transgene can then activate the expression of secondary transgenes that mediate functional perturbations (Branda and Dymecki, 2004; Venken et al., 2011b; Yizhar et al., 2011).

Co-opting a gene's full complement of regulatory elements to faithfully target all the cells that express it has been best achieved by inserting a transgene coding sequence into one of its translated exons (Demir and Dickson, 2005; Diao and White, 2012; Taniguchi et al., 2011). This approach, however, is labor intensive and lacks the convenient modularity of less-precise targeting systems, such as transposon-based systems, in which recombinase-mediated cassette exchange (RMCE) can be used to swap primary transgenes (Gohl et al., 2011).

A targeting method that combines the simplicity of RMCE with the precision of directly coupled transgene and native gene expression for use with fly lines that carry the engineered transposable element MiMIC (i.e., Minos-mediated integration cassette) was recently described in *Drosophila* (Venken et al., 2011a). When a MiMIC insertion is in the 5′ UTR of the gene of interest, RMCE can be used to replace the MiMIC cassette with an artificial exon encoding a primary transgene, preceded by a universal splice acceptor. The splice acceptor insures inclusion of the transgene coding sequence in the mature message of the native gene, and the transgene's start methionine, rather than the native gene's, directs its translation. Although a similar strategy can be used to introduce artificial exons into MiMIC insertions within coding introns, which are two-and-a-half times more numerous than 5′ UTR intron insertions (Venken et al., 2011a), co-translation of these artificial exons produces fusion proteins that will not predictably retain the function of a primary transgene's product. Therefore the MiMIC method's utility for gaining genetic access to cell types of interest is currently limited to genes with MiMIC insertions in 5′ UTR introns, and most *Drosophila* genes lack any MiMIC insertion.

To overcome these limitations, we have created an integrated toolkit of artificial exons that capitalize on the ability of the viral T2A peptide to promote the translation of a second protein product from a single transcript (Diao and White, 2012; Tang et al., 2009). Incorporation of the T2A sequence permits transcriptional effectors encoded by our

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synthesized from the coding sequence of the *VAChT* gene, which is co-expressed from the same genomic locus as *Cha* (Kitamoto et al., 1998). T7 RNA polymerase (New England Biolabs) was used to generate the riboprobe, and *VAChT* mRNA was visualized using a sheep anti-DIG-POD antibody (Roche). See the Supplemental Experimental Procedures for more details. Fluorescent imaging of larval and adult CNS preparations was performed with a Nikon C-1 confocal microscope using a 20× objective, and the images shown are composites of separately acquired volume-rendered images. A Leica TCS SP5 confocal microscope with a 63× oil-immersion objective was used for imaging late embryonic and early larval ventral nerve cords.

ACCESSION NUMBERS

The GenBank accession numbers for pC-(loxP2-attB2-SA(0)), pC-(loxP2-attB2-SA(1)), pC-(loxP2-attB2-SA(2)), pC-(lox2-attB2-SA-Hsp70)3, pT-GEM(0), pT-GEM(1), and pT-GEM(2) are KP686436, KP686437, KP686438, KP686439, KP686440, KP686441, and KP686442, respectively.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.01.059.

AUTHOR CONTRIBUTIONS

Fengqiu Diao and B.H.W. conceived the technique, planned experiments, and drafted the manuscript; Fengqiu Diao, Feici Diao, H.I., H.L., and E.M. made and characterized constructs and fly lines; Fengqiu Diao, H.I., M.L., C.J.P., and W.S. planned and conducted experiments; J.E. contributed unpublished reagents; and C.J.P., M.L., H.I., and J.E. made intellectual contributions and contributed to the manuscript.

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Research Article

Pore dimensions and the role of occupancy in unitary conductance of Shaker K channels

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K channels mediate the selective passage of K^+ across the plasma membrane by means of intimate interactions with ions at the pore selectivity filter located near the external face. Despite high conservation of the selectivity filter, the K^+ transport properties of different K channels vary widely, with the unitary conductance spanning a range of over two orders of magnitude. Mutation of Pro475, a residue located at the cytoplasmic entrance of the pore of the small-intermediate conductance K channel Shaker (Pro475Asp (P475D) or Pro475Gln (P475Q)), increases Shaker's reported ~20-pS conductance by approximately six- and approximately threefold, respectively, without any detectable effect on its selectivity. These findings suggest that the structural determinants underlying the diversity of K channel conductance are distinct from the selectivity filter, making P475D and P475Q excellent probes to identify key determinants of the K channel unitary conductance. By measuring diffusion-limited unitary outward currents after unilateral addition of 2 M sucrose to the internal solution to increase its viscosity, we estimated a pore internal radius of capture of ~0.82 Å for all three Shaker variants (wild type, P475D, and P475Q). This estimate is consistent with the internal entrance of the Kv1.2/2.1 structure if the effective radius of hydrated K^+ is set to ~4 Å. Unilateral exposure to sucrose allowed us to estimate the internal and external access resistances together with that of the inner pore. We determined that Shaker resistance resides mainly in the inner cavity, whereas only ~8% resides in the selectivity filter. To reduce the inner resistance, we introduced additional aspartate residues into the internal vestibule to favor ion occupancy. No aspartate addition raised the maximum unitary conductance, measured at saturating $[K^+]$, beyond that of P475D, suggesting an ~200-pS conductance ceiling for Shaker. This value is approximately one third of the maximum conductance of the large conductance K (BK) channel (the K channel of highest conductance), reducing the energy gap between their K^+ transport rates to ~1 kT. Thus, although Shaker's pore sustains ion translocation as the BK channel's does, higher energetic costs of ion stabilization or higher friction with the ion's rigid hydration cage in its narrower aqueous cavity may entail higher resistance.

INTRODUCTION

K channels elicit the passage of K^+ across the hydrophobic core of the membrane, with high transport rates and exquisite discrimination among cations having similar ionic radii and valence (Parsegian, 1969; Harris et al., 1998; Hille, 2001; Zhou et al., 2001; Long et al., 2005). This property appears to rise from a key feature common to members of the K channel family: the "signature sequence" of eight amino acidic residues (TMxTVGYG) forming the narrowest section of the conduction pathway. As proposed in the early 1970s and confirmed by crystallographic data in 2001, the oxygen atoms of carbonyl groups of what we know now as the "selectivity filter" coordinate the incoming K^+, acting as water surrogates, lowering the energetic cost to place a partially dehydrated ion within the membrane realms (Bezanilla and Armstrong, 1972; Heginbotham et al., 1994; Zhou et al., 2001; but see Yu et al., 2010). Considering the high degree of conservation of the signature sequence, it is surprising to find that single-channel conductance displays wide variability among K channels, which ranges between 2 and 250 pS in standard experimental conditions (around 100 mM of symmetric potassium). Such dispersion gives rise to the general division between small and large conductance K (BK) channels (Latorre and Miller, 1983; Blatz and Magleby, 1986; Carvacho et al., 2008; Moscoso et al., 2012).

Electrostatic calculations on MthK, a large conductance open bacterial K channel structure (Protein Data Bank [PDB] accession no. 1LNQ), showed that the transpore electric field is highly focused along the selectivity filter (Jiang et al., 2002). Thus, ion transit across this narrow region is expected to be the rate-limiting steps for K^+ translocation. In addition, the voltage-dependent Ag^+ accessibility to the CNG channel pore indicates that

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Abbreviation used in this paper: BK, large conductance K.

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the size of a water molecule. Thus, there is enough space for "ball-bearing sliding."

We have proposed a common experimentally derived parameter, the sectional electrical resistance, to account for the diversity in single-channel conductances despite the high conservation in the selectivity filter structure among the potassium channel superfamily. In this scheme, the diverse unitary conductances reside in the cavity's contribution to the total resistance. The low conductance of Shaker channels could be caused by: (a) the high resistance associated with the energetic cost of placing a cation in its narrower aqueous cavity immersed in a low dielectric, or (b) to a higher friction delivered by a rigid hydration cage bumping with cavity walls. Charge addition to the Shaker cavity made it more ion friendly and reduced its resistance. Table 2 shows that the P475D cavity resistance is ~1/70 that of Shaker-WT, reaching a value comparable to that of BK channels. Thus, within this perspective, the addition of negative charges to the cavity circumvents the excess energy required to introduce an ion in it. The negative charge additions to the conduction pathway increased significantly the pore occupancy but did not increase maximum unitary conductance beyond the ~200-pS ceiling (Figs. 3 and 4). This limit indicates the existence of additional barriers or steps for K^+ permeation to overcome in Shaker-type channels in order to match BK channels. These barriers could be, for example, friction, dehydration step(s) to enter/exit the selectivity filter, or simply threefold slower translocation across the selectivity filter, which in activation energy terms is equivalent to ~1 kT.

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RESEARCH ARTICLE

Alzheimer's Disease-Related Protein Expression in the Retina of *Octodon degus*

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Abstract

New studies show that the retina also undergoes pathological changes during the development of Alzheimer's disease (AD). While transgenic mouse models used in these previous studies have offered insight into this phenomenon, they do not model human sporadic AD, which is the most common form. Recently, the *Octodon degus* has been established as a sporadic model of AD. Degus display age-related cognitive impairment associated with Aβ aggregates and phosphorylated tau in the brain. Our aim for this study was to examine the expression of AD-related proteins in young, adult and old degus retina using enzyme-linked or fluorescence immunohistochemistry and to quantify the expression using slot blot and western blot assays. Aβ4G8 and Aβ6E10 detected Aβ peptides in some of the young animals but the expression was higher in the adults. Aβ peptides were observed in the inner and outer segment of the photoreceptors, the nerve fiber layer (NFL) and ganglion cell layer (GCL). Expression was higher in the central retinal region than in the retinal periphery. Using an anti-oligomer antibody we detected Aβ oligomer expression in the young, adult and old retina. Immunohistochemical labeling showed small discrete labeling of oligomers in the GCL that did not resemble plaques. Congo red staining did not result in green birefringence in any of the animals analyzed except for one old (84 months) animal. We also investigated expression of tau and phosphorylated tau. Expression was seen at all ages studied and in adults it was more consistently observed in the NFL-GCL. Hyperphosphorylated tau detected with AT8 antibody was significantly higher in the adult retina and it was localized to the GCL. We confirm for the first time that Aβ peptides and phosphorylated tau are expressed in the retina of degus. This is consistent with the proposal that AD biomarkers are present in the eye.

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ORIGINAL RESEARCH ARTICLE
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Acetylcholine induces GABA release onto rod bipolar cells through heteromeric nicotinic receptors expressed in A17 amacrine cells

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Acetylcholine (ACh) is a major retinal neurotransmitter that modulates visual processing through a large repertoire of cholinergic receptors expressed on different retinal cell types. ACh is released from starburst amacrine cells (SACs) under scotopic conditions, but its effects on cells of the rod pathway have not been investigated. Using whole-cell patch clamp recordings in slices of rat retina, we found that ACh application triggers GABA release onto rod bipolar (RB) cells. GABA was released from A17 amacrine cells and activated postsynaptic $GABA_A$ and $GABA_C$ receptors in RB cells. The sensitivity of ACh-induced currents to nicotinic ACh receptor (nAChR) antagonists (TMPH ~ mecamylamine > erysodine > DhβE > MLA) together with the differential potency of specific agonists to mimic ACh responses (cytisine >> RJR2403 ~ choline), suggest that A17 cells express heteromeric nAChRs containing the β_4 subunit. Activation of nAChRs induced GABA release after Ca^{2+} accumulation in A17 cell dendrites and varicosities mediated by L-type voltage-gated calcium channels (VGCCs) and intracellular Ca^{2+} stores. Inhibition of acetylcholinesterase depolarized A17 cells and increased spontaneous inhibitory postsynaptic currents in RB cells, indicating that endogenous ACh enhances GABAergic inhibition of RB cells. Moreover, injection of neostigmine or cytisine reduced the b-wave of the scotopic flash electroretinogram (ERG), suggesting that cholinergic modulation of GABA release controls RB cell activity *in vivo*. These results describe a novel regulatory mechanism of RB cell inhibition and complement our understanding of the neuromodulatory control of retinal signal processing.

Keywords: acetylcholine, A17 amacrine cell, GABA, GABA receptors, nicotinic receptor, retina, rod bipolar cell, rod pathway

INTRODUCTION

Nicotinic acetylcholine receptors are widely distributed throughout the central nervous system and play essential roles in learning, cognition and addiction (Dani and Bertrand, 2007). Central nAChRs are pentameric cationic channels assembled as homomers of α_7–α_9 subunits or by combinations of α_2–α_6 and β_2–β_4 subunits (Millar and Gotti, 2009). This heterogeneity endows nAChRs with different physiological and pharmacological properties and therefore diverse functional roles in neuronal networks (Mansvelder et al., 2006; Dani and Bertrand, 2007; Albuquerque et al., 2009).

In the mammalian retina, ACh is synthesized and released from starburst amacrine cells (SACs) that form narrowly defined cholinergic plexuses in the inner plexiform layer (IPL) (Voigt, 1986). Release of ACh may activate different classes of cholinergic receptors present in bipolar, amacrine and ganglion cells (AC and GCs) (Keyser et al., 2000; Dmitrieva et al., 2001, 2003, 2007; Moretti et al., 2004; Marritt et al., 2005; Strang et al., 2010). Indeed, functional nAChRs are expressed in GCs that stratify close to SAC dendrites (Kittila and Massey, 1997; Fried et al., 2005; Reed et al., 2005; Strang et al., 2007; Briggman et al., 2011) as well as in cells whose processes are located far from these cholinergic bands (Masland and Ames, 1976; Ariel and Daw, 1982; Schmidt et al., 1987; Strang et al., 2003, 2005). Activation of nAChRs also modulates the ON bipolar cell-dependent b-wave of the electroretinogram (ERG) (Jurklies et al., 1996; Varghese et al., 2011; Moyano et al., 2013), suggesting that ACh may influence signal transmission at stages preceding GC activation, but its specific targets and mechanisms of action remain largely unknown.

ACh release occurs under a broad range of illuminations (Masland and Livingstone, 1976; Massey and Neal, 1978, 1979b; O'Malley and Masland, 1993), including scotopic conditions when luminous signals detected by rods are mainly processed by the classic rod pathway. The first steps in this circuit involve the sequential activation of two dedicated cell types, RB and AII-ACs. The latter form chemical and electric synapses with cone bipolar cells to convey rod signals to GCs (Bloomfield and Dacheux,

Abbreviations: AC, amacrine cell; ACh, acetylcholine; CP-AMPAR, calcium permeable AMPA receptor; CICR, calcium induced calcium release; GC, ganglion cell; IPL, inner plexiform layer; nAChR, nicotinic acetylcholine receptor; RB, rod bipolar.

the observed effects of nicotine in humans (Jurklies et al., 1996; Varghese et al., 2011) and shows that activity of bipolar cells is actively modulated by nAChRs *in vivo*, although the exact nature and dynamic properties of this modulation require further assessment.

CONCLUSION

We are still far from understanding the complexities of the retinal cholinergic neurotransmitter system as the functional relevance of the widespread expression of cholinergic receptors has been elusive, with the notable exceptions of ACh effects on direction-selective GCs (Grzywacz et al., 1998; Fried et al., 2005; Reed et al., 2005) and during development (Feller, 2002). This study demonstrates that in the adult rat retina, ACh is a major player in the regulation of GABAergic inhibition of RB cells. We hypothesize that non-synaptic nAChR activation slowly depolarizes A17 cells, which facilitates GABA release via L-type VGCCs enhancing its gain control function of the RB-AII cell synapse (Dong and Hare, 2002a,b). This cholinergic control provides A17 cells with a modulatory system independent from the activity of RB cells, their main excitatory input and exclusive output, an advantageous situation that would greatly improve the adaptability and computational capabilities of A17 amacrine cells.

AUTHOR CONTRIBUTIONS

Claudio Elgueta designed, performed and analyzed the experiments, designed acquisition and analytical tools and wrote the paper. Oliver Schmachtenberg and Adrian G. Palacios designed the experiments and wrote the paper. Alex H. Vielma performed experiments and analyzed the data.

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SUPPLEMENTARY MATERIAL

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Chapter 1
Biophysical and Molecular Features of Thermosensitive TRP Channels Involved in Sensory Transduction

Gonzalo Ferreira, Natalia Raddatz, Yenisleidy Lorenzo, Carlos González and Ramón Latorre

Abstract Temperature is one of the physical variables that cells and biological organisms constantly monitor to achieve homeostasis and maintain chemical reactions at a suitable speed for the living environment to which they are adapted. In order to monitor and maintain temperature on a constant basis, thermosensitive molecules were selected during evolution. One of the most remarkable sets of molecules acting as sensors is constituted by thermosensitive transient receptor potential channels (thermoTRP channels). TRP channels are a superfamily of non-selective tetrameric cation channels closely related to the classic superfamily of voltage-gated channels, having a set of distinctive sequence elements in common, while acting as polymodal receptors. This latter ability is what makes them suitable for integrating many kinds of signals in different cells, ranging from chemical to physical stimulation (*i.e.*: temperature-, mechano- and chemo-sensitivity). These channels act as allosteric proteins modifying sensitivity to one stimulus in the presence of another, and thus allowing the integration of many different signaling processes that are critical for sensing the extracellular and intracellular environment and for maintaining homeostasis. This ability has made them vital for life support. Several subfamilies of TRP channels have been described. From these subfamilies, some types of channels have been distinguished as being temperature-sensitive, such as TRPV1–4, TRPM 2–5/8, TRPA1 and TRPC5. In this chapter, thermosensitivity will be defined. Then, we will describe the thermosensitive molecules identified so far, focusing our analysis on ion channels, particularly on thermosensitive TRP channels involved in sensory transduction. Their gating and permeation properties and

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1.4 Concluding Remarks

In this chapter we have reviewed the structural, biophysical and evolutionary details of thermoTRP channels, relevant for sensory transduction. Regarding their biophysics and physiology, their allosteric polymodal nature confers them, among other proteins, an enormous advantage for sensing and transducing signals. The wide repertoire of chemical and physical agents that are able to gate these channels provides sophisticated fine-tuning for cell and organism living conditions. Their non-selective cation permeation properties and their mild voltage gating, which are strongly modulated by chemical and physical agents that can interact allosterically, make them unique machineries for cell homeostasis. Regarding their structure, their organization as tetramers with interrelated sensing modules has been revealed through structure-function studies. More recently, 3D models have shown that they have large cytoplasmic interacting regions, making them suitable for monitoring intracellular changes interacting with many ligands and proteins. All these features are reflected in evolution as they appear in molecules within more complex organisms. In line with the structural and biophysical details known to date, analyses of their properties in different organisms have revealed that they are one of the key factors involved in adapting to distinct thermal conditions. As such, these unique properties are conferred to cells and organisms expressing these molecules, indicating a clear advantage that makes them suitable for building complex interactions and networks. Network analysis of these channels in physiology and diseases has just begun (Chun et al. 2013) and have contributed in shedding light on our understanding as to how these channels play important roles in life within the context of systems biology.

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ORIGINAL ARTICLE

Keratitis-Ichthyosis-Deafness Syndrome-Associated Cx26 Mutants Produce Nonfunctional Gap Junctions but Hyperactive Hemichannels When Co-Expressed With Wild Type Cx43

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Mutations in Cx26 gene are found in most cases of human genetic deafness. Some mutations produce syndromic deafness associated with skin disorders, like the Keratitis-Ichthyosis-Deafness syndrome (KID). Because in the human skin connexin 26 (Cx26) is co-expressed with other connexins, like Cx43 and Cx30, and as the KID syndrome is inherited as autosomal dominant condition, it is possible that KID mutations change the way Cx26 interacts with other co-expressed connexins. Indeed, some Cx26 syndromic mutations showed gap junction dominant negative effect when co-expressed with wild-type connexins, including Cx26 and Cx43. The nature of these interactions and the consequences on hemichannels and gap junction channel (GJC) functions remain unknown. In this study, we demonstrate that syndromic mutations, at the N terminus segment of Cx26, change connexin oligomerization compatibility, allowing aberrant interactions with Cx43. Strikingly, heteromeric oligomer formed by Cx43/Cx26 (syndromic mutants) shows exacerbated hemichannel activity but nonfunctional GJCs; this also occurs for those Cx26 KID mutants that do not show functional homomeric hemichannels. Heterologous expression of these hyperactive heteromeric hemichannels increases cell membrane permeability, favoring ATP release and Ca^{2+} overload. The functional paradox produced by oligomerization of Cx43 and Cx26 KID mutants could underlie the severe syndromic phenotype in human skin.

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INTRODUCTION

Gap junction channels (GJCs) allow metabolic and electrical coupling between adjacent cells and are formed by the oligomerization of connexin (Cx) protein subunits. Cxs oligomerize to form hexamers called hemichannels (HCs), which reach the appositional plasma membrane and dock with other complementary HCs provided by an adjacent cell to form gap junction plaques (Segretain and Falk, 2004). In non-appositional plasma membrane, HCs connect the cytoplasm with the external milieu, allowing paracrine and autocrine signaling mediated by ATP and Ca^{2+}, among others (Stout *et al.*, 2004; Sáez *et al.*, 2005). HCs can be homomeric, if all subunits are the same Cxs or heteromeric if they are formed by two or more different Cxs; however, some Cxs are incompatible to form heteromeric channels, like Cx26 and Cx43 (Gemel *et al.*, 2004; Martínez *et al.*, 2011; Jara *et al.*, 2012). Indeed, heteromeric channels have different functional properties compared with homomeric channels; therefore, regulation of Cx–Cx interaction could be important in controlling intercellular communication (Martínez *et al.*, 2002; Martinez *et al.*, 2011; Jara *et al.*, 2012).

Mutations in the human Cx26 gene account for about 50% of genetic deafness. To date, more than 100 Cx26 mutations have been identified, but only 16 of them, mainly located at the N terminus and the transition between the first transmembrane segment and the extracellular loop segment, cause the syndromic phenotype (Martínez *et al.*, 2009). Although syndromic Cx26 mutations are sparse, they have an autosomal dominant inheritance pattern that affects proliferation and

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Abbreviations: CX, connexin; DCF-HBSS, divalent cation-free Hanks' balanced salt solution; GFP, green fluorescent protein; GJC, gap junction channels; HC, hemichannel; hCx26, human Cx26; KID, Keratitis-Ichthyosis-Deafness syndrome

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and cells were incubated for 10 minutes at 37 °C. Subsequently, 5 µl samples of extracellular milieu were mixed with 45 µl ATP-mix solution in a 96-well plaque. Accumulated ATP was determined using an Appliskan Luminometer (Thermo Electro; Thermo Fisher Scientific) based on a calibration curve range from 1 nM to 1 µM ATP. Data were collected with the SkanIT software (Thermo Electro).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Author Contributions

IEG and ADM conceived the present study; IEG and ADM designed the research; IEG, JM, OJ, RC, PO, and AP-M, JR, performed research; TP-A contributed to paper discussion and tool analysis; IEG, JCS, JEC, and ADM analyzed data; IEG, JCS, CG, JEC, and ADM wrote the paper.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Interaction between the Linker, Pre-S1, and TRP Domains Determines Folding, Assembly, and Trafficking of TRPV Channels

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SUMMARY

Functional transient receptor potential (TRP) channels result from the assembly of four subunits. Here, we show an interaction between the pre-S1, TRP, and the ankyrin repeat domain (ARD)-S1 linker domains of TRPV1 and TRPV4 that is essential for proper channel assembly. Neutralization of TRPV4 pre-S1 K462 resulted in protein retention in the ER, defective glycosylation and trafficking, and unresponsiveness to TRPV4-activating stimuli. Similar results were obtained with the equivalent mutation in TRPV1 pre-S1. Molecular dynamics simulations revealed that TRPV4-K462 generated an alternating hydrogen network with E745 (TRP box) and D425 (pre-S1 linker), and that K462Q mutation affected subunit folding. Consistently, single TRPV4-E745A or TRPV4-D425A mutations moderately affected TRPV4 biogenesis while double TRPV4-D425A/E745A mutation resumed the TRPV4-K462Q phenotype. Thus, the interaction between pre-S1, TRP, and linker domains is mandatory to generate a structural conformation that allows the contacts between adjacent subunits to promote correct assembly and trafficking to the plasma membrane.

INTRODUCTION

Transient receptor potential (TRP) cationic channels are important cellular sensors of the environment due to their role in the transduction of physical and chemical stimuli (Voets et al., 2005). Each subunit of the TRP family contains six transmembrane segments (S1–S6) with a pore region between S5 and S6, and intracellular N- and C-terminal tails (Montell, 2005). The classical (TRPC) and vanilloid (TRPV) subfamilies as well as the TRPA1 channel present several N-terminal ankyrin repeat domains (ARDs) (Montell, 2005). A C-terminal TRP box domain immediately after S6 is reported for members of the TRPV, TRPC, and melastatin (TRPM) subfamilies. Similar to voltage-gated K^+ channels, TRP channels assemble as tetramers with 4-fold symmetry and a central ion permeation pore (Huynh et al., 2014; Liao et al., 2013; Maruyama et al., 2007; Mio et al., 2007; Moiseenkova-Bell et al., 2008; Shigematsu et al., 2010). Heteromeric TRP channels formed within the same or different subfamilies are possible (Cheng et al., 2010; Hoenderop et al., 2003; Schaefer, 2005), adding functional diversity to a family of channels already characterized by their polymodal nature.

The proper folding and assembly of different ion-channel subunits is likely mediated by multiple domains generating intra- and intersubunit interactions (Green and Millar, 1995). In this context, TRP tetramerization involves transmembrane segments (Hellwig et al., 2005), ARD (Amiges et al., 2006; Erler et al., 2004; Hellwig et al., 2005), and different regions of the N tails (Chang et al., 2004; Myeong et al., 2014; Pertusa et al., 2014) and C tails (Becker et al., 2008; Erler et al., 2006; Hellwig et al., 2005; Lei et al., 2013; Zhang et al., 2011), including the TRP box (García-Sanz et al., 2004).

Within the vanilloid subfamily of TRP channels, the heat-activated TRPV1 and TRPV4 channels present a high degree of similarity in their sequence and biophysical properties (Owsianik et al., 2006). The TRPV4 cationic channel is widely distributed and participates in the transduction of osmotic (Amiges et al., 2004; Liedtke et al., 2000; Tian et al., 2009), mechanical (Andrade et al., 2005; Liedtke et al., 2003; Suzuki et al., 2003), heat (García-Elías et al., 2013; Güler et al., 2002; Watanabe et al., 2002a), and UVB stimuli (Moore et al., 2013). TRPV1 is expressed primarily on nociceptive neurons and can be activated by capsaicin, noxious heat, and protons (Caterina et al., 1997).

In the present study we have addressed structural determinants governing TRPV subunit folding and assembly, focusing mainly on TRPV4 but also on TRPV1. We have shown how the electrostatic interactions of a triad of residues within the ARD-S1 linker, pre-S1, and TRP box govern the overall channel architecture.

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chemicals were obtained from Sigma-Aldrich except HC-067047 (Tocris Biosciences) and Fura-2 (Invitrogen).

Electrophysiological and Ratiometric Ca^{2+} Recordings

Patch-clamp whole-cell currents were recorded at room temperature (~24°C, unless otherwise indicated) as previously described (Fernandes et al., 2008). Cells were perfused at 0.8 ml/min. Cytosolic Ca^{2+} signals, relative to the ratio (340/380) measured prior to cell stimulation, were obtained from cells loaded with 4.5 μM fura-2 AM as previously described (Fernandes et al., 2008).

Western Blot, Co-Immunoprecipitation, and Deglycosylation with PNGaseF and EndoH

To compare the expression levels of different TRPV4 proteins, 40 μg of total protein was obtained from HeLa cells harvested 24–48 hr after transfection with TRPV4-WT and different mutants. The protein was separated on a pre-cast polyacrylamide gel NuPAGE (4%–12%, Invitrogen). Co-immunoprecipitation experiments were run as previously described (Fernandes et al., 2008). Analysis of TRPV4 glycosylations was carried out as previously described (Arniges et al., 2006). Total protein was extracted from HeLa cells 24 hr after transfection. Cells were lysed in a buffer containing 150 mM NaCl, 5 mM EDTA, 1% NP-40, 1 mM sodium orthovanadate, 1 mM PMSF, 1 mM DTT; 0.05% aprotinin (1 hr at 4°C). The lysis buffer was supplemented with a Complete Mini protease inhibitor cocktail (1:7 v/v; Roche). The nuclear fraction was pelleted by centrifugation at 12,000 rpm for 15 min. Following the manufacturer's instructions, 20 μg of total protein were digested with 2 μl of EndoH or PNGaseF (New England Biolabs) for 1.5 hr at 37°C. Proteins were subjected to SDS-PAGE (8%) and subsequently electroblotted onto nitrocellulose membranes. Incubation with anti-human TRPV4 antibody (1:500) for 1 hr at room temperature was followed by incubation for 1 hr at room temperature with the horseradish peroxidase-conjugated donkey anti-rabbit immunoglobulin G (Amersham Biosciences) at a dilution of 1:2,000. Detection was done with a SuperSignal West chemiluminescent substrate (Pierce).

Confocal Microscopy

Cells transiently transfected with hTRPV1 and hTRPV4 WT and/or mutants (pcDNA3.1) were probed with a polyclonal affinity-purified anti-human TRPV4 (1:1,000) (Arniges et al., 2004, 2006; Fernandes et al., 2008) and/or mouse anti-calreticulin (1:500, BD Biosciences). Anti-TRPV1 antibody was a free gift from Alomone. In cross-complementation assays, cells were co-transfected with pcDNA3.1 TRPV4-WT-Flag and pcDNA3.1-YFP constructs and probed with a rabbit anti-Flag (1:500, Sigma-Aldrich). Alexa Fluor 488 goat anti-rabbit or Alexa 555 goat anti-mouse (Molecular Probes, 1:2,000) were used as secondary antibodies. For membrane detection, cells were stained for 20 min, then fixed in ice with 100 μg/ml concanavalin A tetramethylrhodamine (Invitrogen). Digital images were taken and analyzed using a Leica TCS SP2 and the NIH ImageJ software (http://rsb.info.nih.gov/ij/).

FRET Measurements

Acceptor photobleaching FRET measurements of HeLa cells transfected with TRPV4-WT and mutants with a YFP or CFP tag were carried out in a Leica TCS SP2 confocal microscope (Leica) attached to an inverted microscope. FRET efficiencies were expressed as the increase of the FRET donor CFP after bleaching the FRET acceptor YFP (Arniges et al., 2006; Garcia-Elias et al., 2013).

TRPV4 Molecular Structure and Molecular Dynamics Simulations

The sequence of hTRPV4 from V148 to A755 (UniProt: Q9HBA0) was aligned along the cryo-electron microscopic structure of the rTRPV1 (PDB: 3J5P, closed conformation) of 3.2 Å resolution (Liao et al., 2013). Given a high similarity in the transmembranal zone, 69% of sequence identity was achieved from the whole sequence alignment. Afterward, five molecular models were obtained by using Modeller v9.10 (Eswar et al., 2006) and the model with the lowest DOPE energy was selected for the next stage. Four molecular systems were set up: rTRPV1 WT, rTRPV1 K425Q, hTRPV4 WT, and hTRPV4 K462Q. The protein structures were embedded into a phosphatidyloleoyl phosphatidylcholine (POPC) bilayer. To mimic the experimental conditions, the systems were solvated with the TIP3P water model (Boiteux and Bernèche, 2011), then neutralized and ionized with 0.11 M KCl. The CHARMM36 force fields for lipids (Klauda et al., 2010) and proteins (Huang and MacKerell, 2013) were applied. The final dimensions of the systems were ~170 × ~170 × ~170 $Å^3$. The initial systems were subjected to a standard energy minimization and then equilibrated for 3.6 ns. Position restraints of 1 (kcal/mol $Å^2$) were assigned to the alpha carbons, which were diminished by 0.2 (kcal/mol $Å^2$) for 0.5 ns to reach 0.0 (kcal/mol $Å^2$). The molecular dynamics simulations were performed with periodic boundary conditions. The systems were run using an isobaric-isothermal ensemble, and the temperature and pressure applied were 300 K and 1 atm, respectively. The temperature was controlled using Langevin dynamics with a damping coefficient of 1 ps^{-1}. The computation of long-range electrostatic interactions was calculated with the particle-mesh Ewald method (Darden et al., 1993). The motion equations were integrated with a time step of 2, 2, and 4 fs for bonded, short-range, and long-range non-bonded interactions, respectively. An 8-Å spherical cut-off was used for short-range non-bonded interactions, including a switching function from 7 Å for the van der Waals term and shifted electrostatics (Wells et al., 2012). Once the systems were totally unrestricted, 100 ns of production data were collected for each system. Measurements of the bending angle in the hinge formed by preS1-S1 of the last state of TRPV4-WT and TRPV4-K462Q of molecular dynamics simulations were calculated using the VMDBendix package (Dahl et al., 2012).

Statistical Analysis

Data are expressed as mean ± SEM (or mean ± SD in Figure S7) of n experiments. Statistical analysis was assessed with Student unpaired test or one-way ANOVA and Bonferroni post hoc using Sigma-Plot software.

SUPPLEMENTAL INFORMATION

Supplemental Information includes eight figures and two movies and can be found with this article online at http://dx.doi.org/10.1016/j.str.2015.05.018.

AUTHOR CONTRIBUTIONS

A.G.-E., A.B.-E., F.G.-N., and M.A.V. designed research; A.G.-E., A.B.-E., F.R.-M., S.M., C.P.-P., R.V.S., and R.V. performed research; A.G.-E., A.B.-E., R.V.S., F.G.-N., and M.A.V. analyzed data; and M.A.V. wrote the paper. All authors collaborated in editing the manuscript. The authors declare no conflict of interest.

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Time-frequency methods for studying non-stationary auditory responses

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Abstract—**The study of temporal processing of acoustic signals by the auditory system becomes increasingly important for the development of new strategies for diagnosis and treatment of people who are deaf or hearing impaired. Objectives: To evaluate three time-frequency methods (Short Time Fourier Transform, Morlet Wavelet Transform, and a newly proposed Chirp Analyzer) for the reliable estimation of non-stationary auditory electrophysiological responses, which could be used in the study/diagnosis of hearing problems in clinical practice. Methods: Using simulated and real data, we compare the robustness and reliability of the three methods for different levels of noise and response forms, as well as with different physiological response delay. Results: In general, the three methods provide a fairly reliable estimate of the physiological response when there are low levels of noise and response latency is small. The Chirp Analyzer is faster and more robust to noise, while continuous Morlet Wavelet Transform is very sensitive to noise but more reliable when the responses appears with a considerable delay. Conclusions: Results suggest that the Chirp Analyzer is a promising tool for estimating non-stationary auditory electrophysiological responses, although future validation is needed.**

Keywords— **Auditory temporal processing, steady state, time-frequency analysis, envelope following response**

I. Introducción

The processing of amplitude modulations of acoustic signals by the auditory system is crucial for the correct functioning of the mechanisms of categorization and discrimination of sounds that comprise the oral communication [1]. The study of this type of coding allows the detection and characterization of difficulties in the auditory temporal processing that potentially influence the development of speech. Traditionally, these analysis have been carried out using psychophysical tests, which include the measurement of the temporal modulation transfer function (TMTF) [2]. The objective representation of this function through evoked potentials is essential for studying the auditory temporal processing in subjects that do not respond accurately in behavioral tests (e.g. babies).

Usually, the electrophysiological TMTF is obtained by using many auditory stimuli with different modulation frequencies. This implies performing a long and tiring experiment for the subject. Alternatively, this function can be obtained by using stimuli that are modulated with a continuous sweep of modulation frequencies. Due to the non-stationary nature of the response for this type of stimuli, this evoked potential has been named as Envelope Following Response (EFR) [3,4].

The analysis of the EFR must be performed by means of time-frequency methods that allow to study non-stationary signals. These methodologies provide a complex representation of the EFR, characterizing both the amplitude and phase, of the signal for each frequency and time point [4]. Two of the most popular and practical methods are the Short Time Fourier Transform (STFT) and the Continuous Wavelet Transform (CWT) using Morlet complex functions [4]. Another method is the Fourier Analyzer, which consists in a faster variant of the STFT where instead of estimating the Fourier coefficients for the whole frequency range, it only estimates the coefficient corresponding to the instantaneous modulation frequency (IMF) of the stimulus in every time points [5]. Contrary to other types of evoked potentials, currently there is not a standard methodology for the estimation of the EFR. This is partly because of known theoretical and practical advantages and disadvantages offered by the different methods in different scenarios. To our knowledge, studies of the relative efficacy of each method for the analysis of different electrophysiological signals have not been carried out yet.

In this work, we introduce a Chirp Analyzer (CA) methodology as a new tool for the reliable estimation of non-stationary auditory electrophysiological responses. This is similar to the Fourier Analyzer, but using non-stationary reference functions (chirps) instead of the classical Fourier basis. We compare the relative robustness of this method for studying the EFR, with respect to the responses obtained by using standard time-frequency methodologies, such as Short Time Fourier Transform and Morlet Wavelet Transform. For this purpose, we used simulated data and illustrate the performance on real data of auditory responses of adult rats.

II. Materials and Methods

A. Simulated and real datasets

The stimuli used in this work, consisted in a carrier tone with a fixed frequency, modulated by a sinusoidal signal

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Neurobiology of Disease

Hemichannels Are Required for Amyloid β-Peptide-Induced Degranulation and Are Activated in Brain Mast Cells of APPswe/PS1dE9 Mice

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Mast cells (MCs) store an array of proinflammatory mediators in secretory granules that are rapidly released upon activation by diverse conditions including amyloid beta (Aβ) peptides. In the present work, we found a rapid degranulation of cultured MCs through a pannexin1 hemichannel (Panx1 HC)-dependent mechanism induced by $A\beta_{25-35}$ peptide. Accordingly, $A\beta_{25-35}$ peptide also increased membrane current and permeability, as well as intracellular Ca^{2+} signal, mainly via Panx1 HCs because all of these responses were drastically inhibited by Panx1 HC blockers and absent in the MCs of Panx1 $^{-/-}$ mice. Moreover, in acute coronal brain slices of control mice, $A\beta_{25-35}$ peptide promoted both connexin 43 (Cx43)- and Panx1 HC-dependent MC dye uptake and histamine release, responses that were only Cx43 HC dependent in Panx1 $^{-/-}$ mice. Because MCs have been found close to amyloid plaques of patients with Alzheimer's disease (AD), their distribution in brain slices of APPswe/PS1dE9 mice, a murine model of AD, was also investigated. The number of MCs in hippocampal and cortical areas increased drastically even before amyloid plaque deposits became evident. Therefore, MCs might act as early sensors of amyloid peptide and recruit other cells to the neuroinflammatory response, thus playing a critical role in the onset and progression of AD.

Key words: **Alzheimer's disease; amyloid peptide; degranulation; hemichannels; mast cells**

Introduction

In the brain, mast cells (MCs) are found mainly in the meningeal layers and within the cerebral parenchyma close to the blood vessels lying between the blood–brain barrier and astrocyte end-feet (Silver et al., 1996; Khalil et al., 2007). MCs contain an array of proinflammatory mediators (e.g., histamine, ATP, cytokines, leukotrienes, and proteases) stored in secretory granules and they rapidly release their content the extracellular milieu upon activation (Metcalfe et al., 1997). MC degranulation can be induced by a number of agents including amyloid peptides (Niederhoffer et al., 2009). These features, together with their strategic localization demonstrate that MCs can initiate and/or modulate different inflammatory responses.

Migration and activation of MCs at sites of injury occur in various neurodegenerative disorders (Secor et al., 2000; Graves et al., 2004; Lozada et al., 2005; Strbian et al., 2006; Fiala et al., 2010; Sayed et al., 2011). In postmortem studies of Alzheimer's disease (AD) patients, MCs have also been found close to amyloid plaque lesions in different brain regions (Maslinska et al., 2007), but their involvement in the onset and/or progression of the disease remains unknown.

In cultured MCs, amyloid beta (Aβ) peptides ($A\beta_{1-40}$ and $A\beta_{1-42}$) induce a rapid degranulation response (Niederhoffer et al., 2009). This response is mediated through the membrane complex formed by CD47 receptor, β_1 integrin subunit, and G_i protein (Niederhoffer et al., 2009). Activation of this complex leads to a Ca^{2+} influx (Sick et al., 2009) that is essential for MC degranulation. Several membrane channels permeable to Ca^{2+} have been proposed to participate in this Ca^{2+} influx (Ma and Beaven, 2011), particularly calcium release activated channels (CRAC) and store-operated Ca^{2+} channels (Ma and Beaven, 2011), but the possible participation of hemichannels (HCs) has not been evaluated. Because HCs are membrane pores permeable to Ca^{2+} (Vanden Abeele et al., 2006; Sánchez et al., 2009;

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RESEARCH ARTICLE

Age Progression of Neuropathological Markers in the Brain of the Chilean Rodent *Octodon degus*, a Natural Model of Alzheimer's Disease

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Keywords
Alzheimer' disease, glial activation, oxidative stress, metabolic sensors, neuronal apoptosis and natural model.

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Abstract

Alzheimer's disease (AD) is the most common neurodegenerative disorder and the leading cause of age-related dementia worldwide. Several models for AD have been developed to provide information regarding the initial changes that lead to degeneration. Transgenic mouse models recapitulate many, but not all, of the features of AD, most likely because of the high complexity of the pathology. In this context, the validation of a wild-type animal model of AD that mimics the neuropathological and behavioral abnormalities is necessary. In previous studies, we have reported that the Chilean rodent *Octodon degus* could represent a natural model for AD. In the present work, we further describe the age-related neurodegeneration observed in the *O. degus* brain. We report some histopathological markers associated with the onset progression of AD, such as glial activation, increase in oxidative stress markers, neuronal apoptosis and the expression of the peroxisome proliferative-activated receptor γ coactivator-1α (PGC-1α). With these results, we suggest that the *O. degus* could represent a new model for AD research and a powerful tool in the search for therapeutic strategies against AD.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia and affects nearly 10% of individuals over the age of 65 and nearly 50% of individuals over the age of 85. The increased longevity in the population, combined with the high incidence of AD in older adults, will only exacerbate the global impact on public health (13, 80). AD was first described over 100 years ago, but the etiology of AD is still not well understood, which limits the pharmacological treatment of the disease (66, 81). Different cellular and histopathological biomarkers have been described, including amyloid plaques, neurofibrillary tangles (NFTs), increased production of reactive oxygen species (ROS), mitochondrial dysfunction, decreased cerebral glucose consumption and altered autophagy processes (13, 29, 36, 40, 54, 75).

Transgenic mice have been the most useful tool in studying the pathological mechanisms of AD. However, these models do not recapitulate the entire spectrum of lesions present in human AD brains (17, 42, 84). Moreover, the overexpression of human transgenes in a nonphysiological scenario strongly influences the onset of histopathological features and cognitive decline observed in AD (16, 58). The poor reliability of the currently available AD models limits our understanding of AD pathophysiology and compromises the translation of preclinical data into human clinical trials. Thus, the identification and validation of a natural, wild-type AD model that can mimic the pathological hallmarks observed in AD patients would be highly useful to unravel the mechanisms of AD and validate potential therapeutic targets. We have previously suggested that the Chilean rodent *O. degus* could represent a natural model to study the onset and progression of AD (4, 10, 60). We have described that the *O. degus* naturally develops extracellular amyloid plaques, NFT, failure in cholinergic transmission and hippocampal disconnection in an age-related manner (5, 8, 34).

In this study, we used the brains of *O. degus* derived from several different aged (12–72 months) individuals to evaluate the

of an altered energy balance (52, 68). Some authors have even defined AD as a "diabetic brain" primarily because of a molecular link between diabetes and AD, which includes brain resistance to insulin (11, 14, 15, 18) and results in the deregulation of memory processes. This "cognitive–metabolic syndrome" might include altered cellular energy sensors, such as AMPK and PGC-1α (25). PGC-1α has been associated with the mitochondrial health status and has been proposed to play a major role in the modulation of mitochondrial fusion–fission events, which provide protection against Aβ-derived oxidative damage (69, 91). An altered expression of the active form of AMPK (pThr172-AMPK) has been observed in AD transgenic mice models, such as the APPswe-PSEN1dE9 (28, 63). We evaluated these markers in the brains of *O. degus* and found increased expression only in the case of PGC-1α, which suggests a compensatory mechanism to overcome Aβ-induced damage and prevent neuronal metabolic stress (22).

A general hypothesis is that the augmentation of autophagy flux capacity could represent a potential mechanism of the clearance of *tau* intracellular deposits (61). LC3 is involved in membrane recruitment, and p62 is involved in auto-phagosomal elongation (41). In our model, both markers (mainlyp62) exhibited similar increases, which replicates the pathology in other animal models of AD (APP/PS1) and in the human post-mortem brain (88). We were not able to find changes in LC3 and p62 markers suggesting a difference with the pathology in other models (88). In conclusion, we measured several tissue biomarkers in *O. degus* of different ages that have been described as characteristic of AD, including the classic hallmarks and emerging markers for inflammation (gliosis and microgliosis), oxidative stress (4-HNE and N-TYR), neuronal death and the metabolic stress marker PGC-1α. A summary of the observed changes is in Figure 7. We present evidence for a high correlation between the pathological changes that occur during the aging process in *O. degus* and the changes described in human AD. Taken together, the previously reported evidence and the research presented here provide a strong platform to consider *O. degus* as a reliable "natural" model to investigate the pathobiology of AD. The potential uses of this model are wide: it could increase our knowledge regarding aging and neurodegeneration, enable the development of more efficient staging, and provide novel therapies to slow down or ameliorate AD progression.

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RESEARCH ARTICLE

Genetic analysis of Eclosion hormone action during *Drosophila* larval ecdysis

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ABSTRACT

Insect growth is punctuated by molts, during which the animal produces a new exoskeleton. The molt culminates in ecdysis, an ordered sequence of behaviors that causes the old cuticle to be shed. This sequence is activated by Ecdysis triggering hormone (ETH), which acts on the CNS to activate neurons that produce neuropeptides implicated in ecdysis, including Eclosion hormone (EH), Crustacean cardioactive peptide (CCAP) and Bursicon. Despite more than 40 years of research on ecdysis, our understanding of the precise roles of these neurohormones remains rudimentary. Of particular interest is EH; although it is known to upregulate ETH release, other roles for EH have remained elusive. We isolated an *Eh* null mutant in *Drosophila* and used it to investigate the role of EH in larval ecdysis. We found that null mutant animals invariably died at around the time of ecdysis, revealing an essential role in its control. Further analyses showed that these animals failed to express the preparatory behavior of pre-ecdysis while directly expressing the motor program of ecdysis. Although ETH release could not be detected, the lack of pre-ecdysis could not be rescued by injections of ETH, suggesting that EH is required within the CNS for ETH to trigger the normal ecdysial sequence. Using a genetically encoded calcium probe, we showed that EH configured the response of the CNS to ETH. These findings show that EH plays an essential role in the *Drosophila* CNS in the control of ecdysis, in addition to its known role in the periphery of triggering ETH release.

KEY WORDS: Neuropeptide, Molting, Behavior, Postembryonic development, Insect

INTRODUCTION

In insects, continuous growth and development requires the exoskeleton to be replaced, which occurs during the molt and culminates with the process of ecdysis. During ecdysis, a precisely timed and concatenated series of behaviors causes the remains of the old exoskeleton to be shed and the new one to be inflated, hardened and pigmented. Research conducted during the last 40 years has revealed that a suite of neuropeptides controls the precise sequence of behaviors and physiological events that allow the insect to transition from one stage to the next (for reviews, see Ewer and Reynolds, 2002; Zitnan and Adams, 2012). These neuropeptides include Ecdysis triggering hormone (ETH), which is produced by peripheral endocrine cells, and the centrally produced neuropeptides, Eclosion hormone (EH), Crustacean cardioactive peptide (CCAP) and Bursicon. Evidence from both Lepidoptera (e.g. Zitnan et al., 1996) and *Drosophila* (e.g. Park et al., 2002) indicates that ETH can turn on the entire ecdysial sequence. Direct targets of ETH include neurons that express FMRFamide, EH and CCAP (some of which also express Bursicon and/or the MIP peptide; Kim et al., 2006a,b), and both their timing of activation after ETH release and functional analyses (Lahr et al., 2012; Honegger et al., 2008; Kim et al., 2006a; Gammie and Truman, 1997a) suggest a role in the control of different phases of ecdysis. Thus, FMRFamide is proposed to regulate the early phase of the behavior, EH and the CCAP neurons that express CCAP or CCAP and MIP would regulate ecdysis proper, and neurons that coexpress CCAP, MIP and Bursicon participate in the postecdysial phases of the behavior.

EH has been implicated in the control of ecdysis since its discovery in Lepidoptera more than 40 years ago (Truman and Riddiford, 1970). In *Manduca* (Truman et al., 1980; Copenhaver and Truman, 1982) and *Bombyx* (Fugo and Iwata, 1983), injections of EH into the hemolymph cause premature ecdysis, and addition of EH to an isolated *Manduca* central nervous system (CNS) can induce the ecdysis motor program (Gammie and Truman, 1999; Zitnan and Adams, 2000), indicating that EH is sufficient for turning on ecdysis. In *Tribolium*, injection of EH interfering RNA causes a severe weakening of pre-ecdysis and a complete suppression of ecdysis (Arakane et al., 2008), suggesting that EH is also necessary for ecdysis. Nevertheless, the precise role of EH in *Drosophila* remains elusive. Indeed, flies bearing targeted ablations of EH neurons express relatively minor defects at larval ecdysis (McNabb et al., 1997; Clark et al., 2004), with about a third of animals reaching adulthood (McNabb et al., 1997). In addition, and most perplexingly, flies lacking EH neurons are insensitive to injections of ETH: in contrast to wild-type animals, for which such injections advance the onset of ecdysis, ETH injections do not change the timing of ecdysis of either larvae or adults bearing targeted ablations of EH neurons (McNabb et al., 1997; Clark et al., 2004).

From these observations it is difficult to propose a unified model for the role of EH in the control of ecdysis beyond its well-accepted role in potentiating ETH release (Ewer et al., 1997; Kingan et al., 1997). Furthermore, the majority of the information from *Drosophila* stems from experiments in which the EH neurons were genetically ablated (McNabb et al., 1997; Baker et al., 1999; Clark et al., 2004). Although this approach has provided valuable insights into the possible role of this neuropeptide at ecdysis, the interpretation of the findings is complicated by the fact that such animals lack the EH neurons in addition to the EH peptide, making it impossible to distinguish between functions subserved by the peptide itself from other roles played by the EH neurons.

We report here on the isolation of a null allele of the *Eh* gene and the characterization of the larval ecdysis phenotype of animals

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RESEARCH ARTICLE

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Creation of genetic deletion that includes *Eh* gene

Exelixis strains *f01683* and *d00811* were used to create a 32 kb genetic deletion that included the *Eh* gene [*Df(3)Eh*$^{-}$; abbreviated in all figures as *Df(Eh)*; cf. Fig. 1A], using the FLP-FRT system as described by Parks et al. (2004). Putative deletion-bearing males were used singly to set up balanced lines; homozygous larvae were then screened by PCR using primer pair EH-F2+EH-R2 (see Table 1) and the limits of resulting deletions verified by PCR. In addition to the *Eh* gene, this deletion also completely removes gene *CG14330* (which encodes a gene of unknown function) and partly removes *CG5873*, a heme peroxidase-encoding gene, which when mutant causes no apparent defects (FlyBase).

Molecular biology

PCR

DNA was obtained from single third instar larvae as described by Gloor et al. (1993), but using 10 µl of 'squish buffer' (0.4 µg/µl proteinase K, 10 mM Tris pH 8, 0.2 mM EDTA and 25 mM NaCl) per fly larva. One microliter of extract was used for each 20 µl PCR, which was run using the following conditions: 94°C (3 min); then 30 cycles of 94°C (45 s), 55°C (0.5 min) and 72°C (1.0 min/kb of product); followed by one cycle at 72°C for 10 min.

Transgenic constructs

UAS-*Eh* construct

Eh cDNA was amplified by RT-PCR from RNA extracted from third instar CNSs following the manufacturer's instructions. The primer pair EH-F3+EH-R3 (Table 1) was used to amplify a 400 bp fragment that includes the entire *Eh* coding region; the 3′ reverse primer included a *Not*I site for subcloning purposes. PCR products obtained from three independent cDNAs were cloned into pGEM-T Easy vector (Promega) and sequenced for verification. The fragment containing the *Eh* cDNA was then cloned into pUAST *P*-element vector (van Roessel and Brand, 2000) and sent to BestGene for germline transformation.

Genomic *Eh* rescue construct

A 4.8 kb fragment of genomic DNA containing the entire *Eh* gene and including 1.9 kb of 5′ regulatory sequences, which is sufficient to drive gene expression faithfully in EH neurons (McNabb et al., 1997), was amplified by PCR from a BAC clone from the RPCI-98 *Drosophila melanogaster* BAC Library (http://bacpac.chori.org/dromel98.htm) using the High Fidelity Expand Long Template PCR system (Roche) following the manufacturer's instructions using primer pair EH-F4+EH-R4 (Table 1). The PCR product was cloned into pGEM-T Easy vector (Promega), subcloned into the *pattB* vector (Venken et al., 2006) by Genewiz and sent to BestGene for germline transformation.

Synthesis of EH

Construction of pMAL-EH

Synthetic EH was produced by *in vitro* expression using the pMAL protein fusion and purification system (pMAL-c2x; New England Biolabs). For this, a 222 bp fragment that encodes the predicted mature EH protein (minus putative leader sequence) was amplified from the EH cDNA (see above) using primer pair EH-F5+EH-R5 (Table 1); forward primer included an *Eco*RI site for subcloning purposes. The PCR product was subcloned into pGEM-T Easy vector (Promega), sequenced for verification, and subcloned in frame into the *Eco*RI site of the pMAL-c2x vector.

EH synthesis

Maltose-binding protein-EH (MBP-EH) fusion protein and MBP alone (control) were expressed following the manufacturer's recommendations in Origami cells (Novagen, Merck) to facilitate disulfide bond formation, which is thought to be a critical component of bioactive EH (Nagasawa et al., 1983; Terzi et al., 1988).

Hormone injections

Synthetic ETH was obtained from Bachem. It was diluted in distilled water and used at a final concentration of 1 mM. EH-MBP and MBP (see above) were diluted in distilled water, and 50-100 nl was injected into pharate second instar larvae using a PV800 pneumatic picospritzer (World Precision Instruments). For ETH, this dose (corresponding to ~50-100 fmoles) is known to cause suprathreshold responses in pharate larvae (Park et al., 2002; Clark et al., 2004). Control injections consisted of the same volume of distilled water (for ETH) and similar concentration of MBP alone (for EH).

Immunostaining

Immunostaining was carried out as described by Clark et al. (2004) using the following antisera: rabbit anti-CCAP, generously provided by Hans Agricola (Friedrich-Schiller University Jena, Jena, Germany), and used at 1:5000; rabbit anti-EH generously provided by James Truman (HHMI Janelia Research Campus, Ashburn, VA, USA) and used at 1:200; rabbit anti-ETH generously provided by Michael Adams (University of California, Riverside, CA, USA) and used at 1:2000.

Quantification of immunolabeling

CCAP and ETH immunoreactivity were quantified as described by Clark et al. (2004), assigning a subjective score of 0 (no staining) to 3 (strongest staining). The person scoring the preparations did not know the genotype or time at which the tissues had been fixed.

Behavioral analyses

Larvae were collected and their ecdysial behaviors recorded as described by Clark et al. (2004). All analyses involving *Eh* mutants were done using hemizygous *Eh*^{exc}/*Df(3)Eh*$^{-}$ larvae; genetic rescue animals were tested in a similar manner in this genetic background.

Imaging of Ca^{2+} dynamics

Imaging of *ex vivo* Ca^{2+} dynamics was carried out as described by Kim et al. (2006a), using CNSs from second instar larvae at the DVP ('double vertical plate') stage, approximately 30 min prior to ecdysis (Park et al., 2002). Preparations were imaged under an Olympus DSU Spinning Disc microscope using a 40× (NA 0.80) water immersion lens. They were first imaged every 5 s for 5 min, and preparations showing spontaneous activity (~5% of the preparations) were discarded. They were then stimulated with 1 µM synthetic ETH1 (Bachem) and GFP fluorescence captured every 5 s for 90 min. Resulting recordings were analyzed using Cell^R Olympus Imaging Software (version 2.6).

Statistical analyses

Statistical significance was evaluated using the Prism v. 6.0 (GraphPad Software). Quantitative results were compared by ANOVA followed by Tukey's HSD post hoc analyses. Categorical data based on qualitative measurements were compared by Kruskal–Wallis one-way analysis of variance.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

E.K., W.M. and J.E. planned the experiments; E.K. carried out most experiments; W.M. carried out the calcium imaging; E.C.L. and E.C.J. provided reagents. E.K. and J.E. analyzed the results; J.E. wrote the manuscript. All authors read and commented on the manuscript and approved the final version.

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MOLECULAR PHARMACOLOGY

MINIREVIEW—EXPLORING THE BIOLOGY OF GPCRs: FROM IN VITRO TO IN VIVO

Model Organisms in G Protein–Coupled Receptor Research

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ABSTRACT

The study of G protein–coupled receptors (GPCRs) has benefited greatly from experimental approaches that interrogate their functions in controlled, artificial environments. Working in vitro, GPCR receptorologists discovered the basic biologic mechanisms by which GPCRs operate, including their eponymous capacity to couple to G proteins; their molecular makeup, including the famed serpentine transmembrane unit; and ultimately, their three-dimensional structure. Although the insights gained from working outside the native environments of GPCRs have allowed for the collection of low-noise data, such approaches cannot directly address a receptor's native (in vivo) functions. An in vivo approach can complement the rigor of in vitro approaches: as studied in model organisms, it imposes physiologic constraints on receptor action and thus allows investigators to deduce the most salient features of receptor function. Here, we briefly discuss specific examples in which model organisms have successfully contributed to the elucidation of signals controlled through GPCRs and other surface receptor systems. We list recent examples that have served either in the initial discovery of GPCR signaling concepts or in their fuller definition. Furthermore, we selectively highlight experimental advantages, shortcomings, and tools of each model organism.

Introduction

G protein–coupled receptor (GPCR) pharmacology began in earnest with Raymond Ahlquist's conjecture that there must be two types of adrenotropic receptors to account for excitatory and inhibitory effects of the sympathetic adrenergic mediator, epinephrine. This conclusion was based on a set of experiments that characterized the effect of biogenic amines on a roster of vegetative functions in dogs, cats, rats, and rabbits (Ahlquist, 1948). Most interestingly, the proposal of adrenoceptor subtypes was achieved before the era of molecular biology, before receptors transformed from a physiologic concept into a molecular fact (De Lean et al., 1980; Dixon et al., 1986; Palczewski et al., 2000; Rasmussen et al., 2007).

Ahlquist's work illustrates one advantage of animal models in pharmacological research: the ability to learn about receptor functions on cellular, organ, and organismic states without

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ABBREVIATIONS: AC, adenylyl cyclase; aGPCR, adhesion G protein–coupled receptor; DREADD, designer receptors exclusively activated by designer drug; ECM, extracellular matrix; ETH, ecdysis triggering hormone; FRET, fluorescence resonance energy transfer; GPCR, G protein–coupled receptor; GPS, G protein–coupled receptor proteolysis site; LNv, ventrolateral clock neuron; MB, mushroom body; PDF, pigment dispersing factor; PDFR, pigment dispersing factor receptor; TM, transmembrane.

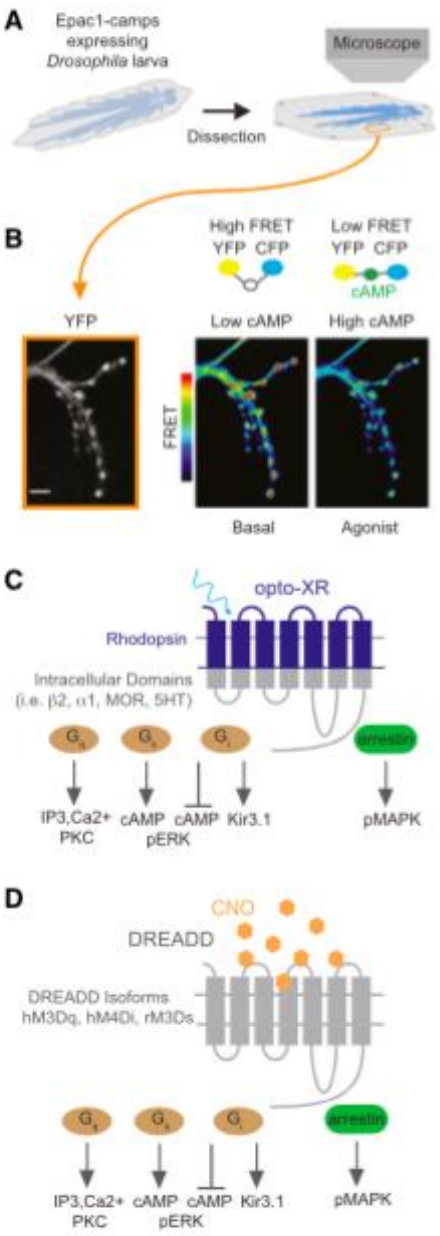

Fig. 3. Modern tools for investigating GPCR signaling in vivo. (A) Representation of intact (right) and dissected (left) *Drosophila* larva expressing the FRET-based sensor for monitoring cAMP changes, Epac1-camps. (B) YFP and time-resolved pseudocolor FRET images of *Drosophila* motoneurons expressing Epac1-camps. The application of agonist mediates the activation of the endogenous receptors, which results in production of cAMP. This cAMP increase is revealed by a loss in FRET caused by a conformational rearrangement of the sensor, induced by cAMP binding. (C) Cartoon depicting the chimeric "Opto-XR" approach, whereby rhodopsin cDNA is fused with wild-type GPCR cDNA intracellular loops and tail to generate a photosensitive receptor system capable of spatiotemporal engagement of canonical GPCR signaling pathways such as G_{i}, G_{s}, and G_{i} or arrestin recruitment in selected cell types when combined with viral and genetic approaches in vivo. (D) Cartoon representing chemogenetic GPCRs termed DREADDs, which selectively respond to the ligand CNO to engage canonical GPCR signaling pathways in selected cell types when also combined with viral or mouse genetic approaches. CFP, cyan fluorescent protein; CNO, clozapine–nitreous oxide; ERK, extracellular signal-regulated kinase; IP3, inositol trisphosphate; MAPK, mitogen-activated protein kinase; PKC, protein kinase C; YFP, yellow fluorescent protein. Bar, 10 μm.

metabotropic activity differed substantially. Activation of octopamine receptors by octopamine elicited a generalized cAMP signal in the region of the MB involved in appetite modulation. By contrast, dopamine stimulation triggered a localized cAMP signal in parts of the MB that modulate aversive learning. This demonstrates how knowledge of cellular location and dynamics of GPCR-generated signals in the nervous system helps to unravel complex brain functions, unlocking an organ- and behavior-specific context to GPCR function.

Optogenetics and Designer Receptors Exclusively Activated by Designer Drugs for Studying GPCR Function In Vivo. The advent of optogenetics has transformed the ability of biologists to selectively interrogate neural circuits, cell types, and pathways critical for behavior and disease. Several recent advances are underway to use the advantages of light's spatial-temporal characteristics to selectively engage GPCR signaling in a cell-type selective manner in vivo.

Investigators have turned to utilizing vertebrate and non-mammalian rhodopsin receptors to mimic G protein signaling both in vitro and in vivo using a variety of approaches (Zemelman et al., 2002; Schroll et al., 2006; Zhang et al., 2007). A recent study showed that bovine rhodopsin and class A GPCRs could be made into receptor chimeras, composed of extracellular and hydrophobic light-sensitive rhodopsin domains and intracellular loops targeted to couple to specific G-protein pathways (Airan et al., 2009). The authors used β_2-adrenergic and α_1-adrenergic receptor intracellular loop and carboxy tail components fused to bovine rhodopsin to achieve $G\alpha_s$ and $G\alpha_q$ signaling, respectively (Fig. 3C). This technique allows experimenters to use fiber optic or wireless light-emitting diode technology (Yizhar et al., 2011; Kim et al., 2013) to activate GPCR signaling within selected cell types in the mammalian brain of awake behaving animals. Elegant extensions of this approach have also been used in modifications of vertebrate rhodopsin with components of the 5HT1a or 5HT2c serotonin receptor for examining neural circuits and signaling in anxiety behavior (Masseck et al., 2014; Spoida et al., 2014). Recent work has also shown that chimeric opsin–wild-type receptors can be used to optically mimic opioid receptor signaling (Siuda et al., 2015) (Fig. 3C). Other studies used opsin GPCRs in cellular models for achieving remarkable spatial-temporal control of signaling gradients and cell migration (Karunarathne et al., 2015). These approaches allow the experimenter to precisely control the spatial components of subcellular GPCR signaling without activating receptor signaling in other microdomains or at different cellular stations (O'Neill and Gautam, 2014). Using plant cryptochrome domains (CRY2/CIB1), one can light-trigger recruitment of regulators of G protein signaling or Gbg, allowing for the selected sequestering of GPCR signaling. Similar approaches have used short-wavelength opsins and invertebrate opsins from jellyfish to achieve subcellular spatial-temporal control.

Another line of technology to interrogate GPCR signals in vivo regards the recent wide adoption of designer receptors

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ORIGINAL PAPER

Biological Cybernetics

Modeling neural activity with cumulative damage distributions

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Abstract Neurons transmit information as action potentials or spikes. Due to the inherent randomness of the inter-spike intervals (ISIs), probabilistic models are often used for their description. Cumulative damage (CD) distributions are a family of probabilistic models that has been widely considered for describing time-related cumulative processes. This family allows us to consider certain deterministic principles for modeling ISIs from a probabilistic viewpoint and to link its parameters to values with biological interpretation. The CD family includes the Birnbaum–Saunders and inverse Gaussian distributions, which possess distinctive properties and theoretical arguments useful for ISI description. We expand the use of CD distributions to the modeling of neural spiking behavior, mainly by testing the suitability of the Birnbaum–Saunders distribution, which has not been studied in the setting of neural activity. We validate this expansion with original experimental and simulated electrophysiological data.

Keywords Birnbaum–Saunders and inverse Gaussian distributions · Integrate-and-fire model · Inter-spike intervals · Maximum likelihood method · Model selection and goodness of fit

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1 Introduction

The modeling of neural activity may help to understand the neural code, whereby information is transmitted, processed and stored in the nervous system (Tuckwell 1989). The information transmitted by neurons is encoded by series of action potentials or neural spikes (called spikes hereafter), which are the key units used in neural transmission (Truccolo et al. 2005). Due to the uniformity of the action potentials, spiking behavior can be described by the inter-spike intervals (ISI), that is, through the time elapsed between spikes of a neuron or group of neurons (Brown et al. 2002). For example, it has been shown that the frequency of spikes, and their ISIs, encode sensory stimuli faithfully in afferent sensory nerves (Mountcastle et al. 1966). A deterministic model that has been widely used to analyze neural activity is the integrate-and-fire model (see Burkitt 2006, and references therein). However, due to the inherent randomness of neural activity, spike trains for identical stimuli may vary, and therefore, stochastic versions of the integrate-and-fire model are better suited to describe neural behavior (Burkitt 2006; La Camera et al. 2008).

This randomness of the ISIs allows them to be considered as random variables (RVs), which can be described by probabilistic models, whose parameters may be linked to values with biological interpretation (Levine 1991; Inoue et al.

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Table 8 BF (B_{12}) values in pair-wise comparison (M_1 vs M_2) of the indicated distribution and data set

Set	M_1	M_2	$2\log(B_{12})$	Evidence in favor of M_1
ES1	BS	IG	0.015	Weak
	BS	LN	1.548	Weak
	BS	ER	20.329	Very strong
	BS	EX	122.426	Very strong
ES2	IG	BS	0.684	Weak
	IG	LN	2.220	Positive
	IG	ER	8.150	Strong
	IG	EX	142.990	Very strong
ES3	IG	BS	0.686	Weak
	IG	LN	2.391	Positive
	IG	ER	9.394	Strong
	IG	EX	113.057	Very strong
ES4	IG	BS	0.076	Weak
	IG	LN	1.108	Weak
	IG	ER	23.835	Very strong
	IG	EX	103.700	Very strong

family of cumulative damage distributions has been highly useful for describing time-related cumulative processes and has allowed us to consider certain deterministic principles in the modeling of inter-spike intervals but from a probabilistic perspective. Specifically, from the theoretical viewpoint, we have derived the Birnbaum–Saunders distribution from the discretization of a stochastic differential equation, which has allowed us to provide a probabilistic version of specific dynamical models. By considering that the increments of the ionic currents are positive with a high probability, we have shown that the parameters of this distribution match the biological parameters very well. From the practical viewpoint, we have conducted a simulation study to evaluate the performance of our expansion and illustrated its effectiveness for fitting inter-spike interval data extracted from original electrophysiological recordings. Overall, the application of goodness-of-fit methods and model selection criteria to simulated and experimental inter-spike interval data sets has shown that the Birnbaum–Saunders and inverse Gaussian distributions fit better these data sets than the exponential and lognormal distributions.

6.2 Future works

Cumulative damage distributions can model neural activity resulting from excitatory or inhibitory (neurotransmitter) inputs, which increase or decrease the probability that a neuron fires an action potential, respectively. Thus, models for repairable systems may be an interesting avenue for future studies related to the present paper, enabling our extension to admit both damage and repair, making an analogy between models for repairable systems and inhibitory inputs. This would allow our work to be linked to reliability models (Magloczky and Freundemail 2005; Nikulin et al. 2010; Peng et al. 2010). Researchers often use statistical distributions different from the well-known normal or Gaussian distribution for modeling both neural networks and artificial neural networks, because it has been shown that recurrent connections can induce spiking activity to be different from the usual probabilistic behavior. Some recent papers that relate ISI and fatigue life distributions to neural networks are attributed to Chen and Nitz (2011), Figueira and Andrade (2011) and Steimer and Douglas (2013). The distributions considered in the present study can also be used for modeling neural networks, possibly providing better fittings, as shown in the experimental data analysis presented here. In addition, the expansion proposed in this paper, based on cumulative damage, can be useful for comparing if two neurons (or the same neuron in two different conditions) are firing with the same pattern. Thus, the behavior of a neuron in a network may also be modeled with cumulative damage distributions. Therefore, the practicality of the proposed expansion in the setting of neural networks remains open and will be addressed by the authors in future studies. Also, multivariate aspects of cumulative damage distributions could be considered (see Marchant et al. 2015, and references therein).

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PLOS | ONE

RESEARCH ARTICLE

Biomimetics: From Bioinformatics to Rational Design of Dendrimers as Gene Carriers

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Abstract

Biomimetics, or the use of principles of Nature for developing new materials, is a paradigm that could help Nanomedicine tremendously. One of the current challenges in Nanomedicine is the rational design of new efficient and safer gene carriers. Poly(amidoamine) (PAMAM) dendrimers are a well-known class of nanoparticles, extensively used as non-viral nucleic acid carriers, due to their positively charged end-groups. Yet, there are still several aspects that can be improved for their successful application in *in vitro* and *in vivo* systems, including their affinity for nucleic acids as well as lowering their cytotoxicity. In the search of new functional groups that could be used as new dendrimer-reactive groups, we followed a biomimetic approach to determine the amino acids with highest prevalence in protein-DNA interactions. Then we introduced them individually as terminal groups of dendrimers, generating a new class of nanoparticles. Molecular dynamics studies of two systems: PAMAM-Arg and PAMAM-Lys were also performed in order to describe the formation of complexes with DNA. Results confirmed that the introduction of amino acids as terminal groups in a dendrimer increases their affinity for DNA and the interactions in the complexes were characterized at atomic level. We end up by briefly discussing additional modifications that can be made to PAMAM dendrimers to turned them into promising new gene carriers.

Introduction

Biomimetics is the implementation of principles from Nature to the development of new materials or systems. Evolutionary pressure has driven the optimization of efficiency in natural systems; thus, it is valuable to use this knowledge as a source of inspiration to solve existing problems[1]. One of the areas that can benefit from biomimetics is nanomedicine, which provides platforms to understand, build and use structures with biomedical applications at nanometric scale.

THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

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Allosteric Communication Pathways and Thermal Rectification in PDZ-2 Protein: A Computational Study

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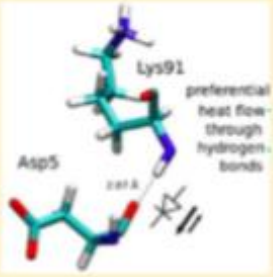
ABSTRACT: Allosteric communication in proteins is a fundamental and yet unresolved problem of structural biochemistry. Previous findings, from computational biology (Ota, N.; Agard, D. A. *J. Mol. Biol.* **2005**, *351*, 345−354), have proposed that heat diffuses in a protein through cognate protein allosteric pathways. This work studied heat diffusion in the well-known PDZ-2 protein, and confirmed that this protein has two cognate allosteric pathways and that heat flows preferentially through these. Also, a new property was also observed for protein structures: heat diffuses asymmetrically through the structures. The underling structure of this asymmetrical heat flow was a normal length hydrogen bond (~2.85 Å) that acted as a thermal rectifier. In contrast, thermal rectification was compromised in short hydrogen bonds (~2.60 Å), giving rise to symmetrical thermal diffusion. Asymmetrical heat diffusion was due, on a higher scale, to the local, structural organization of residues that, in turn, was also mediated by hydrogen bonds. This asymmetrical/symmetrical energy flow may be relevant for allosteric signal communication directionality in proteins and for the control of heat flow in materials science.



INTRODUCTION

Allosteric communication is an unresolved problem in biochemistry. This phenomenon involves important physiological and cellular functions and is implicated in several human diseases.[1−3] Allosteric communication involves signal transmission along protein structures on both short (3 Å) and long (100 Å) distances, and is studied using both experimental[4−12] and theoretical[8,13−19] techniques. Today, its occurrence is an accepted phenomena.

Several questions surround the phenomenology of signal transduction and energy flow in proteins. This paper assesses how energy is transported from one site to another in proteins. In particular, directionality for energy flow in proteins was empirically observed. The structure underlying this directional energy flow was the normal length hydrogen bond (~2.85 Å). This result suggests that a normal length hydrogen bond is the minimal chemical structure that can operate as a thermal rectifier in biomolecules. Another source of asymmetrical heat diffusion was found on a higher scale in the local structural organization of residues, which were also mediated by hydrogen bonds. In contrast, the thermal rectification effect seemed to be suppressed in short hydrogen bonds (~2.60 Å), giving rise to symmetrical thermal diffusion. Asymmetrical energy flow may be relevant for allosteric signal communication directionality in protein structures and for heat flow control in the field of materials science.

Hydrogen bonds have been extensive matter of research for many years.[20] Their role in stabilization of protein structure has been clearly stated.[21] They additionally have a relevant role in chemical reactivity in proteins[22−24] and, as earlier from the realm of physics, can operate as a supporting structure for vibrational energy flow in protein structures.[25] Recent studies have shown that hydrogen bonds are also determinant in the diffusion of thermal energy across the β-sheet structure of the spider silk protein[26] and in α-helices.[27] An experimental study[28] presents for the first time detailed experimental evidence of ATD in a protein, where hydrogen bonding makes important contributions. Computational work on green fluorescent protein and water also highlights the importance of hydrogen bonds in the energy transport network in this particular system.[29]

As a protein folds to reach its biologically active state, it generates a complex and intricate network of contacts. Computational analysis has shown that vibrational energy runs following to the physical connections of this network,[30] with propagation velocities of the order of approximately 10 Å ps^{-1}.[24] This flow of energy is similar to the energy transport in percolation clusters, in which thermal energy flows anisotropically, e.g., faster through connected channels and slower along the routes that lead to dead-ends.[14] The anisotropic heat flow in proteins was empirically demonstrated by Ota and Agard using a computational pump−probe method termed Anisotropic Thermal Diffusion (ATD).[32] Anisotropic thermal diffusion is a nonequilibrium molecular dynamics (MD)

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it to Gln43, while the heating of Gln43 mainly transferred back to residue Asp15. This residue was the individual heat connection of group 13−17 to Gln43. All the previous data suggest a relevant role of hydrogen bonds in vibrational coupling among segments of the secondary structure in this system and, therefore, in the constitution of allosteric pathways.

In summary, this work analyzed the vibrational energy diffusion in PDZ-2 and the correlation with cognate allosteric pathways for this system. Examples of asymmetrical heat propagation in protein structures that give rise to signal directionality were also offered. Thermal diffusion maps showed a high degree of similarity with connectivity maps, thus supporting the idea that Type I allosteric communication is the connectivity network that defines the allosteric route and its directionality. Taken together, it is suggested that hydrogen bonds perform a relevant role as a transport structure for heat and thermal rectification in the allosteric communication pathways of proteins.

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Notes

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Hydrogen bonds and asymmetrical heat diffusion in α-helices. A computational analysis

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ABSTRACT

In this work, we report the heat rectifying capability of α-helices. Using molecular dynamics simulations we show an increased thermal diffusivity in the C-Terminal to N-Terminal direction of propagation. The origin of this effect seems to be a function of the particular orientation of the hydrogen bonds stabilizing these α-helices. Our results may be relevant for the design of thermal rectification devices for materials science and lend support to the role of normal length hydrogen bonds in the asymmetrical energy flow in proteins.

1. Introduction

Hydrogen bonds have been an inexhaustible source of research for decades [1]. Their role as stabilizing agents of protein structures has been clearly established [2]. These also modulate the chemical reactivity of enzymes through changes in the physicochemical properties of the surrounding structural elements [3–5]. Likewise, the role of hydrogen bonds in thermal conduction in proteins has recently been reported, revealing their importance in heat diffusion across α-helices [6] and the β-sheet structure of spider silk [7]. Proteins are an interesting source of molecular examples for thermal control. Phenomena such as conformational changes, enzyme catalysis, allosteric cooperativity, and intermolecular affinities, among other processes [8], require of a high degree of control over thermal energy flow. Interestingly, heat flow in protein structures has been recently correlated to the known pathways for allosteric communication. This means that heat propagates preferentially along the same structural pathway that defines the allosteric communication route in proteins [10–13].

Understanding heat flow at the molecular and nanoscale levels is currently of high interest in the development of phononics [14–16]. Control of heat flow in materials science is highly desirable for implementing thermal devices such thermal logic gates [17]; thermal memories [18], and acoustic and thermal cloakers [14]. Fundamental for the implementation of these phononic devices is the thermal diode that rectifies thermal energy passage in a predetermined direction [14,19]. Experimental heat flow rectification has been achieved using mass-graded carbon and boron nitrite nanotubes [20], as well as with vanadium dioxide composites [21] in which a symmetry-breaking structure joins the thermal source with the thermal drain. The rectifying capability of symmetry-breaking structures has also been computationally demonstrated in carbon nanotube intramolecular junctions [22], carbonano-cones [23], and in asymmetric graphene ribbons [24].

In this work, we report the rectifying capability of a set of α-helices, that is, the capability of heat transfer with preferential directionality. Using molecular dynamics simulations, energy flux was studied by vibrational excitation on the N- or C-terminal sides of the α-helices, independently (Figure 1). Increased thermal diffusivity was found in the C-Terminal to N-Terminal direction of heat diffusion, showing that α-helices may act as thermal rectifiers. Heat flow in both directions of propagation was analyzed in terms of the global temperature for each structure; average power; and the kinetic and potential energy of the atoms and bonds involved in selected hydrogen bonds. All of the results suggest that backbone hydrogen bonds, and their natural asymmetry (–C=O···H–N–), are a symmetry-breaking element that gives rise to the observed thermal rectification. The presented results are consistent with computational reports about hydrogen-bond mediated directional thermal energy transport in functionalized hydrophobic and hydrophilic silica–water interfaces [25].

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4. Conclusions

Motivated by a previous report about the role of hydrogen bonds in heat diffusion in α-helices [6] and by the observations of Schoen et al. [25] about a possible rectifying role of hydrogen bonds in functionalized materials, we decided to investigate the effects of applying heat to a set of α-helices at the opposite side than that previously reported. The particular set of α-helices used in these previous and this present study are stabilized by c.a. 30 hydrogen bonds connected in a series along the helix backbone. This characteristic makes them very suitable models to study the effects of hydrogen bond orientation in structure thermalization in the 'direct' (N-term → C-Term) and 'inverse' (N-Term ← C-Term) directions.

The results showed that the inverse direction had faster thermalization while the direct direction had slower thermalization. Thermalization rates were also followed by measuring the average power, heat flux and a respective R values at the O, N, C, and Cα atoms of residues 28 and 6 for the direct and inverse heating procedures, respectively. Comparison with other theoretical determinations, such us in alkane chains placed between azulene and antracene moieties [30], exhibit a similar R values, with a similar temperature difference (300 K). Experimentally, similar values of R are observed for a solid state thermal rectifier operating bellow 200 K which, however, require a smallest difference in temperature (about 50 K) to achieve a value for R approximately of 1.5 [31]. A room temperature thermal rectifier, based in cobalt oxides, has been developed showing R values in the range 1.02–1.07 [20].

Kinetic and potential energy analyses of selected hydrogen bonds in both directions suggested that these act as the underlying chemical motif for thermal rectification. It seems that the thermal mechanism follows the intrinsic asymmetry of the hydrogen bonds, –C=O···H–N–. When the heat injected into the N-terminal side (direct direction) first reached the C=O moiety of each hydrogen bond, this point acted as a kind of 'hard' end that trapped heat, which was reflected by the increased kinetic and potential energy of this bond. In the inverse direction, when heat was injected into the C-terminal side, it first reached the H–N moiety of each hydrogen bond. This moiety seemed to act as a kind of 'soft' end that was less effective than its counterpart (C=O moiety) at trapping heat. Due to its lower spring constant, the H–N moiety could not trap energy in the same way as the C=O moiety. The overall effect was that heat diffused more efficiently than in the inverse situation, and the favored heat diffusion direction of C=O ← H–N held.

We conclude that the preferential direction of heat diffusion is directed by the particular orientation of hydrogen bonds. In α-helices, this orientation of hydrogen bonds is conserved along the structure, and the effect is therefore multiplied by the number of hydrogen bonds that support the helical structure. Our findings are consistent with the proposal of Schoen et al. [25], in which hydrogen bonds may act as thermal diodes. Recent evidence has shown a correlation between the heat diffusion pathways and the known allosteric communication pathways in proteins [10–13]. The present results support the role of hydrogen bonds in heat diffusion in proteins [6,7], the function of normal length hydrogen bonds as thermal rectifiers in proteins and that these bonds can operate a source of directional energy flow in proteins [13]. The study and understanding of heat flow directionality in materials – phonon rectification – is desirable in materials science for the development of thermal gates [17], thermal memories [18], and thermal cloakers [14]. In this respect, the inclusion of α-helices in the designs of materials science may be a useful alternative for implementing these thermal devices.

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ROLE OF ION CHANNELS IN SALT SECRETION BY ATLANTIC SALMON GILLS DURING ACCLIMATION TO SEAWATER

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ABSTRACT

Smoltification, also called parr-smolt transformation, is a complex developmental process that consists of a number of independent, but coordinated changes, in the biochemistry, physiology, morphology and behavior of juvenile salmon in their transition from freshwater to seawater life. A key component of smoltification is represented by the physiological adaptations that enable smolts to thrive in hyperosmotic environments. Instrumental to this process is the ability of smolt gills to gradually become capable of actively secreting salt through specialized cells known as mitochondria-rich (MR) cells, ionocytes or chloride cells. NaCl secretion by teleost gills is therefore accomplished via the secondary active transport of Cl^- and the passive transport of Na^+. The driving force for active transport is provided by Na^+/K^+ ATPase, which maintains low intracellular Na^+ and high intracellular K^+ concentrations. However, this NaCl secretion mechanism needs at least two different ion channels: A CFTR type chloride channel for the passive exit of Cl^- and a potassium channel to recycle extracellular K^+, which is a thermodynamic prerequisite to work under conditions imposed by high extracellular salinity in seawater. The characteristics of K^+ channels required for NaCl secretion from MR cells into seawater are still unknown for *Salmo salar* and only recently have begun to be studied in other teleosts.

Keywords: smoltification, salt secretion, ion channels, atlantic salmon

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rectification. Kir1.1 presents a tight regulation by internal pH, at physiological range, which could be important for the smoltification process [28].

2.1.2. BK channels

The high conductance voltage- and Ca^{2+}-activated K^+ channel is one of the most broadly expressed channels in metazoans. The name 'Big K' stems from its single-channel conductance that can be as large as 250 pS under symmetrical 100 mM K^+ condition [29, 30]. BK channels are homotetramers formed by its α-subunit, encoded by the *slo-1* gene (KNCMA1), it is member of the voltage-gated potassium (Kv) channel superfamily [31]. BK channel architecture is different from typical Kv channel, it has an extra transmembrane segment (S0), thus each monomer presents seven transmembrane segments instead of six [32].
This channel presents an exquisite regulation by intracellular Ca^{2+}, achieving its maximum activity (Po ~ 1) at 100 μM [33]. The structural motif that confers to BK Ca^{2+} sensitivity is the **R**egulator of **C**onductance of **K+** (RCK) domain, which is part of the intracellular regulatory domain called gating ring conformed by the N and C-terminus (see part 2.1.3) (Fig. 2C) [34, 35]. BK channels have been implicated in a variety of physiological processes, from the regulation of smooth muscle tone [36] to the modulation of hormone and neurotransmitter release [37]. Interestingly, BK channels are also involved in modulating K^+ transport in the mammalian kidney [38, 39], pulmonary epithelium [40] and colon epithelium [41]. These channels have been characterized in different species, from *Drosophila* to humans, nonetheless detailed studies about their properties and functions in teleosts fish are lacking, with the exception of earlier work from Rohmann and colleagues, which identified BK currents as one of the major outward currents in teleost fish hair cells [23]. The genomic organization of *slo-1* has only recently been reported in the zebrafish (*Danio rerio*) [42]. BK channel transcripts have also been detected in the intestinal epithelium of the European eel (*Anguilla Anguilla*) [43], in the nervous system of different teleost fish and in gills of the midshipman fish (*Porichthys notatus*) [23] and the Mozambique tilapia (*Oreochromis mossambicus*) [22].

3. Coda

The smoltification process is the consequence of an adaptive response of the fish in order to survive the change of its environment, from freshwater to seawater. This tightly regulated process encompasses from huge morphological to subtle physiological changes in the fish. Within this changes, one of the most important is the change in the expression pattern of different set of membrane proteins and ion channels in specialized cells in the gills, called MR cells. The different biophysical properties of the channels mentioned above, help the fish to achieve the osmoregulation in different osmotic conditions (freshwater and seawater). Understanding the functioning of these channels is key to understand the smoltification process in salmonids, which is important for different productive processes, depending on the smoltification, like fish farming.

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Review

Voltage-dependent BK and Hv1 channels expressed in non-excitable tissues: New therapeutics opportunities as targets in human diseases

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ABSTRACT

Voltage-gated ion channels are the molecular determinants of cellular excitability. This group of ion channels is one of the most important pharmacological targets in excitable tissues such as nervous system, cardiac and skeletal muscle. Moreover, voltage-gated ion channels are expressed in non-excitable cells, where they mediate key cellular functions through intracellular biochemical mechanisms rather than rapid electrical signaling. This review aims at illustrating the pharmacological impact of these ion channels, highlighting in particular the structural details and physiological functions of two of them – the high conductance voltage- and Ca^{2+}-gated K^+ (BK) channels and voltage-gated proton (H_v1) channels- in non-excitable cells.

BK channels have been implicated in a variety of physiological processes ranging from regulation of smooth muscle tone to modulation of hormone and neurotransmitter release. Interestingly, BK channels are also involved in modulating K^+ transport in the mammalian kidney and colon epithelium with a potential role in the hyperkalemic phenotype observed in patients with familial hyperkalemic hypertension type 2, and in the pathophysiology of hypertension. In addition, BK channels are responsible for resting and stimulated Ca^{2+}-activated K^+ secretion in the distal colon.

H_v1 channels have been detected in many cell types, including macrophages, blood cells, lung epithelia, skeletal muscle and microglia. These channels have a central role in the phagocytic system. In macrophages, H_v1 channels participate in the generation of reactive oxygen species in the respiratory burst during the process of phagocytosis.

Contents

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Table 2
Examples of drugs acting on BK and Hv channels and their potential pharmacological use for treatments of diseases.

Channel	Drugs	Effect on channel	Disease	Physiological effect	Ref.
BK channels	Paxilline	Inhibitor	Epilepsy	Reduces seizure duration and severity	[116–118]
	NS1619 and isopimaric acid	Activators	Tinnitus	Reduce hyperactivity of spontaneously active auditory networks	[79]
	BMS-204352/compound 1 and *Bulfuzi* -cerebrosides	Activators	Acute ischemic stroke	Cortical neuroprotection by suppressing excess excitability, reducing neurotransmitter release, energy expenditure and attenuating infarct growth.	[84,86]
	NS-8 and Glo5lo-5R family[a]	Activators	Overactive bladder	Suppression of the micturition reflex by decrease of afferent pelvic nerve activity. Inhibition of spontaneous contraction of bladder smooth muscle.	[88,91]
	Isopropyl unoprostone[a]	Activator	Retinitis pigmentosa Glaucoma and Intraocular Pressure	Improvement of retinal sensitivity by opening the BK channels, Increasing of outflow facility through the trabecular meshwork	[92]
	Andolast*	Activator	Asthma	Decreases cell excitability and promotes smooth muscle relaxation.	[93]
	Emodepside	Activator	Anthelmintic	Suppression of neuronal and muscle activity	[95]
Hv channels	2GBI (Guanidine derivative)	Inhibitor	Breast cancer cells Ischemic stroke	Reduce cell proliferation and invasiveness Protective effect from brain damage after stroke	[99]

The drugs without marks are either discontinued or in pre-clinical phase.
[a] Currently in Phase III clinical.

However there are not many BK channel modulators in the market as a therapeutic treatment [88]. Only unoprostone isopropyl (unoprostone), a prostanoid and synthetic docosanoid approved for the treatment of open-angle glaucoma and ocular hypertension [89]. In fact, in 2012 it was shown that this BK activator delayed the decrease in the central retinal sensitivity in patients with Retinitis Pigmentosa in a Phase 2Clinical Study [75]. A few others activators have advanced into clinical trials. BMS-204532 was tested in the treatment of stroke but it failed to show efficacy when compared to placebo in a Phase III study [90]. Another BK opener to enter to clinical trials was NS-8 for the treatment of overactive bladder but it lacked of efficacy at a therapeutic dose [91]. Today, Andolast for the treatment of asthma is the only BK opener currently in clinical trials [93]. Finally, other studies have found BK channel to be incidentally involved in the effects of sildenafil in patients with Class II and III pulmonary arterial hypertension [45] and the benefit of sildenafil in this kind of patients has been validated [94].

A last example of the broad pharmacological possibilities of BK channels as target, it is emodepside, a broad spectrum anthelmintic. Its unique mode of action suggests that it acts to inhibit neuronal and muscle activity of nematodes by increasing the opening of BK channels [95]. Also, there is evidence of direct action of emodepside on the BK channel by rescue experiments [95,96].

In the case of Hv1 channels possible uses of inhibitors have been envisaged in different pathological conditions, ranging from diseases with a strong activation of the phagocyte NADPH oxidase (e.g., stroke) to infertility, osteoporosis, and cancer [97]. Recently, it has been shown that some guanidine derivatives block H_v1 channels by accessing to the core of the VSD from the intracellular side when the channels are in an open conformation [98,99].

The quest for drugs targeting BK or Hv channels is still ongoing; in perspective this is an important endeavor to provide additional therapeutic alternatives for several human pathologies

Conflict of interest

None

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Article

Zebrafish $Ca_V2.1$ Calcium Channels Are Tailored for Fast Synchronous Neuromuscular Transmission

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ABSTRACT The $Ca_V2.2$ (N-type) and $Ca_V2.1$ (P/Q-type) voltage-dependent calcium channels are prevalent throughout the nervous system where they mediate synaptic transmission, but the basis for the selective presence at individual synapses still remains an open question. The $Ca_V2.1$ channels have been proposed to respond more effectively to brief action potentials (APs), an idea supported by computational modeling. However, the side-by-side comparison of $Ca_V2.1$ and $Ca_V2.2$ kinetics in intact neurons failed to reveal differences. As an alternative means for direct functional comparison we expressed zebrafish $Ca_V2.1$ and $Ca_V2.2$ α-subunits, along with their accessory subunits, in HEK293 cells. HEK cells lack calcium currents, thereby circumventing the need for pharmacological inhibition of mixed calcium channel isoforms present in neurons. HEK cells also have a simplified morphology compared to neurons, which improves voltage control. Our measurements revealed faster kinetics and shallower voltage-dependence of activation and deactivation for $Ca_V2.1$. Additionally, recordings of calcium current in response to a command waveform based on the motorneuron AP show, directly, more effective activation of $Ca_V2.1$. Analysis of calcium currents associated with the AP waveform indicate an approximately fourfold greater open probability (P_O) for $Ca_V2.1$. The efficient activation of $Ca_V2.1$ channels during APs may contribute to the highly reliable transmission at zebrafish neuromuscular junctions.

INTRODUCTION

At central synapses, the $Ca_V2.1$ and $Ca_V2.2$ calcium channels commonly co-mediate neurotransmission, but their contributions to release vary greatly, particularly among GABAergic and glutamatergic synapses (1). Evidence continues to accumulate in support of the idea that they play distinctly different roles in synaptic transmission.

For example, cholecystokinin-expressing interneurons in the rat dentate gyrus release GABA (γ-aminobutyric acid) in a highly asynchronous manner when compared to the parvalbumin interneurons. Cholecystokinin neurons rely principally on $Ca_V2.2$ whereas parvalbumin neurons utilize $Ca_V2.1$ (2), leading to the proposal that $Ca_V2.2$ may play a greater role in asynchronous release.

Weaker coupling to fast release on the part of the $Ca_V2.2$ is also reflected in the channel's delayed recruitment, which was associated with action-potential (AP) broadening during repetitive stimulation (3). The stronger coupling of the $Ca_V2.1$ channel to release has been ascribed to a closer proximity to the release site (4), which might be expected to promote exocytosis by providing either greater access to calcium (2,5) or through physical interaction with the exocytotic machinery (6–10).

One more idea, however, arose from modeling studies, suggesting that functional advantages offered by $Ca_V2.1$ facilitated high-frequency signaling (3). As a test of this idea, we sought to compare the function of heterologously expressed zebrafish $Ca_V2.2$ and $Ca_V2.1$ isoforms under conditions of high-quality voltage-clamp wherein pharmacological inhibition of additional isoforms is not required. This avoids potential contribution by additional isoforms due to incomplete inhibition.

Finally, we also capitalized on the ability to use the AP waveform recorded from the zebrafish primary motor neuron in vivo, which mediates fast synchronous neuromuscular transmission solely through $Ca_V2.1$ calcium channels (11).

MATERIALS AND METHODS

Human embryonic kidney cells (HEK293T) were transfected (Lipofectamine 2000; Invitrogen, Carlsbad, CA) with an equal molar cDNA ratio coding for the EGFP-tagged zebrafish α-subunit, rat $\alpha_2\delta_1$ subunit (addgene accession:AF286488; Addgene, https://www.addgene.org/), and zebrafish β_{1b} subunit (11). Within 24–48 h posttransfection the cells were mechanically lifted, replated, and given 1 h to reattach. Calcium currents were recorded in the whole-cell configuration with an EPC-9 amplifier under PATCHMASTER software control (HEKA Instruments, Darmstadt, Germany). Patch electrodes with resistances of 3–5 MΩ contained 115 mM Cs-methanesulfonate, 15 mM CsCl, 5 mM BAPTA, 4 mM Mg-ATP, and 10 mM Cs-HEPES, pH 7.2. The bath solution contained 134 mM NaCl, 2.9 mM KCl, 1.2 mM $MgCl_2$, 2.1 mM $CaCl_2$, 10 mM Glucose, and 10 mM Na-HEPES, pH 7.8. Whole-cell currents were sampled at 100 kHz except for the AP waveform that that was sampled at 50 kHz.

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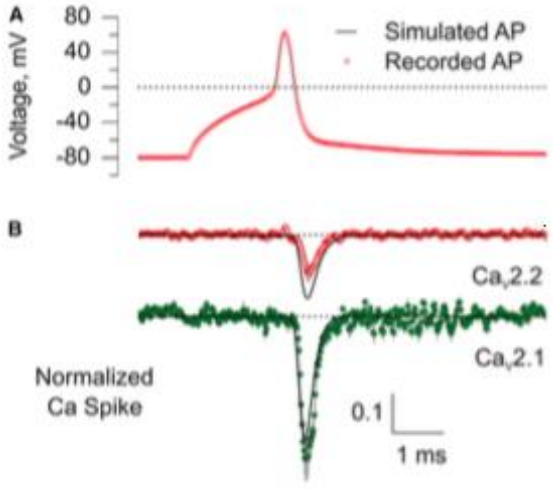

FIGURE 4 Calcium currents for both channel types in response to the motor neuron AP waveform. (*A*) Comparison of a recorded AP waveform from a CaP motor neuron in vivo (*red open circles*) with the one used to simulate calcium currents with Scheme 1 (*solid line*). The simulated AP waveform was constructed from 27 voltage ramps concatenated to match the recorded waveform. (*Dashed line*) 0 mV. (*B*) Experimentally measured average $Ca_V2.2$ ($n = 8$) and $Ca_V2.1$ ($n = 7$) calcium currents elicited in response to a neuronal AP. To obtain average values, each recording was normalized to the total available calcium current in that cell by using the peak tail current during a step from +70 mV to −80 mV. (*Solid lines*) Predicted calcium currents with the GHK permeation regime in response to the simulated AP based on Markov chain simulations using the software IonChannelLab (12). Parameters were based on Scheme 1 and extra- and intracellular Ca^{2+} concentrations were set to 2 mM and 4 μM, respectively, to yield a calcium reversal potential of +80 mV. (*Calibration bar*) 0.1 normalized calcium current; (*dashed traces*) 0 current level.

tb204a mutant line (11). Our assignment of zebrafish β4 was based on published expression patterns in zebrafish larvae. Seven genes have been identified in the zebrafish genome encoding voltage-dependent calcium channel β-subunits with distinct expression patterns during development (21). We chose to express β4 together with other calcium channel subunits in this study because it has been shown to express in spinal cord in a segmental fashion, consistent with the location of primary motor neurons. Both α- and β-cDNAs used in the study represent the most abundant splice variants in larval fish. Coexpression with $\alpha_2\delta_1$ subunits was based on the requirement for functional expression and their known role in stabilizing the core complex (22). While HEK cells do not express calcium current, it remains formally possible that they contribute some unidentified functional component to the expressed channel because this cell type does express a few neuron-specific genes (23). In light of the uncertainty as to molecular composition at mammalian nerve terminals, difference in composition between the two preparations remains a viable candidate.

Second, the differences between studies could reflect incomplete voltage control and limited speed provided by conventional patch-clamp recordings that vary with experimental context. The functional distinctions that we identified between channel types expressed in HEK cells placed the highest demands on voltage control. The functional distinctions reported for morphologically simplified HEK cells may be even further obscured in morphologically complex nerve terminals owing to compromised voltage control.

Third, the differences could reflect the evolutionary distance between fish and mammals or alternatively a difference in $Ca_V2.1$ function in the peripheral nervous system compared to the central nervous system. In sum, the answer to this question will have to await further comparative analyses.

CONCLUSIONS

Both mossy fiber and zebrafish studies support the prediction that $Ca_V2.1$ channels open more effectively during brief APs than the $Ca_V2.2$ counterpart. Our findings, as well as with those from mossy fiber terminals, show that prolongation of the AP reduces the difference in P_O for the two isoforms. This further points to a specific role of $Ca_V2.1$ in fast neuronal firing (3). This functional bias toward speed for $Ca_V2.1$ is in line with studies showing that $Ca_V2.2$ underlies slow release in chromaffin cells and asynchronous release in certain central neurons (1,2,24). The slow release process depends on calcium accumulation that is associated with repetitive firing (3,25).

$Ca_V2.1$ and $Ca_V2.2$ are the substrates for different modulation resulting in altered AP waveforms and associated changes in calcium entry (26,27). In addition to functional distinctions, there likely exist additional important differences between calcium channel isoforms, such as sensitivity to modulatory agents and physical coupling to release machinery for specific isoforms (6,27–29). Overall, evidence is mounting in support of very different functional roles played by these two channel types, perhaps answering, in part, the question as to why they are coexpressed at so many synapses.

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Chapter 10
Mathematical Modeling of TRPM8 and the Cold Thermoreceptors

Erick Olivares and Patricio Orio

Abstract The role of TRPM8 channel in thermotransduction involves several aspects of complexity that make it difficult to understand intuitively. First, it is activated by several stimuli (cold, voltage, agonists and intracellular signaling) that interact with each other, raising the question of how these interactions occur. Experimental evidence in this type of polymodal channel may be misinterpreted if the consequences of a working hypothesis are not considered carefully. Second, in parallel with the identification of TRPM8 as the main molecular transducer of cold temperatures in cold thermoreceptors of the somatosensory system, a list of other ion channels have been shown to be involved in the activity of cold-sensitive neurons and nerve endings. The variety of firing patterns observed at cold sensitive nerve endings arises from a complex interaction of ion channels that operate on different time scales. Mathematical modeling has been instrumental in understanding these phenomena, showing the consequences of the hypotheses raised. Here we review some of the models that have been proposed in these two areas: the activation of TRPM8 and TRPV1 by voltage and temperature, and the generation of firing patterns of cold thermoreceptors. We finish this chapter with a mathematical model showing how the calcium-dependent adaptation of TRPM8 may account for the response of cold thermoreceptors to rapid changes in temperature.

Keywords Mathematical modeling · Cold thermoreceptors · TRPM8

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10.5 Concluding Remarks

Our model shows that the only element necessary to display a dynamic response in a model of cold thermoreceptor is a cold-activated channel (resembling TRPM8) with a slow activity-dependent adaptation. Moreover, and in tune with the discussion presented in the first part of this chapter, a simple two-state model of TRPM8 does the job, as all that is needed for this purpose is a phenomenological representation of the channel behavior within the physiological ranges of temperature and voltage.

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RESEARCH ARTICLE

TRPM8-Dependent Dynamic Response in a Mathematical Model of Cold Thermoreceptor

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Abstract

Cold-sensitive nerve terminals (CSNTs) encode steady temperatures with regular, rhythmic temperature-dependent firing patterns that range from irregular tonic firing to regular bursting (static response). During abrupt temperature changes, CSNTs show a dynamic response, transiently increasing their firing frequency as temperature decreases and silencing when the temperature increases (dynamic response). To date, mathematical models that simulate the static response are based on two depolarizing/repolarizing pairs of membrane ionic conductance (slow and fast kinetics). However, these models fail to reproduce the dynamic response of CSNTs to rapid changes in temperature and notoriously they lack a specific cold-activated conductance such as the TRPM8 channel. We developed a model that includes TRPM8 as a temperature-dependent conductance with a calcium-dependent desensitization. We show by computer simulations that it appropriately reproduces the dynamic response of CSNTs from mouse cornea, while preserving their static response behavior. In this model, the TRPM8 conductance is essential to display a dynamic response. In agreement with experimental results, TRPM8 is also needed for the ongoing activity in the absence of stimulus (i.e. neutral skin temperature). Free parameters of the model were adjusted by an evolutionary optimization algorithm, allowing us to find different solutions. We present a family of possible parameters that reproduce the behavior of CSNTs under different temperature protocols. The detection of temperature gradients is associated to a homeostatic mechanism supported by the calcium-dependent desensitization.

Introduction

In mammals, cold is detected by specific cutaneous thermoreceptor neurons of the somatosensory system. The transduction of cold stimuli into electrical impulses takes place in the free endings of the thermoreceptor fibers, corresponding to the axonal endings of cold-sensitive neurons from trigeminal and dorsal root ganglion [1,2].

Cold thermoreceptors frequently have spontaneous firing of action potentials at resting skin temperature (33–34°C). Their response to temperature displays two essential features: The

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ORIGINAL RESEARCH
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Restraint stress increases hemichannel activity in hippocampal glial cells and neurons

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Stress affects brain areas involved in learning and emotional responses, which may contribute in the development of cognitive deficits associated with major depression. These effects have been linked to glial cell activation, glutamate release and changes in neuronal plasticity and survival including atrophy of hippocampal apical dendrites, loss of synapses and neuronal death. Under neuro-inflammatory conditions, we recently unveiled a sequential activation of glial cells that release ATP and glutamate via hemichannels inducing neuronal death due to activation of neuronal NMDA/P2X$_7$ receptors and pannexin1 hemichannels. In the present work, we studied if stress-induced glia activation is associated to changes in hemichannel activity. To this end, we compared hemichannel activity of brain cells after acute or chronic restraint stress in mice. Dye uptake experiments in hippocampal slices revealed that acute stress induces opening of both Cx43 and Panx1 hemichannels in astrocytes, which were further increased by chronic stress; whereas enhanced Panx1 hemichannel activity was detected in microglia and neurons after acute/chronic and chronic stress, respectively. Moreover, inhibition of NMDA/P2X$_7$ receptors reduced the chronic stress-induced hemichannel opening, whereas blockade of Cx43 and Panx1 hemichannels fully reduced ATP and glutamate release in hippocampal slices from stressed mice. Thus, we propose that gliotransmitter release through hemichannels may participate in the pathogenesis of stress-associated psychiatric disorders and possibly depression.

Keywords: hemichannels, connexins, pannexins, stress, hippocampus, glia, neuron

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Introduction

Major depression disorder (MDD) is a disabling illness that adversely affects subject's family, behavior, mood, activity and physical health. In developed countries, around 3% of MDD patients commit suicide, whereas several studies show that around 60% of all suicide victims had previously suffered from MDD (Arsenault-Lapierre et al., 2004). Interestingly, ample evidence indicates that stressful life events increase the risk for MDD, including acute and chronic stress (Kessler, 1997; Kendler, 1998; Hammen, 2005; Hammen et al., 2009). The term stress defines all physiological and/or psychological responses to events that

to unveil the exact mechanisms by which chronic stress affects hemichannels in glia and neurons and what the contribution of GCs on this process really is.

Although our working model does not recapitulate the mechanisms underlying the brain abnormalities induced by major depression and stress-associated psychiatric disorders, it allows us to dissect the specific contribution of hemichannels expressed by individual brain cell types. It must be noted that both chronic restraint stress and chronic GC administration are effective models for obtaining depressive-like symptoms in rodents (Levinstein and Samuels, 2014). In consequence, it is possible that hemichannel activation induced by chronic restraint stress may also contribute to the pathogenesis of depressive-like symptoms. Therefore, these findings may shed light into the early phases of neuronal dysfunction associated to stress, which may lead to major depression, post-traumatic stress disorder and other anxiety disorders. Our findings brings new vistas on the role of gliotransmitters on chronic stress and how hemichannels could arise as possible targets for developing novel pharmacological strategies to ameliorate different mental disorders associated to stress, anxiety and depression.

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Molecular Determinants of Phosphatidylinositol 4,5-Bisphosphate ($PI(4,5)P_2$) Binding to Transient Receptor Potential V1 (TRPV1) Channels*

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Background: The mode of action of $PI(4,5)P_2$ in TRPV1 is controversial.
Results: Positively charged amino acids in the S4-S5 linker and in the TRP box form the $PI(4,5)P_2$ binding site.
Conclusion: $PI(4,5)P_2$ is a TRPV1 agonist and induces a conformational change of the internal gate.
Significance: The molecular nature of the $PI(4,5)P_2$ binding site in TRPV1 is defined.

Phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) has been recognized as an important activator of certain transient receptor potential (TRP) channels. More specifically, TRPV1 is a pain receptor activated by a wide range of stimuli. However, whether or not $PI(4,5)P_2$ is a TRPV1 agonist remains open to debate. Utilizing a combined approach of mutagenesis and molecular modeling, we identified a $PI(4,5)P_2$ binding site located between the TRP box and the S4-S5 linker. At this site, $PI(4,5)P_2$ interacts with the amino acid residues Arg-575 and Arg-579 in the S4-S5 linker and with Lys-694 in the TRP box. We confirmed that $PI(4,5)P_2$ behaves as a channel agonist and found that Arg-575, Arg-579, and Lys-694 mutations to alanine reduce $PI(4,5)P_2$ binding affinity. Additionally, *in silico* mutations R575A, R579A, and K694A showed that the reduction in binding affinity results from the delocalization of $PI(4,5)P_2$ in the binding pocket. Molecular dynamics simulations indicate that $PI(4,5)P_2$ binding induces conformational rearrangements of the structure formed by S6 and the TRP domain, which cause an opening of the lower TRPV1 channel gate.

Of the thermo-TRP channels known to date, the transient receptor potential vanilloid 1 (TRPV1),[4] which was the first to be cloned, is activated by temperature increases in the noxious range and by capsaicin (CAP), which is the pungent ingredient of chili peppers (1, 2). TRPV1 channels, which are found in a subset of neurons in the dorsal root and trigeminal ganglia, have been implicated in thermal nociception, specifically in inflammation-induced thermal hyperalgesia (3–5). TRPV1 is a non-selective cation channel that works as a polymodal signal integrator of chemical and physical stimuli, such as activation by CAP, anandamide, heat, and protons. At the structural level, TRPV1 is a homotetramer, where each subunit has six transmembrane segments (*i.e.* S1-S6) with the N and C termini located in the intracellular region (6, 7). Recently, a three-dimensional structure of the TRPV1 channel has been determined at a resolution of 3.3 Å by cryo-electron microscopy. Moreover, the structure of TRPV1 in complex with two potent agonists, namely resiniferatoxin (RTX; Ref. 1) and the spider double-knot toxin (Ref. 8; DkTx), as well as TRPV1 in complex with CAP were determined at resolutions of 3.8 and 4.2 Å, respectively (9, 10).

With regard to phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), while it is agreed that the C terminus contains a $PI(4,5)P_2$ binding site (9, 11–13), there is still debate as to whether $PI(4,5)P_2$ behaves as an activator (13, 14) or as a TRPV1 channel antagonist, as recently proposed by (15). Regardless of whether $PI(4,5)P_2$ activates TRPV1 or exerts an inhibitory effect, it may be safe to assume that the negatively charged headgroups of $PI(4,5)P_2$ should be in contact with positively charged regions of the TRPV1 channel. A molecular simulation showed that a $PI(4,5)P_2$ headgroup made contact with positively charged res-

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[4] The abbreviations used are: TRPV, transient receptor potential vanilloid; $PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; CAP, capsaicin; MD, molecular dynamics; PME, particle-mesh Ewald.

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Habituation of auditory steady state responses evoked by amplitude-modulated acoustic signals in rats

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Abstract

Generation of the auditory steady state responses (ASSR) is commonly explained by the linear combination of random background noise activity and the stationary response. Based on this model, the decrease of amplitude that occurs over the sequential averaging of epochs of the raw data has been exclusively linked to the cancelation of noise. Nevertheless, this behavior might also reflect the non-stationary response of the ASSR generators. We tested this hypothesis by characterizing the ASSR time course in rats with different auditory maturational stages. ASSR were evoked by 8-kHz tones of different supra-threshold intensities, modulated in amplitude at 115 Hz. Results show that the ASSR amplitude habituated to the sustained stimulation and that dishabituation occurred when deviant stimuli were presented. ASSR habituation increased as animals became adults, suggesting that the ability to filter acoustic stimuli with no-relevant temporal information increased with age. Results are discussed in terms of the current model of the ASSR generation and analysis procedures. They might have implications for audiometric tests designed to assess hearing in subjects who cannot provide reliable results in the psychophysical trials.

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Key words: auditory temporal processing, evoked potential, habituation, stationary response, rats.

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Introduction

Auditory steady state responses (ASSR) are periodic electrical brain oscillations evoked by acoustic stimuli sinusoidally modulated in amplitude-and/or frequency.[1,2] Traditionally, the extraction of these responses from the measured signal rely on averaging across stimulus-locked epochs of the raw data. Although successful, such a manipulation assumes that electrophysiological signals represent a linear superposition between the random ongoing background and the highly stereotyped and repeatable auditory response.

In practice, the amplitude of the ASSR progressively decreases over the sequential averaging of epochs.[3,4] This phenomenon has been associated with relatively high contribution of the un-averaged noise to the measurements in the first epochs of the recording, which is gradually attenuated as the electrical signal is averaged in the time-domain.[1,3,5-9]

Alternatively to the noise cancelation hypothesis, the time-dependent decrease of the ASSR amplitude might represent the non-stationary response of the ASSR generators. Specifically, it could be a consequence of the progressive reduction of the synchronic response of the ASSR neural generators following the high presentation rate of acoustic stimuli. This phenomenon can be referred as habituation of the ASSR.

Habituation of the ASSR might have implications for the clinical practice. So far, methodologies for optimizing the detection of ASSR have included the use of different types of stimulus and averaging procedures.[3,10,11] In addition, the use of multichannel electroencephalogram recordings and appropriate statistical tests for different samples of stimulus-related epochs has been evaluated.[12-14] Recently, attention has been focused on other factors affecting the detection of the ASSR such as the optimum recording length and the stopping criteria of the recordings.[4,8,15] The habituation of the response might be then a

Critical Review

Carbon Monoxide: A New Player in the Redox Regulation of Connexin Hemichannels

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Abstract

Carbon monoxide (CO) is a gaseous transmitter that is known to be involved in several physiological processes, but surprisingly it is also becoming a promising molecule to treat several pathologies including stroke and cancer. CO can cross the plasma membrane and activate guanylate cyclase, increasing the cGMP concentration and activating some kinases, including PKG. The other mechanism of action involves induction of protein carbonylation. CO is known to directly and indirectly modulate the function of ion channels at the plasma membrane, which in turn have important repercussions in the cellular behavior. One group of these channels is hemichannels, which are formed by proteins known as connexins (Cxs). Hemichannel allows not only the flow of ions through their pore but also the release of molecules such as ATP and glutamate. Therefore, their modulation not only impacts cellular function but also cellular communication, having the capability to affect tissular behavior. Here, we review the most recent results regarding the effect of CO on Cx hemichannels and their possible repercussions on pathologies. © 2015 IUBMB Life, 67(6):428–437, 2015

Keywords: *connexins; hemichannels; redox potential; gap junction channels; post-translational modification; gaseous transmitters*

Introduction

Connexins (Cxs) are a family of proteins that share a common plasma membrane topology: four transmembrane domains, two extracellular loops, one intracellular loop, and both the C- and N-termini located on the cytoplasmic side (Fig. 1A). At least 20 isoforms have been described in mammals (1), which are named according to their predicted molecular weight (*i.e.*, Cx46 is predicted to have a MW of 46 kDa). Cx isoforms exhibit considerable homology; however, the C-terminus is the most variable region, which in addition varies in length between isoforms. Thus, Cx23 presents a very short C-terminus when compared with Cx62, which has the longest one. Moreover, the C-terminus contains a number of regulatory sites, including consensus phosphorylation (2–5), oxidation (6–9), protein–protein interaction (10–12), and cleavage sites (13,14). Similar to the C-terminus of Cx channel, some post-translational modifications have been reported in the N-terminus and the intracellular loop (15). These modifications include ubiquitination (16), SUMOylation (17), acetylation (18), and hydroxylation (19). It is worth mentioning that the N-terminus is projected into the channel pore, which means that it forms part of the channel pore (only probed for Cx26; ref.

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interesting because extracellular Ca^{2+} modulates the voltage dependence of loop gating (39,40). Therefore, CO could be somehow modulating this so-called slow gating or loop gating (41) through modifications in the mobility of some segments of the extracellular Cxs loops. In summary, CO inhibits Cx hemichannels in *Xenopus laevis* oocytes in a wide range of concentrations (1–100 μM); however, the effect in mammalian cells is more complex, because it presents a dual response depending on CO concentration. The CO effect can be reverted by a reducing agent–dependent process; however, the molecular mechanism is still unknown. The CO effect seems to influence the loop gating of hemichannels; however, much more evidence is needed to test this hypothesis. Finally, experiments to verify if the inhibitory effect observed in HeLa cells is reverted and if CO affects GJC expressed in *Xenopus oocytes* and HeLa cells are needed.

Possible Interplay Between CO and NO in Cx Hemichannel Activity

CO and NO are two gaseous transmitters that activate similar intracellular pathways. Thus, it is well known that both CO and NO stimulate soluble guanylyl cyclase to produce cGMP, which in turn activates PKG (133). Therefore, it is possible to suggest that these two gases can at some point interact and modulate their cellular effect. Accordingly, it has been shown that both CO and NO act as a safety mechanism in renal afferent arteriolar vasoconstriction regulation. Thus, in the presence of NO, CO does not induce evident changes in arterial diameter, but when NO production is inhibited, CO is able to induce vasodilatation (133). In this case, CO and NO are performing similar effects in renal arteries. However, this is not always the case. For example, when iNOS is activated, an increase of NO occurs, which in turn increases cell expression of HO-1 with the consequent production of CO (134). An increase of CO will have a negative effect on iNOS activity, thus decreasing the levels of NO (134). In general terms, an increase of CO concentration has been associated to protective cellular effects (135,136), whereas increases in NO concentration have been associated to deleterious effects (137). According to this, the addition of NO donors to astrocytes in culture induces a massive Cx43 hemichannel opening (6); however, when a CO donor is added to HeLa cells, Cx43 hemichannel became closed (24). However, it is unknown whether astrocytes exposed to CO donors also close their Cx43 hemichannels, and whether the effect of NO and CO over Cx43 hemichannels is synergic or antagonic is also unknown. The final effect of these two gases *in vivo* could also be affected by the intracellular distribution of enzymes that produce NO and CO, thus it is known that HO-1 isoform is located mainly in the endoplasmic reticulum (138) and nucleus (139), whereas HO-2 is mainly located in the endosomes (140). On the other hand, the endothelial nitric oxide synthase (eNOS) is mainly located at the plasma membrane and Golgi apparatus (141), the neuronal type (nNOS) is mainly in the cytoplasm (142), and the inducible form is located mainly in the cytoplasm (143). The differences in localization of enzymes that produce NO and CO will certainly have a differential impact in Cx regulation.

Another type of interaction between NO and CO could occur at the molecular level. NO interacts with Cys groups inducing protein S-nitrosylation (144), whereas CO can induce secondary carbonylation in Cys as well (145). Thus, both can compete for Cys groups and exert their modulation in a competitive way. Obviously, this competition for Cys groups will be affected by the concentration and localization of HO and NOS enzymes. Coimmunoprecipitation and high-resolution confocal studies are needed to understand the interactions of these enzymes and Cx hemichannels and thus understand the overall effect of these two gaseous transmitters in the intercellular communication based on Cxs.

Future Directions

It is known that CO is neuroprotective in cerebral ischemia (146,147); however, the molecular mechanisms are not well understood. Hemichannels are massively open in ischemia/metabolic inhibition conditions as observed in astrocytes (6,60) and neurons (148), and this in turn affects neuronal viability (149). We propose that CO could induce Cx36 and/or Cx43 hemichannel closing and, thus, prevent cell death. Additionally, pannexin channels (Panx), which also form channels at the plasma membrane with similar characteristic as Cx hemichannels (150), are involved in neuronal death in ischemia episodes (151,152). It would be interesting to study if Panx are also affected by CO. Another example of the use of CO as treatment for a pathological condition would be for cancer (153,154). Recently, it has been proposed that Cx hemichannels have a role in cancer progression (155), where these channels could increase the P2X/Y signaling that in turn would affect the intracellular Ca^{2+} concentration (155), which is a powerful signaling in cancer cells (156). Therefore, it is plausible to speculate that CO may affect hemichannels in cancer cells and thus modulate intracellular calcium levels.

There are many other human pathologies where CO is used for their treatment (157), and in which hemichannels could be involved. Thus, the study on the effect of CO on Cx-based hemichannels, GJCs, and Panx will help to understand the underlying molecular mechanism of action involving CO in pathological as well as in physiological conditions.

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frontiers in Cellular Neuroscience

REVIEW
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Diseases associated with leaky hemichannels

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Hemichannels (HCs) and gap junction channels (GJCs) formed by protein subunits called connexins (Cxs) are major pathways for intercellular communication. While HCs connect the intracellular compartment with the extracellular milieu, GJCs allow the interchange of molecules between cytoplasm of two contacting cells. Under physiological conditions, HCs are mostly closed, but they can open under certain stimuli allowing the release of autocrine and paracrine molecules. Moreover, some pathological conditions, like ischemia or other inflammation conditions, significantly increase HCs activity. In addition, some mutations in Cx genes associated with human diseases, such as deafness or cataracts, lead to the formation of more active HCs or "leaky HCs." In this article we will revise cellular and molecular mechanisms underlying the appearance of leaky HCs, and the consequences of their expression in different cellular systems and animal models, in seeking a common pattern or pathological mechanism of disease.

Keywords: connexins, leaky hemichannels, mutations, gap junction channels, cell death, disease

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Introduction

Connexins (Cxs) are a family of transmembrane (TM) proteins formed by 21 members (Eiberger et al., 2001; Söhl and Willecke, 2004) named according to their predicted molecular weight (i.e., Cx43 has ~43 kDa). Cxs are expressed in almost every cell type in the human body (Bruzzone et al., 1996). However, there are some differences. Thus, for example, there are Cxs widely expressed such as Cx43, which is found in the brain, kidneys, heart and reproductive organs, among others (Beyer et al., 1987, 1989; Sáez et al., 2003), or restricted to myelin-forming glial cells, as in the case of Cx29 (Söhl et al., 2001). Cxs form two types of channels; hemichannels (HCs) and gap junction channels (GJCs). HCs are formed by the oligomerization of six Cxs monomers and travel in vesicles to the plasma membrane (Vinken et al., 2006). The Cx topology in cell membrane is depicted in **Figure 1** and includes four TM segments (TM1-4), which are connected through two extracellular loops (EL1-EL2) and one intracellular loop (IL); and the N-terminal (NT) and C-terminal (CT) segments oriented to the cytosol (Kumar and Gilula, 1996). HCs can form GJC in the appositional membranes of contacting cells or stay as "free" HCs anywhere on the plasma membrane (**Figure 2**). Free HCs are mostly closed under physiological conditions (Contreras et al., 2003), that is because they have low open probability (OP) due to one or more of the following mechanisms: (i) a blockage by extracellular Ca^{2+} and Mg^{2+} in the mM range, (ii) a negative membrane potential that closes most Cx HCs and (iii) posttranslational modification (i.e., phosphorylation) of some Cxs (Contreras et al., 2003; Gómez-Hernández et al., 2003; Johnstone et al., 2012). However, HCs can open under physiological conditions allowing communication between extracellular and intracellular space (Sáez et al., 2010). On the other

Cx43, which have been correlated with neuronal malfunctioning and death (Orellana et al., 2012). When an ischemic episode occurs, astrocytes open their Cx43 HCs (Contreras et al., 2002; Retamal et al., 2006), probably due to dephosphorylation and S-nitrosylation of Cx43 (Retamal et al., 2006). The previous conditions induce a massive opening of astrocyte Cx43 HCs allowing the release of high amounts of ATP and glutamate from astrocytes (Orellana et al., 2011a; Li et al., 2015). This increment in extracellular ATP and glutamate concentration could induce the opening of neuronal Pannexin1 channels, perturbing neuron homeostasis causing cell death (Orellana et al., 2011a). Consistently, administration of Cx43 mimetic peptides, to block HCs, improved brain recovery after ischemia in fetal sheep (Davidson et al., 2012) and neonatal rats (Li et al., 2015).

Hyperactive HCs may also be involved in other brain diseases. Lysosomal storage diseases (LSDs) encompass a large group of inherited metabolic disorders characterized by the accumulation of storage material within lysosomes and HCs seems to have a relevant role in the progression of these diseases (Bosch and Kielian, 2014). In this line, an enhanced Cx43 HC activity was observed in astrocytes from a mouse model of LSD ($CLN3^{\Delta ex7/8}$; Finn et al., 2011; Burkovetskaya et al., 2014) which could importantly contribute to neuronal deterioration as mentioned above. On the other hand, opening of HCs could also contribute to brain deterioration in Alzheimer's disease. Orellana et al. (2011b) reported that Aβ peptide induces massive HC opening in astrocytes, microglia, and neurons, either in culture and in hippocampal slices (Orellana et al., 2011b). This increase of HC activity is correlated with augmented release of neuroactive molecules, such as glutamate and ATP, with induction of cellular death (Orellana et al., 2011b; Bosch and Kielian, 2014). Accordingly, blockage of HCs improved memory impairment in a mouse model of Alzheimer's disease (Takeuchi et al., 2011). Other neurodegenerative diseases in which HC have been involved are: HIV encephalitis (Eugenin and Berman, 2013; Orellana et al., 2014), amyotrophic lateral sclerosis (Boillee et al., 2006; Yamanaka et al., 2008; Takeuchi et al., 2011), Parkinson's disease (Rufer et al., 1996; Kawasaki et al., 2009), Rasmussen encephalitis (Cepeda et al., 2015) and epilepsy (Mylvaganam et al., 2014). A common milestone of these diseases is the inflammation condition, where cytokines and reactive oxygen species (ROS) can activate HCs in glial cells (astrocytes and microglia; Retamal et al., 2007) increasing the extracellular concentration of compounds, like ATP and glutamate, that could indirectly open Pannexin1 channels leading to neuronal death (Orellana et al., 2012; Bosch and Kielian, 2014; Takeuchi and Suzumura, 2014).

Future Directions

When opened in a controlled fashion, Cx HCs allow physiological paracrine and autocrine communication between neighboring cells. However, under certain pathological conditions, these HCs open more frequently, inducing ionic imbalance and cell lysis. In particular, specific missense mutations in Cx genes associated with human genetic disease produce leaky HCs, a condition that perturbs ionic cell homeostasis, increases ATP release and Ca^{2+} influx, which in the extreme condition leads to cell death. Probably, the major problem in the study of Cx- based channels is the lack of specific pharmacological tools able to block or open these channels. Thus, for example, one of the most used HC blockers is La^{3+} (usually used at 200 μM), but this lanthanide also blocks TRP channels (Zhao et al., 2015), cGMP-activated currents (Wang et al., 2013b) and Ca^{2+} channels (Nelson et al., 1984). Fortunately, in the last years new tools have been developed for the study of Cx- HCs. These are based on small peptides that mimic some regions of a given Cx (Iyyathurai et al., 2013). Through the use of these mimetic peptides it has been possible to study *in vitro*/*in vivo* the role of HCs in a much more specific way. Because of their specificity and high affinity, they could be used for the treatment of diseases associated with leaky HCs. In this line of thought, mimetic peptides Gap26 or Gap27 have been observed to block cardiomyocyte death induced by ischemic-like conditions *in vitro* (Shintani-Ishida et al., 2007) as well as *in vivo* (Hawat et al., 2012). In the NS, Gap26 and Gap27 peptides blocked Cx43 HC opening induced by inflammatory conditions (Retamal et al., 2007; Froger et al., 2010), while Gap27 reduced spontaneous epileptiform activity in organotypic hippocampal slice cultures and cell death associated with this activity (Samoilova et al., 2008; Yoon et al., 2010). On the other hand, mimetic peptide Gap26 inhibits the spread of damage from the trauma zone to the penumbra in an *in vitro* model (Rovegno et al., 2015). Similar results have been observed *in vivo* in a model of spinal cord injury (Huang et al., 2008; O'Carroll et al., 2008) and post-ischemic brain injury (Davidson et al., 2012). Moreover, inhibition of Cx43 HCs with mimetic peptides in the spinal cord, significantly reduced the sensitization to neuropathic pain (Chen et al., 2014), which suggests that opening of HCs could result in an excessive release of neuroactive molecules in chronic pain. Accordingly, exposure of sensory ganglia to mimetic peptides, to block Cx43 HCs of satellite glial cells, reduced sensory neuron activity (Retamal et al., 2014a,b). Therefore, mimetic peptides could be used as the starting point to develop new and more specific pharmacologic agents to inhibit HCs to treat human diseases associated to leaky HCs.

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ORIGINAL RESEARCH
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Pannexin channels mediate the acquisition of myogenic commitment in C_2C_{12} reserve cells promoted by P2 receptor activation

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The acquisition of myoblast commitment to the myogenic linage requires rises in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). Putative cell membrane pathways involved in these $[Ca^{2+}]_i$ increments are P2 receptors (P2Rs) as well as connexin (Cx) and/or pannexin (Panx) hemichannels and channels (Cx HChs and Panx Chs), respectively, which are known to permeate Ca^{2+}. Reserve cells (RCs) are uncommitted myoblasts obtained from differentiated C_2C_{12} cell cultures, which acquire commitment upon replating. Regarding these cells, we found that extracellular ATP increases the $[Ca^{2+}]_i$ via P2Rs. Moreover, ATP increases the plasma membrane permeability to small molecules and a non-selective membrane current, both of which were inhibited by Cx HCh/Panx1Ch blockers. However, RCs exposed to divalent cation-free saline solution, which is known to activate Cx HChs (but not Panx Chs), did not enhance membrane permeability, thus ruling out the possible involvement of Cx HChs. Moreover, ATP-induced membrane permeability was inhibited with blockers of P2Rs that activate Panx Chs. In addition, exogenous ATP induced the expression of myogenic commitment and increased MyoD levels, which was prevented by the inhibition of P2Rs or knockdown of Panx1 Chs. Similarly, increases in MyoD levels induced by ATP released by RCs were inhibited by Panx Ch/Cx HCh blockers. Myogenic commitment acquisition thus requires a feed-forward mechanism mediated by extracellular ATP, P2Rs, and Panx Chs.

Keywords: calcium signal, membrane permeability, MyoD, ATP, purinergic receptors, pannexons, myogenesis

Introduction

During skeletal muscle ontogeny and regeneration, pluripotential mesodermal or satellite cells acquire myogenic commitment, which involves the expression of myogenic determination factors such as MyoD, Myf-5, and myogenin, transforming these cells into proliferative myoblasts (Charge and Rudnicki, 2004).

The acquisition of myogenic commitment requires increases in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), which promote the activation of calcineurin (a Ca^{2+}-dependent protein phosphatase) that, in turn, induces the expression of the Myf5 transcription

observed in ATP-treated RCs. As a result of the latter, Panx1 Chs could be activated via a cytoplasmic factor (i.e., PKC and/or calmodulin/Ca^{2+}-dependent kinase) (Barbe et al., 2006), allowing for more ATP release. The positive loop may be inhibited in differentiated cultures of C_2C_{12}, which could provide a possible explication for the reduction of MyoD levels in mononucleated cells, since the extracellular medium is known to contain high levels of phosphatase activity (Sandona et al., 2004). The latter is directly related to the expression of α-sarcoglycan, which is a proteoglycan with ATP binding domains and phosphatase activity (Sandona et al., 2004). In this way, both the ATP tone and MyoD levels could be diminished. In support of this putative mechanism, replated RCs with low levels of myotube contamination, and consequently, low levels of phosphatases, would allow for ATP accumulation in the extracellular medium, which would induce the acquisition of myogenic commitment.

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RESEARCH ARTICLE

Connexin43 Hemichannels Mediate Secondary Cellular Damage Spread from the Trauma Zone to Distal Zones in Astrocyte Monolayers

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The mechanism of secondary damage spread after brain trauma remains unsolved. In this work, we redirected the attention to astrocytic communication pathways. Using an *in vitro* trauma model that consists of a scratch injury applied to an astrocyte monolayer, we found a significant and transient induction of connexin43 (Cx43) hemichannel activity in regions distal from the injury, which was maximal ~1 h after scratch. Two connexin hemichannel blockers, La^{3+} and the peptide Gap26, abolished the increased activity, which was also absent in Cx43 KO astrocytes. In addition, the scratch-induced increase of hemichannel activity was prevented by inhibition of P2 purinergic receptors. Changes in hemichannel activity took place with a particular spatial distribution, with cells located at ~17 mm away from the scratch presenting the highest activity (dye uptake). In contrast, the functional state of gap junction channels (dye coupling) was not significantly affected. Cx43 hemichannel activity was also enhanced by the acute extracellular application of 60 mM K^+. The increase in hemichannel activity was associated with an increment in apoptotic cells at 24 h after scratch that was totally prevented by Gap26 peptide. These findings suggest that Cx43 hemichannels could be a new approach to prevent or reduce the secondary cell damage of brain trauma.

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Key words: traumatic brain injury, connexins, P2 receptors, astroglia, apoptosis

Introduction

Traumatic brain injury (TBI) is a leading cause of morbidity and death, especially for people under 45 years of age. In the United States, 1.4 million incidents of TBI occur annually resulting in 235,000 hospitalization, 50,000 death, and USD $60 billons in costs (Langlois et al., 2006).

TBI is characterized by a primary damage zone at the impact site, which propagates to neighboring zones because of ischemia, excitotoxicity, cellular tumefaction, and inflammation (Werner and Engelhard, 2007). In the past decade, all clinical trials designed to test possible neuroprotective protocols have failed (Jain, 2008). Consequently, an effective neuroprotective drug is still lacking, mainly because relevant target molecules have not been identified. These negative results can be explained in part by a neuron-centered approach, which could lead to overlooking the participation of other cell types and pathogenic mechanisms (Rovegno et al., 2012). Alternatively, cell types that could play a relevant role in neuronal survival are glia and particularly astrocytes, because they play major roles both in physiologic and

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

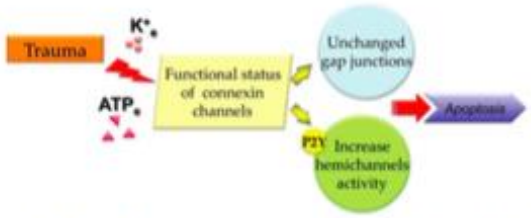

FIGURE 5: Model of propagation of a trauma *in vitro* induced damage mechanism, which results in the increase of connexin HC activity, leading to cell death. Trauma through the extracellular elevation of K^+ and ATP mediates the induction in Cx43 HC activity, without changes in GJC mediated coupling, at early stages post injury. It depends on a direct action of $[K^+]_e$ on the activation of Cx43 HC and ATP signaling pathway, probably mediated by a P2Y receptor. The increased HC activity disturbs cell homeostasis and leads to apoptosis. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

controversy might be explained by differences in cell heterogeneity; a cell line (rat C6 glioma cell line) is likely to be more homogenously susceptible to death than primary cultures of cortical astrocytes.

Notably, extracellular ATP and elevated $[K^+]_e$ induced apoptosis at similar levels than in the injury scratch wounding model used in the present work. These findings are consistent with a common pathway regarding secondary injury propagation, namely that extracellular elevation of K^+ and ATP enhance the scratch-associated induction of Cx43 HC activity resulting in cellular apoptosis. Activation of connexin HC carries a potential danger to cells, a fact known for other connexin types, and also for Cx43 (Decrock et al., 2009).

Inflammation is a well-established secondary response associated with acute brain injury. In brain trauma, infiltrated and local cells (i.e., microglia and astrocytes) trigger an inflammatory cascade involving intercellular communication mediated by cell–cell interactions and pro-inflammatory molecules (Bennett et al., 2012; Finnie, 2013; Orellana et al., 2009). Inflammatory changes appear with a delay of several hours, and peaks from 12 h to 2 days after trauma (Helmy et al., 2011a). In the trauma model used here, only a discrete inflammatory response was detected as evidenced by elevation of nitrite levels at 48 h after the scratch injury, but no significant increment in TNF-α was found. These findings contrast with those of Lau and Yu (Lau and Yu, 2001), who described a significant increment of inflammatory cytokines after scratching astrocyte monolayers. However, the two models have important differences in the traumatized area. Whereas ~40% of the culture surface was scratched in the study by Lau and Yau, it was less than ~1% in our model. That could explain the differences in the inflammatory outcome. In fact, we considered that the use of a model with almost no inflammation was an advantage. Inflammation induces drastic changes on connexin-based channels functions. Particularly, 24 h of treatment with TNF-α and IL-1β increase the Cx43 HC activity and decrease cell–cell coupling in astrocytes (Morita et al., 2007; Retamal et al., 2007). Here, we demonstrated the existence of an early modification of connexin HC function that explains the spread of secondary damage. Furthermore, in our study stimulation with TNF-α and IL-1β did not increase connexin HC activity above the scratch-induced effect at 1 or 24 h of treatment.

Although these results were obtained from astrocyte-enriched cultures, they provide new insight about the early mechanisms that take place during the cellular spread of secondary damage. As illustrated in Fig. 5, we highlighted how intrinsic components of trauma, namely elevated $[K^+]_e$ and ATP, which are part of the primary damage of cells, can activate a connexin HC-dependent pathway by means of a direct action of $[K^+]_e$ and ATP via P2 receptors. Accordingly, it has been shown that Cx43 HCs can contribute to the propagation of necrosis in models of *in vitro* ischemia (Contreras et al., 2002; Orellana et al., 2010) as well as propagation of apoptosis induced by cytochrome C (Decrock et al., 2009). To our knowledge, this is the first demonstration on extracellular ATP-mediated activation of connexin HC leading to post trauma cell death propagation. Therefore, inhibition of Cx43 HCs could reduce the spread of secondary cell damage and thus could open a glia-centered approach for therapeutic applications in brain trauma.

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Cellular and Molecular Life Sciences

MULTI-AUTHOR REVIEW

Regulation of pannexin and connexin channels and their functional role in skeletal muscles

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Abstract Myogenic precursor cells express connexins (Cx) and pannexins (Panx), proteins that form different membrane channels involved in cell-cell communication. Cx channels connect either the cytoplasm of adjacent cells, called gap junction channels (GJC), or link the cytoplasm with the extracellular space, termed hemichannels (HC), while Panx channels only support the latter. In myoblasts, Panx1 HCs play a critical role in myogenic differentiation, and Cx GJCs and possibly Cx HCs coordinate metabolic responses during later steps of myogenesis. After innervation, myofibers do not express Cxs, but still express Panx1. In myotubes and innervated myofibers, Panx1 HCs allow release of adenosine triphosphate and thus they might be involved in skeletal muscle plasticity. In addition, Panx1 HCs present in adult myofibers mediate adenosine triphosphate release and glucose uptake required for potentiation of muscle contraction. Under pathological conditions, such as upon denervation and spinal cord injury, levels of Panx1 are upregulated. However, Panx1 $^{-/-}$ mice show similar degree of atrophy as denervated wild-type muscles. Skeletal muscles also express Cx HCs in the sarcolemma after denervation or spinal cord injury, plus other non-selective membrane channels, including purinergic P2X7 receptors and transient receptor potential type V2 channels. The absence of Cx43 and Cx45 is sufficient to drastically reduce denervation atrophy. Moreover, inflammatory cytokines also induce the expression of Cxs in myofibers, suggesting the expression of these Cxs as a common factor for myofiber degeneration under diverse pathological conditions. Inhibitors of skeletal muscle Cx HCs could be promising tools to prevent muscle wasting induced by conditions associated with synaptic dysfunction and inflammation.

Keywords Pannexons · Connexons · Myoblasts · Myotubes · Striate muscles · Myogenesis · Denervation

Abbreviations

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
Cx	Connexin
GJC	Gap junction channel
HC	Hemichannel
P2R	Purinergic P2 receptor
Panx	Pannexin

Introduction

Satellite cells or their precursors, pluripotent mesenchymal stem cells, become committed to myogenic differentiation and fuse to form skeletal muscle fibers after muscle injury during either regeneration after muscle injury or in development of skeletal muscles. This process requires the expression of myogenic transcriptional factors, including myogenic differentiation 1 protein, myogenic factor 5 and

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increase in Panx1 HC activity seen during potentiation of muscle contraction due to increase in opening probability of available channels in the cell membrane or is due to recruitment of more Panx1 HCs to the sarcolemma? (3) What mechanisms restore the activity of Panx1 HCs to the basal state? Similarly, the atrophy observed in adult denervated or inflamed skeletal muscles can be largely prevented in muscles that do not express Cx43 and Cx45. Is this a mechanism that obeys a hierarchical expression of calcium-permeable channels or, instead, might all of them be involved such that each contribute to reach a critical increase in intracellular free calcium concentration?

In general, the field of Cx-based and Panx-based channel research is rather limited, but offers numerous relevant questions to be answered to further increase our knowledge in skeletal muscle physiology and pathophysiology. Moreover, Cx HCs might be potential molecular targets to reduce muscle atrophy in diverse circumstances. The mechanisms by which expression of sarolemmal Cx HCs is repressed in innervated and noninflamed muscles remains unknown. In addition, the development of potent and selective Cx HC blockers might be useful to reduce muscle degeneration induced by spinal cord injury and many other pathological conditions in which the integrity of nerve muscle contact and functional regulation has been altered.

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Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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Role of Akt and Ca^{2+} on cell permeabilization via connexin43 hemichannels induced by metabolic inhibition

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ABSTRACT

Connexin hemichannels are regulated under physiological and pathological conditions. Metabolic inhibition, a model of ischemia, promotes surface hemichannel activation associated, in part, with increased surface hemichannel levels, but little is known about its underlying mechanism. Here, we investigated the role of Akt on the connexin43 hemichannel's response induced by metabolic inhibition. In HeLa cells stably transfected with rat connexin43 fused to EGFP (HeLa43 cells), metabolic inhibition induced a transient Akt activation necessary to increase the amount of surface connexin43. The increase in levels of surface connexin43 was also found to depend on an intracellular Ca^{2+} signal increase that was partially mediated by Akt activation. However, the metabolic inhibition-induced Akt activation was not significantly affected by intracellular Ca^{2+} chelation. The Akt-dependent increase in connexin43 hemichannel activity in HeLa43 cells also occurred after oxygen-glucose deprivation, another ischemia-like condition, and in cultured cortical astrocytes (endogenous connexin43 expression system) under metabolic inhibition. Since opening of hemichannels has been shown to accelerate cell death, inhibition of Akt-dependent phosphorylation of connexin43 hemichannels could reduce cell death induced by ischemia/reperfusion.

1. Introduction

Connexins (Cxs) constitute a protein family ubiquitously expressed in chordates that form two types of structures: hemichannels (HCs), pore-like structures in the cell surface that communicate the intracellular and extracellular media, and gap junction channels (GJCs), the result of the docking of two HCs from adjacent cells that communicate the cytosol of contiguous cells [1]. Once HCs are opened, free movement of ions and small molecules occurs down their concentration gradients. Because of the big size and low selectivity of HC pores, for many years it was believed that they were always closed, but currently it is known that they open under specific physiological and pathological conditions [2], including ischemia [3].

Ischemia increases the open probability of connexin43 (Cx43) HCs [4,5]. This has been associated with dephosphorylation or nitrosylation of the protein subunit and accelerated cell death [3,6–8]. Moreover, Cx43 seems to be essential for ischemic preconditioning (IP), a mechanism in which an organ is exposed to a non-lethal ischemia/reperfusion (I/R) insult, resulting in protection against a subsequent more harmful I/R episode [9]. Metabolic inhibition (MI), a controlled ischemic-like condition, increases cell membrane permeability through Cx43 HCs in rat cardiomyocytes [10–12], astrocytes [5,7] and kidney epithelial cells [13]. Elevated Cx HC activity may be accomplished by either increasing the open probability or the number of HCs at the cell surface. MI has been proved to activate both of them. Some of the mechanisms that lead to the opening of Cx43 HCs and are activated during MI are: decreased phosphorylation status [3,7,8], reduced redox potential [8], increased intracellular $[Ca^{2+}]$ [14,15] and decreased extracellular $[Ca^{2+}]$ [16,17]. MI also increases the cell surface levels of Cx32 and Cx43 [8,18,19], but the mechanism behind this phenomena is still unknown.

The structure of Cx43 has multiple phosphorylation sites that are dynamically modified and affect membrane insertion and degradation [20] as well as the activity of Cx43 GJCs and HCs [1,21,22]. Akt is a Ser/Thr kinase activated during I/R with an important role during IP [23]. It can phosphorylate Cx43 on Ser369 and Ser373 [24], affecting the size of Cx43 gap junctions during hypoxia/reperfusion [25] and Cx43 HC activity induced by mechanical stress [26], but a similar mechanism operating

Abbreviations: Cx43, connexin43; Cx, connexin; HC, hemichannel; GJC, gap junction channel; OGD, oxygen-glucose deprivation; MI, metabolic inhibition; I/R, ischemia/reperfusion; IP, ischemic preconditioning; Etd, ethidium.

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amount of Cx43 HCs at the cell surface. Our data showed that Akt-dependent increase in Cx43 HC activity is a mechanism also present in cells endogenously expressing Cx43 (e.g., astrocytes and osteoblasts). Moreover, they suggest that Akt can be added to those regulators of Cx43 HC activity [5,7,8,52]. Using a different experimental approach to deplete intracellular ATP consisting in hypoxia, nutrient deprivation, high K^+ and acidic pH to mimic the extracellular milieu of ischemic cells, our results showed that Akt-mediated regulation of Cx43 HCs was also present in this experimental model. Considering that linoleic acid, a pro-inflammatory unsaturated fatty acid, also increase Cx43 HC activity by a Ca^{2+}/Akt dependent pathway [29], we propose that this mechanism is activated in general inflammatory states regardless the stimuli. Considering our results and those reported previously, Akt-dependent regulation of Cx43 HCs and gap junctions seems to be a general mechanism operating in physiological (mechanic stress) [26] as well as pathologic conditions (hypoxia/reoxygenation, MI) [25,45], but further experiments should be done to test this hypothesis.

Taking our results together, we propose the following mechanism: MI activates Akt, which in turn increases intracellular Ca^{2+} levels. Both signals increase the amount of cell surface Cx43 HCs, thus increasing the cell membrane permeability and finally affecting cell survival. The biological consequence should depend on features of the insult such as intensity and duration. Accordingly, brief and/or low intensity (sublethal insult) could be associated to preconditioning possibly due to enhanced adenosine release via Cx43 HCs [53], whereas, long and/or intense insult lead to cell death associated to Ca^{2+} overload possibly related to persistent Cx43 HC opening [7].

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbadis.2015.03.004.

Transparency document

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Abstract

The dental pulp in human primary teeth is densely innervated by a plethora of nerve endings at the coronal pulp-dentin interface. This study analyzed how the physiological root resorption (PRR) process affects dental pulp innervation before exfoliation of primary teeth. Forty-four primary canine teeth, classified into 3 defined PRR stages (early, middle, and advanced) were fixed and demineralized. Longitudinal cryosections of each tooth were stained for immunohistochemical and quantitative analysis of dental pulp nerve fibers and associated components with confocal and electron microscopy. During PRR, axonal degeneration was prominent and progressive in a Wallerian-like scheme, comprising nerve fiber bundles and nerve endings within the coronal and root pulp. Neurofilament fragmentation increased significantly during PRR progression and was accompanied by myelin degradation and a progressive loss of myelinated axons. Myelin sheath degradation involved activation of autophagic activity by Schwann cells to remove myelin debris. These cells expressed a sequence of responses comprising dedifferentiation, proliferative activity, GAP-43 overexpression, and Büngner band formation. During the advanced PRR stage, increased immune cell recruitment within the dental pulp and major histocompatibility complex (MHC) class II upregulation by Schwann cells characterized an inflammatory condition associated with the denervation process in preexfoliative primary teeth. The ensuing loss of dental pulp axons is likely to be responsible for the progressive reduction of sensory function of the dental pulp during preexfoliative stages.

Keywords: Wallerian degeneration, myelin, Schwann cell, neuroplasticity, pain, immune system

Introduction

Human teeth have evolved with a huge neurosensory system predominantly supplied by sensory nerve fibers of the trigeminal ganglion (Fried and Gibbs 2014). The dental pulp in both primary and permanent teeth is richly innervated by myelinated and unmyelinated axons (Itoh 1976; Johnsen and Johns 1978). Sensory nerve fibers run from the tooth apex in bundles to the crown, where they branch to innervate the peripheral dental pulp (Rodd and Boissonade 2002; Byers et al. 2003; Monteiro et al. 2009). At the pulp-dentin interface, a dense network of nerve fibers forms the subodontoblastic plexus of Raschkow, from where branched nerve endings project radially through the odontoblastic layer and enter the predentin (Rapp et al. 1967; Itoh 1976; Rodd and Boissonade 2001). These sensory nerves mediate nociception and are involved in tooth pain. Their total number and density within the pulp suggest that sensory innervation is crucial for tooth protection, immune defense, and dental pulp repair (Byers and Narhi 1999; Rodd and Boissonade 2002; Couve et al. 2014).

Physiological root resorption (PRR) of human primary teeth is an odontoclastic resorption process that progressively reduces the root and dental pulp tissue prior to exfoliation, to allow the replacement of primary teeth by permanent teeth (Moorrees et al. 1963). PRR in primary teeth proceeds as an asymptomatic process without evidence of bacterial infection (Bolan and Rocha 2007; Zhu et al. 2013). A decrease in dental pulp innervation within primary teeth has been described during advanced stages of root resorption (Johnsen and Johns 1978). However, primary teeth affected with caries show similar neural branching responses within the dental pulp as permanent teeth, even at advanced root resorption stages (Rodd and Boissonade 2001; Rajan et al. 2014).

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A supplemental appendix to this article is published electronically only at http://jdr.sagepub.com/supplemental.

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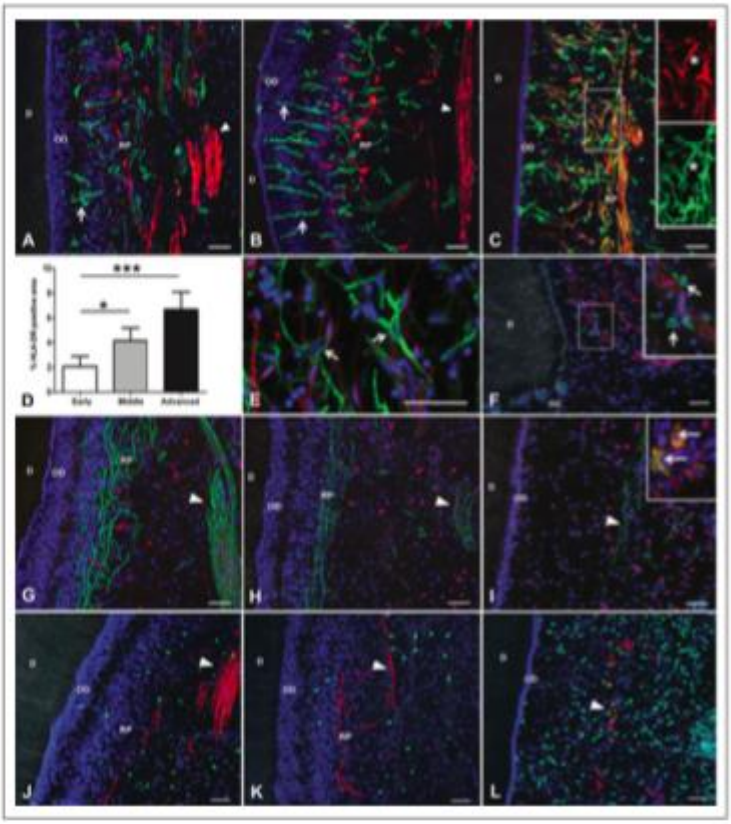

Figure 4. Immunocompetent dendritic cells (**A–C**, green) and leukocytes (**G–I**, red; **J–L**, green) within the dental pulp during physiological root resorption (PRR) progression. (A–C) Double immunolabeling of dendritic cells (HLA-DR, green, arrows) and Schwann cells (S100, red, arrowheads). Dendritic cell density increases during PRR progression. At advanced stages of PRR, Schwann cells display extensive colocalization with HLA-DR, a specific marker for antigen-presenting cells. The insets show double labeling of a Schwann cell (asterisks) by HLA-DR and S100. (**D**) Quantitative results showing a significant increase in HLA-DR immunoreactivity with PRR progression (*$P < 0.05$, ***$P < 0.001$). (**E**) Double immunolabeling of mature dendritic cells (CD83, green, arrows) and Schwann cells (S100, red) from an advanced PRR stage. (**F**) Double immunolabeling of macrophages (CD68, green) and Schwann cells (S100, red) showing the prominent presence of macrophages at the resorption site during advanced PRR stages. (G–I) Double immunolabeling of leukocytes (CD45, red) and neurofilament (NF-200, green, arrowheads) reveals a scarce number of leukocytes present in the pulp during early and middle PRR stages, while leukocyte infiltration becomes extensive at advanced stages of PRR. Note the progressive reduction in neurofilament labeling. The inset in (I) displays the presence of macrophages (arrows) charged with NF-200–positive inclusions, in proximity to leukocytes (red). (J–L) Double immunolabeling of leukocytes (CD45, green) and myelin basic protein (MBP, red, arrowheads) displays a progressive increase of leukocytes accompanied by a reduction in myelin marker expression at advanced stages of PRR. D, dentin; OC, odontoclasts; OD, odontoblast layer; RP, Raschkow plexus. Scale bars: 50 µm.

only within putative dendritic cells at advanced stages of PRR but not by Schwann cells (Fig. 4E). On the other hand, immune cells labeled for CD45 (leukocyte common antigen [LCA]) infiltrated the dental pulp as PRR progressed (Fig. 4G–I), while myelinated fibers disappeared (Fig. 4J–L). At the root resorption front, putative macrophages were frequently detected in the vicinity of nerve fibers remnants, with neurofilament markers inside, supporting their participation in the neuronal clearing process (Fig. 4F).

Discussion

The data shown here demonstrate that axonal degeneration characterizes the progressive denervation of dental pulp in human primary canine teeth, which occurs during the physiological root resorption progress. We propose that the mechanisms of axonal degeneration of dental pulp nerves during PRR in primary teeth reflect the sequence of major events that characterize and define Wallerian degeneration (Coleman 2005; Gaudet et al. 2011; Neukomm and Freeman 2014). After nerve fiber injury, axons distal to the lesion undergo a temporally degenerative sequence, characterized by initial degradation of the axonal cytoskeleton, and a robust activation of Schwann cells that dedifferentiate, remove myelin, and stimulate macrophage and lymphocyte recruitment (Gaudet et al. 2011).

During the eruption process of permanent teeth, the onset of PRR in the overlying primary teeth triggers such a Wallerian-like degeneration process of peripheral nerves, which is known as an active synchronized process with axons suffering progressive degeneration and myelin sheath loss (Stoll and Muller 1999; Kirsch et al. 2009). During Wallerian degeneration, activation of Schwann cells displays a stereotypical sequence of reactions to create a microenvironment that supports nerve repair and nerve target remodeling (Jessen and Mirsky 2005; Allodi et al. 2012; Arthur-Farraj et al. 2012). After nerve injury, Schwann cells upregulate GFAP expression, acquire proliferative and migratory properties, and form bands of Büngner that facilitate peripheral axon regeneration (Cheng and Zochodne 2002; Griffin and Thompson 2008). The present study demonstrates that Schwann cell dedifferentiation is characterized by increased expression of GFAP and PH3, suggesting cellular activity and proliferation to support remodeling of the peripheral nerve network. These reactions are principally observed during early and middle stages of PRR (Zhu et al. 2013). Moreover, the expression of GAP-43 within affected nerve fibers confirms the expression of specific neurotrophic factors that promote

frontiers in Cellular Neuroscience

ORIGINAL RESEARCH
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Neural progenitor cells isolated from the subventricular zone present hemichannel activity and form functional gap junctions with glial cells

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The postnatal subventricular zone (SVZ) lining the walls of the lateral ventricles contains neural progenitor cells (NPCs) that generate new olfactory bulb interneurons. Communication via gap junctions between cells in the SVZ is involved in NPC proliferation and in neuroblast migration towards the olfactory bulb. SVZ NPCs can be expanded *in vitro* in the form of neurospheres that can be used for transplantation purposes after brain injury. We have previously reported that neurosphere-derived NPCs form heterocellular gap junctions with host glial cells when they are implanted after mechanical injury. To analyze functionality of NPC-glial cell gap junctions we performed dye coupling experiments in co-cultures of SVZ NPCs with astrocytes or microglia. Neurosphere-derived cells expressed mRNA for at least the hemichannel/gap junction channel proteins connexin 26 (Cx26), Cx43, Cx45 and pannexin 1 (Panx1). Dye coupling experiments revealed that gap junctional communication occurred among neurosphere cells (incidence of coupling: 100%). Moreover, hemichannel activity was also detected in neurosphere cells as evaluated in time-lapse measurements of ethidium bromide uptake. Heterocellular coupling between NPCs and glial cells was evidenced in co-cultures of neurospheres with astrocytes (incidence of coupling: 91.0 ± 4.7%) or with microglia (incidence of coupling: 71.9 ± 6.7%). Dye coupling in neurospheres and in co-cultures was inhibited by octanol, a gap junction blocker. Altogether, these results suggest the existence of functional hemichannels and gap junction channels in postnatal SVZ neurospheres. In addition, they demonstrate that SVZ-derived NPCs can establish functional gap junctions with astrocytes or microglia. Therefore, cell-cell communication via gap junctions and hemichannels with host glial cells might subserve a role in the functional integration of NPCs after implantation in the damaged brain.

Keywords: subventricular zone, microglia, astrocytes, gap junctions, hemichannels, dye coupling, dye uptake

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direct communication via gap junctions between microglia and NPCs might also occur physiologically in the SVZ neurogenic niche with roles in the physiology of both cell types. In pathological conditions, possible scenarios for NPCs-microglia interactions include the use of NPC implants in lesioned brain with microglia activation. The inflammatory response triggered by host microglial cells is known to preserve implanted NPCs in an undifferentiated state in which they can promote CNS tissue healing by the secretion of immunomodulatory and neuroprotective molecules (Martino and Pluchino, 2006). As we have demonstrated *in vitro* direct NPC-microglia coupling, we raise the possibility that functional gap junctions between implanted NPCs and host microglia in the lesioned tissue might also intervene on restorative mechanisms induced by the implants.

Altogether our results demonstrate that postnatal SVZ NPCs cultured as neurospheres present functional hemichannels and gap junctions. In addition, neurosphere-derived cells can establish gap junctional communication with astrocytes and with microglia *in vitro*. Gap junctional communication between NPCs and glial cells might be involved in the behavior of NPCs both in their natural niche and also after implantation in the injured brain.

AUTHOR CONTRIBUTIONS

AMP, ERM and JCS designed research. RT, PF, RE and JCS performed research. RT, PF, RE, AMP, ERM and JCS analyzed data. ERM and JCS wrote the paper.

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Review

The Loss of Scents: Do Defects in Olfactory Sensory Neuron Development Underlie Human Disease?

Kathleen E. Whitlock*

The olfactory system is a fascinating and beguiling sensory system: olfactory sensory neurons detect odors underlying behaviors essential for mate choice, food selection, and escape from predators, among others. These sensory neurons are unique in that they have dendrites contacting the outside world, yet their first synapse lies in the central nervous system. The information entering the central nervous system is used to create odor memories that play a profound role in recognition of individuals, places, and appropriate foods. Here, the structure of the olfactory epithelium is given as an overview to discuss the origin of the olfactory placode, the plasticity of the olfactory sensory neurons, and finally the origins of the gonadotropin-releasing hormone neuroendocrine cells. For the purposes of this review, the development of the peripheral sensory system will be analyzed, incorporating recently published studies highlighting the potential novelties in development mechanisms. Specifically, an emerging model where the olfactory epithelium and olfactory bulb develop simultaneously from a continuous neurectoderm patterned at the end of gastrulation, and the multiple origins of the gonadotropin-releasing hormone neuroendocrine cells associated with the olfactory sensory system development will be presented. Advances in the understanding of the basic mechanisms underlying olfactory sensory system development allows for a more thorough understanding of the potential causes of human disease.

Birth Defects Research (Part C) 105:114–125, 2015.

Key words: anosmia; neural plate; GnRH; Kallmann syndrome; hypothalamus

Structure of the Olfactory Sensory System

The olfactory sensory system is a conserved characteristic in all vertebrates, with other Chordate groups, such as Cephalochordata (*Amphioxus*), having little to no structural conservation in the developmental process leading to the formation of the peripheral olfactory sensory system. The olfactory sensory system is made up of the peripherally located sensory neurons that detect odors and their central targets in the olfactory bulb and pyriform cortex. In terrestrial vertebrates the peripheral olfactory system is divided into two distinct regions: main olfactory epithelia (MOE) and vomeronasal olfactory epithelium (VNO), in contrast to aquatic vertebrates that have the cell types characteristic of the MOE and VNO mixed within one epithelium. Olfactory structures are present in all adult vertebrates except whales: baleen whales (*Mysticeti*: humpback whale) show a reduction but not total loss of olfactory structures, yet toothed whales (*Odontoceti*: sperm whales, dolphins) are greatly reduced to totally lacking olfactory structures (Berta et al., 2014). Support for the diminished olfaction in cetaceans include genetic changes observed across the whales species, correlating with the structural changes: approximately 29–58% of the olfactory receptors in baleen whales are pseudogenes, compared to 74–100% in the toothed whales (Kishida et al., 2007). Finally, like humans, apes, and monkeys, where the vomeronasal organ is nonfunctional or absent (Smith et al., 2014), the vomeronasal organ is also absent in the extant cetaceans (Buhl and Oelschlager, 1986; Oelschlager et al., 1987). The loss of the VNO has implications for interpretations of neuroendocrine defects in humans (discussed below).

Within the stratified olfactory epithelium (OE; Fig. 1), the most prominent cells are the olfactory sensory neurons (OSNs) that have two forms: the ciliated (Fig. 1B) and the microvillar (Fig. 1C) sensory neurons. Ciliated OSNs are found in the MOE of terrestrial vertebrates, and the microvillar are the form found in the vomeronasal epithelia. The OSNs are supported by sustentacular cells, glia, glandular cells, and progenitor cells. Within the stratified epithelium, the basally located cells, the globose basal (Fig. 1, yellow) and horizontal basal cells (Fig. 1, green), are the source of the continually regenerating olfactory epithelium (Suzuki and Osumi, 2015). The Bowman's glands (Fig. 1, blue) lie interspersed within lamina propria, and extend into the upper olfactory mucosa of the olfactory epithelium. The previous cell types are all derived from the olfactory placode during early development. In contrast, outside of the basal aspect of the olfactory mucosa lies the lamina propria, the region where the neural crest derived olfactory ensheathing cells (Jacob, 2015), the glia of the olfactory nerve, (Fig. 1 light purple) are evident. Additionally, it is in this region that neural crest derived neuromodulatory GnRH cells (Whitlock et al., 2003; Whitlock et al.,

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Annex 4
Organization of Scientific Events


International
Gap Junction
Conference
Valparaíso 2015
March 28th - April 2nd
CENTRO INTERDISCIPLINARIO DE
Neurociencia de
Valparaíso


BIOPHYSIC OF HEMICHANNELS AND
GAP JUNCTION CHANNELS:
A THEORETICAL AND PRACTICAL TRAINING
International Workshop
March 24 - 27, 2015
Santiago (Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile)
Valparaíso (Centro Interdisciplinario de Neurociencia de Valparaíso - Universidad de Valparaíso)
Rs1
Rj
V2
Ib
V1
Ia
Cell B
Cell A
Ij
Participants:
Agustín Martínez, Chile
Ramón Latorre, Chile
Carlos González, Chile
Donglin Bai, Canada
Vytautas Verselis, USA
Jorge E.Contreras.USA
Luis C. Barrio, Spain
Mohammed Kreir, Germany
Guillermo Altenberg, USA
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INTERNATIONAL WORKSHOP:
"Biochemical, Spectroscopic and Crystallographic approaches used to study the functional-structural correlations of membrane proteins"
EXPOSITORES: DR. LUIS CUELLO & BEATRIZ VELEZ
TEXAS TECH UNIVERSITY
SÁBADO 25 JULIO AL MIÉRCOLES 29 JULIO
9:00 A 12:30 HRS.
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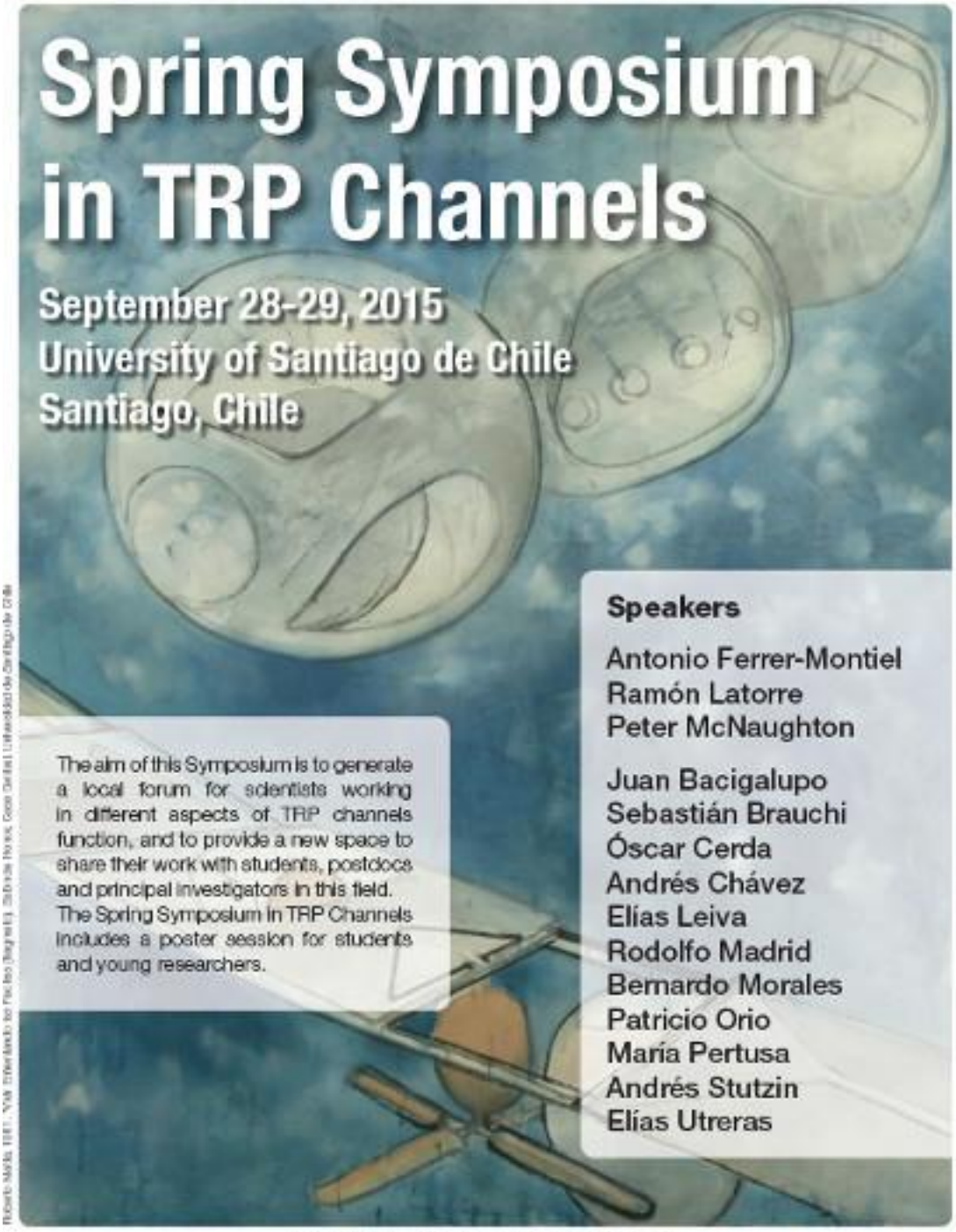
Spring Symposium
in TRP Channels
September 28-29, 2015
University of Santiago de Chile
Santiago, Chile
Speakers
Antonio Ferrer-Montiel
Ramón Latorre
Peter McNaughton
Juan Bacigalupo
Sebastián Brauchi
Óscar Cerda
Andrés Chávez
Elías Leiva
Rodolfo Madrid
Bernardo Morales
Patricio Orio
María Pertusa
Andrés Stutzin
Elías Utreras
The aim of this Symposium is to generate a local forum for scientists working in different aspects of TRP channels function, and to provide a new space to share their work with students, postdocs and principal investigators in this field. The Spring Symposium in TRP Channels includes a poster session for students and young researchers.
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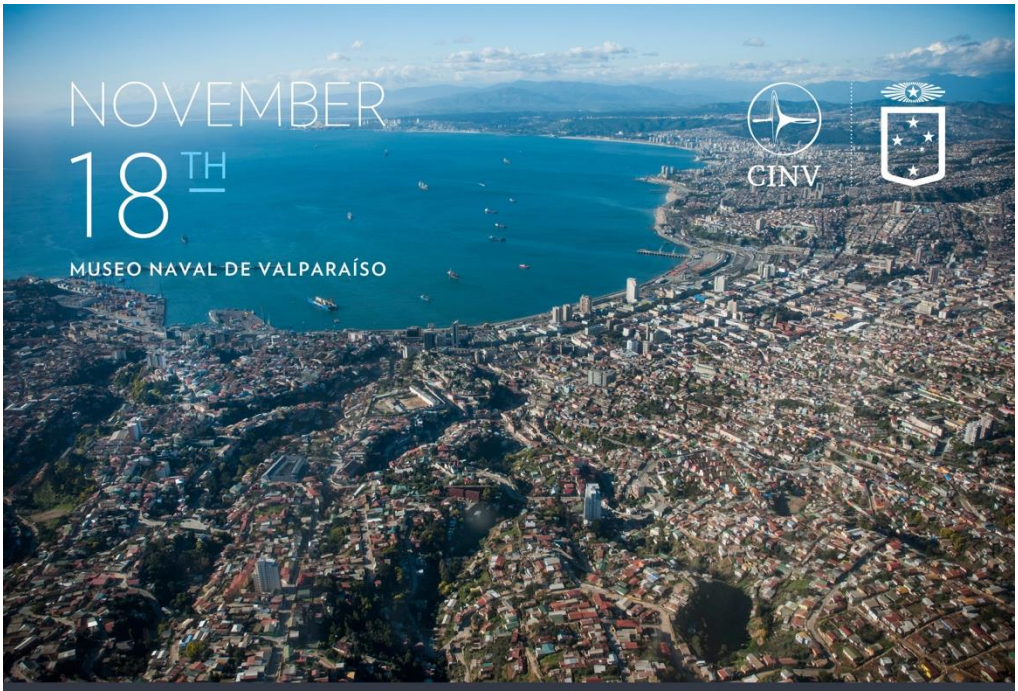

CINV-MAX PLANCK TANDEM GROUP COMPETITION

THE BEGINNING OF A LONG FRIENDSHIP

PARTICIPANTS

Sebastián Brauchi *(Chile)* / **Gonzalo Cancino** *(Canada)* / **Maite Castro** *(Chile)*
Chiayu Chiu *(USA)* / **Alisson Gontijo** *(Portugal)* / **Giovanni González** *(USA)*
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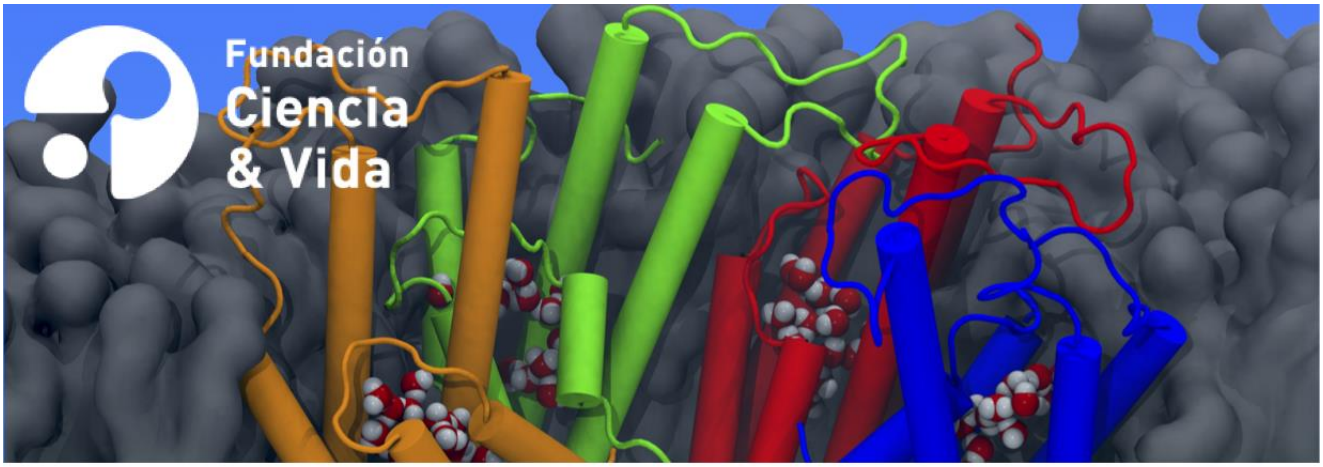
Iniciativa Científica Milenio

Gobierno de Chile



INTERNATIONAL SPRING SCHOOL

Applied Statistical Thermodynamics 2015

from theory to molecular dynamics simulation

Willem F van Gunsteren
Swiss Institute of Technology (ETH), Zürich, Suiza

Chris Oostenbrink
Universität für Bodenkultur Wien (BOKU), Austria

Maria Reif
Technische Universität München (TUM), Germany

Jose Antonio Garate
Fundación Ciencia & Vida (FCV), Santiago, Chile

NOVEMBER 16 - 27 - FUNDACIÓN CIENCIA & VIDA
ZAÑARTU 1482 – ÑUÑOA – SANTIAGO – CHILE

ORGANIZING COMMITTEE
Tomas Perez-Acle | Jose Antonio Garate
Computational Biology Lab - Fundación Ciencia & Vida
info@dlab.cl | http://dlab.cl/courses

INTERNATIONAL WORKSHOP

Molecular dynamics simulations: from theory to applications

Past and future of bio-molecular simulations

Willem F van Gunsteren

Swiss Institute of Technology (ETH), Zürich, Suiza

Ions in computer simulations

Maria Reif

Technische Universität München (TUM), Germany

INSTITUTO DE Sistemas Complejos DE VALPARAÍSO

NOVEMBER 16, 10:30 - 13:00 HRS.
INSTITUTO DE SISTEMAS
COMPLEJOS DE VALPARAISO
SUBIDA ARTILLERIA 470, VALPARAISO

Universidad de Valparaíso CHILE

CONICYT COMISIÓN NACIONAL DE INVESTIGACIÓN CIENTÍFICA Y TECNOLÓGICA

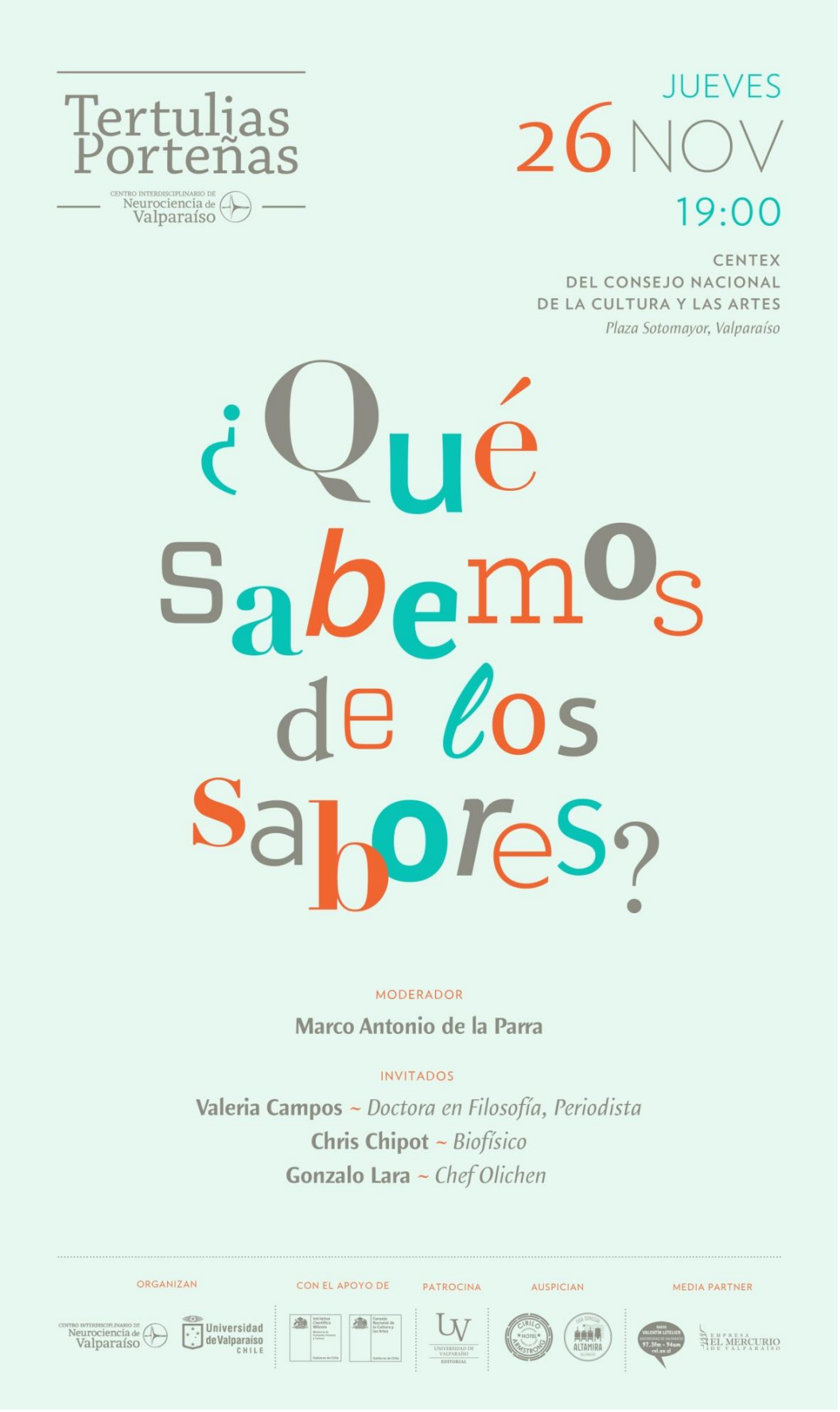
NLHPC National Laboratory for High Performance Computing Chile

Fundación Ciencia & Vida

Annex 7.1
Outreach activities throughout the period


Tertulias Porteñas
CENTRO INTERDISCIPLINARIO DE Neurociencia de Valparaíso
JUEVES
27AGO
19:00
CENTEX
DEL CONSEJO NACIONAL
DE LA CULTURA Y LAS ARTES
Plaza Sotomayor, Valparaíso
¿Qué sabemos del lenguaje?
MODERADOR
Marco Antonio de la Parra
INVITADOS
Eduardo Sabrovsky - Doctor en Filosofía
Francisco Aboitiz - Doctor en Neurociencia
Anamaría Briede - Artista Visual
ORGANIZAN
CON EL APOYO DE
PATROCINA
AUSPICIAN
MEDIA PARTNER
Universidad de Valparaíso CHILE
EL MERCURIO DE VALPARAÍSO


Tertulias Porteñas
CENTRO INTERDISCIPLINARIO DE Neurociencia de Valparaíso
JUEVES
15 OCT
19:00
CENTEX
DEL CONSEJO NACIONAL
DE LA CULTURA Y LAS ARTES
Plaza Sotomayor, Valparaíso
¿Qué sabemos de las emociones?
MODERADOR
Marco Antonio de la Parra
INVITADOS
Andrea Slachevsky ~ Doctora en Ciencias
Elías Cohen ~ Director Teatral y Coreógrafo
Ernesto Pfeiffer ~ Doctor en Literatura y Editor de Libros
ORGANIZAN
APOYAN
PATROCINA
AUSPICIAN
MEDIA PARTNER
Universidad de Valparaíso CHILE
EL MERCURIO DE VALPARAISO


Tertulias Porteñas
CENTRO INTERDISCIPLINARIO DE Neurociencia de Valparaíso
JUEVES
26 NOV
19:00
CENTEX
DEL CONSEJO NACIONAL
DE LA CULTURA Y LAS ARTES
Plaza Sotomayor, Valparaíso
¿Qué Sabemos de los Sabores?
MODERADOR
Marco Antonio de la Parra
INVITADOS
Valeria Campos ~ Doctora en Filosofía, Periodista
Chris Chipot ~ Biofísico
Gonzalo Lara ~ Chef Olichen
ORGANIZAN
CON EL APOYO DE
PATROCINA
AUSPICIAN
MEDIA PARTNER
Universidad de Valparaíso CHILE
EL MERCURIO

Pictures of “Que Tienes en Mente”

Annex 7.3
Articles and Interviews

1) **Interview with Dr. Ramón Latorre on the Abate Molina Building**
   Radio: Cooperativa. Programa GPS conducido por Soledad Oneto
   Date: January 23, 2015
   Link: http://www.audionoticias.net/videos/llambias/llambiasradio.htm
   Scope: National

2) **Regional government will provide $2.500 million to build science center in Valparaíso**
   Web Page: Diario Publimetro
   Date: January 26, 2015
   Link: www.publimetro.cl
   Scope: National

Dr. Ramón Latorre, Director del Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso

Aprueban $2.500 millones para construir edificio de la ciencia en Valparaíso

PUBLICADO : Hoy 10:58 h.

Recomendar 0 Twittear 8 +1 0 Compartir

Con aportes adicionales de Universidad de Universidad de Valparaíso y del Ministerio de Obras Públicas, se reunirán los 5 mil millones de pesos que requiere esta obra. Proyecto impulsado por el Centro Interdisciplinario de Neurociencia busca posicionar a la ciudad como referente de la investigación en Latinoamérica. Sede del primer Congreso en Chile estará abierto a la comunidad y acogerá a más de 150 científicos nacionales y extranjeros.

El Consejo Regional de Valparaíso aprobó recursos por $2.500 millones para la recuperación definitiva del ex edificio La Matriz, futuro edificio Juan Ignacio Molina, impulsado por el Centro Interdisciplinario de Neurociencia de Valparaíso (CINV) con el objetivo de posicionar a la ciudad puerto en la referencia de la investigación científica latinoamericana.

3) **Abate Molina Building Ceremony: interviews to Dr. Ramón Latorre and UV President, Aldo Valle.**
TV Channel: TVN Valparaíso. Noticias 24 Horas
Date: January 28, 2015
Link: http://www.24horas.cl/regiones/valparaiso/valparaiso-construiran-nuevo-edificio-de-neurociencia-1569387
Scope: National

4) **Interview of Dr. Ramón Latorre on construction of Abate Molina building.**
TV Channel: TVN. Noticias 24 Horas
Date: January 29, 2015
Scope: International

5) **Construction of Neuroscience building in Valparaiso's "Barrio Puerto" will start at the end of the year**
Newspaper: El Mercurio de Valparaíso
Date: January 29, 2015

Scope: Regional

CONSTRUCCIÓN DEMANDARÁ UNA INVERSIÓN DE $ 5 MIL MILLONES.

Edificio de Neurociencia en el Barrio Puerto se comenzará a construir a fines de este año

● Ayer se dio el vamos para concretar en el emblemático edificio Juan Ignacio Molina, del Barrio Puerto, el Centro Interdisciplinario de Neurociencia de Valparaíso, cuya construcción se estima comenzará a fines de este año. La ceremonia contó con la presencia de autoridades regionales, encabezadas por el intendente Ricardo Bravo, y académicas de la Universidad de Valparaíso (UV), quienes destacaron la importancia de la obra para la Ciudad Puerto y para la ciencia en Chile. El nuevo centro cuenta con una inversión de $ 5.000 mil millones aportados por el Core, el Ministerio de Obras Públicas y la UV.

6) Proof that meditation reduces aging of the brain
Newspaper: El Mercurio
Date: February 11, 2015
Scope: National

Estudio de la UCLA con 100 pacientes:

Comprueban que la meditación reduce el envejecimiento cerebral

Si bien se comprobó que esta técnica hace más lenta la pérdida de materia gris en el cerebro, falta por definir el tiempo óptimo que debe durar cada sesión y con qué frecuencia practicar.

SEBASTIÁN URBINA

A medida que la esperanza de vida de las sociedades actuales sigue en aumento —superando en muchas de ellas los 80 años—, el gran desafío que plantea esto es el envejecimiento cerebral, ya que esto puede malograr la calidad de vida de los últimos años de una persona.

La evidencia demuestra que, antes de los 30 años, el cerebro ya comienza a reducirse de tamaño y a perder peso. Este cambio causa problemas de funcionamiento en este órgano, lo que se acompaña de un mayor riesgo de enfermedades mentales y problemas neurodegenerativos.

Para hacer frente a esto, los investigadores se han dedicado a identificar cuáles son los factores de riesgo que amenazan al cerebro. Pero se les ha prestado poca atención a los elementos que pueden mejorar su salud.

Ahora, un grupo de científicos de la Universidad de California en Los Angeles (UCLA) demostró que las personas que meditan logran hacer más lento el deterioro de este órgano.

El estudio incluyó a 50 personas que llevaban meditando un promedio de 20 años, y las comparó con otras 50 que no lo hacían. En cada grupo había 28 hombres y 22 mujeres, y la edad promedio de ambos grupos era de 51 años.

El trabajo, que se publica en la revista Frontiers in Psychology, agrega una nueva evidencia de que la meditación sería un potente factor protector del cerebro.

Materia gris

Los investigadores estudiaron los cerebros de los voluntarios usando imágenes de resonancia magnética de última generación. Así pudieron ver que, si bien en todos ellos había una disminución de la sustancia gris que es rica en neuronas, quienes meditaban perdían menos células nerviosas, algo que se observa en todo el cerebro y no solo en algunas zonas.

El doctor Florian Kurth, coautor del estudio y quien trabaja en el Centro de Mapeo Cerebral de la UCLA, dijo que estaban sorprendidos por la magnitud de la diferencia que se observó entre ambos grupos.

"Esperábamos encontrar efectos más pequeños y localizados en regiones que ya sabíamos que se afectan con la meditación", explica Kurth. "En lugar de eso, lo que en realidad vimos fue un efecto generalizado de la meditación, que abarca distintas zonas a través de todo el cerebro", agrega.

Esto es importante en un mundo que envejece y presenta una creciente carga de problemas cognitivos y demencia. "A la luz de esto, parece esencial que la mayor expectativa de vida no se produzca al costo de una menor calidad de vida", advierte la doctora Eileen Luders, autora principal del trabajo y profesora asociada de Neurología en la Escuela de Medicina de la UCLA.

Para el doctor Ramón Latorre, profesor y director del Centro Interdisciplinario de Neurociencia de Valparaíso, del Instituto Científico Milenio, "es claro que la meditación altera la actividad cerebral y hay estudios que nos dicen que este tipo de actividad aumenta el número de conexiones entre las neuronas, disminuye el estrés —que mata células nerviosas— y la presión arterial".

Según este científico, otros estudios también demuestran que hay cambios profundos en el electroencefalograma durante la meditación. Estos cambios incluyen pasar de las ondas beta, que son características de la actividad normal y alerta del cerebro, a las ondas alfa, que son de relajación. "Incluso otros estudios indican que la meditación puede ser una buena terapia contra la depresión", concluye el doctor Latorre.

Para los autores de este estudio, aunque se confirma nuevamente lo beneficiosa que puede ser la meditación, quedan aún muchas dudas que despejar en futuras investigaciones.

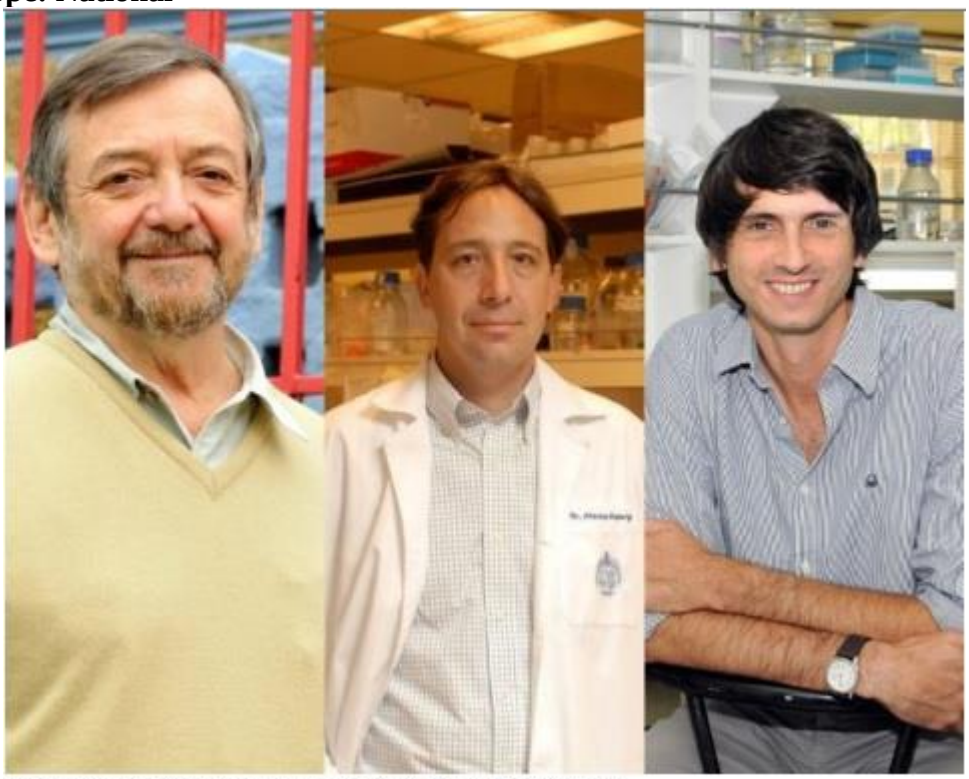
Entre ellas los diferentes efectos que podrían tener los distintos tipos de meditación, el tiempo óptimo diario para meditar y la frecuencia con que se debería hacer para alcanzar los efectos deseados.

Fumar y beber

Si bien fumar acelera el envejecimiento del cerebro, al dejar de hacerlo se puede revertir en parte el daño, recuperación que aumenta con los años. Esto según un estudio con 500 escoceses que se publica en la revista Molecular Psychiatry, del grupo Nature. En tanto, una investigación con ratones de la U. A&M de Texas demostró que el resveratrol, sustancia que se encuentra en la piel de las uvas rojas y en el maní, previene el deterioro mental e incluso, podría ser de ayuda a quienes sufren de mal de Alzheimer.

JOSÉ LUIS RISSETTI

7) **Directors of Centres of Excellence support the creation of a Ministry of Science in Chile.**
Web Page: Diario Publimetro
Date: February 11, 2015
Link: www.publimetro.cl
Scope: National



De izquierda a derecha: Dr. Ramón Latorre, Dr. Alexis Kalergis y Dr. Andrés Couve.

Directores de Centros de Excelencia apoyan la creación de un Ministerio para la Ciencia Chilena

PUBLICADO : Hoy 12:54 h.

Recomendar 0 Twittear 7 +1 0

Compartir

ENLACES RELACIONADOS

Representantes de Institutos Milenios e integrantes de la Comisión Presidencial "Ciencia para el Desarrollo de Chile", llaman a priorizar la investigación básica y aplicada, y a incrementar la inversión de recursos para éstas y otras áreas.

"Necesitamos crear una institucionalidad de las ciencias en Chile, ya sea un Ministerio u otra entidad similar. Lo importante es generar un programa coherente de políticas públicas, de largo plazo y con un claro programa de desarrollo para la ciencia y la innovación tecnológica", comentaron los Doctores Alexis Kalergis, Andrés Couve y Ramón Latorre, directores de Institutos Milenios de: Inmunología e Inmunoterapia, IMII, de Neurociencia Biomédica, BNI, y del Centro Interdisciplinario de Neurociencias, albergados en la Pontificia Universidad Católica de Chile, la Universidad de Chile y la Universidad de Valparaíso, CINV.

8) **Directors of Centres of Excellence demand that there be a political will to change the national scientific landscape**
Web Page: El Mostrador
Date: February 11, 2015
Link: www.elmostrador.cl
Scope: National

Ciudad

Alexis Kalergis, Andrés Couve y Ramón Latorre solicitan un Ministerio de la Ciencia

Directores de Centros de Excelencia exigen determinación política para cambiar el panorama científico nacional

Representantes de Institutos Milenios e integrantes de la Comisión Presidencial "Ciencia para el Desarrollo de Chile", hicieron un llamado a las autoridades para que en el corto plazo se implemente el proyecto de institucionalidad científica en Chile, se incremente el aporte de recursos a la investigación científica y se promueva y valorice aún más, el desarrollo de la ciencia básica y aplicada.

por **CULTURA+CIUDAD, EL MAGAZINE DE EL MOSTRADOR** ENVIAR RECTIFICAR IMPRIM

"¡Necesitamos crear una institucionalidad de las ciencias en Chile!". Así de claros y enfáticos son los doctores Alexis Kalergis, Andrés Couve y Ramón Latorre. Los directores de Institutos Milenios de: Inmunología e Inmunoterapia, de Neurociencias Biomédica, y del Centro Interdisciplinario de Neurociencias opinan que "lo importante es generar un programa coherente de políticas públicas, de largo plazo y con un claro programa de desarrollo para la ciencia y la innovación tecnológica".

9) Chilean squid used as a basis for developing drugs against pain
Newspaper: El Mercurio de Valparaíso
Date: February 17, 2015
Scope: Regional

8 | **Actualidad**

Usan jibia chilena como base de fármacos contra dolores

VALPARAÍSO. *Científicos usan laboratorios de la zona y el abundante recurso de este animal para analizar cómo funcionan los canales del dolor.*

Un grupo de académicos y científicos trabaja en Valparaíso para generar fármacos capaces de inhibir fuertes dolores corporales. Los doctores Francisco Bezanilla, profesor de la Universidad de Chicago, y Ramón Latorre, director del Centro Interdisciplinario de Neurociencia, de la Universidad de Valparaíso (CINV), lideran este trabajo que usa uno de los recursos más abundantes en las costas de la Región: la jibia.

En este calamar gigante, los investigadores están abocados a estudiar los axones de esta especie -aquellas prolongaciones de las neuronas que se conectan con el sistema nervioso- y que, debido a su gran tamaño, hacen posible comprender cómo funciona el impulso nervioso y los procesos eléctricos, comunes a todos los seres vivos.

"Cuando hay cáncer, muchas personas mueren a causa de estos fuertes malestares, y no precisamente producto de la enfermedad".

Ramón Latorre
Científico y director del CINV

El dr. Latorre, Premio Nacional de Ciencias, explica que durante la visita del Dr. Bezanilla, se dedicarán a colectar nervios ópticos de jibia y a estudiar estructuras de las proteínas que median la conducción nerviosa, conocimiento que le permitirá, a otros expertos, poder avanzar en la comprensión y manejo clínico de problemas como el dolor corporal, o bien, de patologías como la epilepsia o la hipertensión.

"Lo que haremos, es tratar de aislar y cristalizar una proteína que está en la membrana de todos los nervios, llamada canal de sodio, y que es la encargada de transmitir las señales eléctricas a través de todo nuestro sistema nervioso. En estos momentos estamos tratando de determinar la estructura de ese canal, lo que a futuro tendrá utilidad en el plano médico", comenta. En tanto, el Dr. Francisco Bezanilla, Doctor Honoris Causa de la U. de Valparaíso y miembro de la Academia de Ciencias de los EE.UU, indica que seguirán utilizando el axón gigante de la jibia "para explorar algunos estados intermedios del ciclo de la bomba de Sodio/Potasio", una proteína presente en todas la membranas celulares, que ayuda al mantenimiento de señales eléctricas.

EL USO DE LA JIBIA EN INVESTIGACIONES CIENTÍFICAS SE REMONTA A VARIAS DÉCADAS.

El rol clave de Montemar en la indagación

◆Camino a Concón, se encuentra el laboratorio de Montemar, faro de la ciencia y del estudio sobre bioelectricidad en Chile, con más de cincuenta años dedicado a la exploración de la jibia y sus mecanismos eléctricos. Situado frente a la Facultad de Ciencias del Mar, de la U. de Valparaíso, este espacio -de la U. de Chile- ..ha sido rescatado por el Centro Interdisciplinario de Neurociencia, CINV, de la mano de su director. Gracias a este esfuerzo, el trabajo con jibias se sigue realizando año tras año. Montemar fue fundado en los años 60' por Mario Luxoro -primer biofísico chileno y Premio Nacional de Ciencias-, época en la que también participaron activamente el Dr. Ramón Latorre, Dr. Francisco Bezanilla y la Dra. Cecilia Hidalgo, otra Premio Nacional en Ciencias Naturales.

MANEJO DEL DOLOR

Según explica el Dr. Latorre, "el dolor" es uno de los problemas más serios que actualmente existen en la medicina: "Cuando hay cáncer, muchas personas mueren a causa de estos fuertes malestares, y no precisamente producto de la enfermedad. Por tanto, si sabemos la estructura y función de ese canal de sodio, vamos a tener mayores facilidades para producir fármacos que lo inhiban, contribuyendo al manejo del dolor".

El director de CINV también comenta que los canales de sodio están involucrados en la hipertensión, enfermedad caracterizada por un incremento continuo de la presión sanguínea. "Algunas fallas en esas proteínas -los canales de sodio- pueden conducir a esta patología. Por esta razón, entender cómo funcionan estas estructuras puede ser de gran ayuda para éste y otros problemas de salud".

Al respecto, hay otra línea de investigación llevada a cabo por el Dr. Bezanilla, el Dr. Latorre, y el científico joven del CINV, Juan Pablo Castillo -junto a investigadores de la Universidad de Chicago-, que aborda el estudio de unos canales de potasio llamados Big K. "Estos canales son muy importantes para el control de la presión arterial y la contracción de las arteriolas", señala Bezanilla.

La jibia de Humboldt, calamar que puede llegar a medir dos metros con sus tentáculos estirados, constituye un motor de la biofísica chilena, desde que hace unos cincuenta años, investigadores nacionales y de otros países, han llegado hasta las costas de la Quinta Región para estudiar sus axones gigantes. OS

10) Agreement between CINV and the Max Planck Society will place Valparaiso in the orbit of world science
Web Page: El Mostrador.
Date: March 20, 2015
Link: www.elmostrador.cl
Scope: National

El acuerdo se firmó en la Escuela de Derecho de la Universidad de Valparaíso

Millonario convenio en Neurociencias ubica a Valparaíso en la órbita de la ciencia mundial

Gracias a una importante alianza entre el Ministerio de Educación e Investigación de Alemania; Institutos Max Planck, Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso, la Universidad de Valparaíso y la Iniciativa Científica Milenio, dos millones de dólares se invertirán en el puerto para estimular la ciencia joven. Para esto se seleccionará a dos científicos jóvenes de excelencia, provenientes de Chile o del extranjero, quienes serán invitados a radicarse en la ciudad porteña y desde allí organizar su investigación en neurociencia, durante cinco años.

por **CULTURA+CIUDAD, EL MAGAZINE DE EL MOSTRADOR** ENVIAR RECTIFICAR IMPRIMI

En la ciudad de Valparaíso se invertirán dos millones de dólares para incentivar la ciencia joven, durante un periodo de siete años. Esta propuesta se materializará gracias a un convenio suscrito en las últimas horas, por representantes del Ministerio de Educación e Investigación de Alemania; Institutos Max Planck; Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso, CINV, Iniciativa Científica Milenio y Universidad de Valparaíso.

11) Two million dollars will be invested to support young science in Valparaíso
Web Page: UCV Radio Valparaíso
Date: March 23, 2015
Link: www.ucvradio.cl
Scope: National

En Valparaíso se invertirán dos millones de dólares para incentivar la ciencia joven

21 de marzo de 2015

Se premiará a dos investigadores de excelencia, quienes desarrollarán libremente sus proyectos durante un período de siete años.

En la ciudad de Valparaíso se invertirán dos millones de dólares para incentivar la ciencia joven, durante un periodo de siete años. Esta propuesta se materializará gracias a un convenio suscrito en las últimas horas, por representantes del Ministerio de Educación e Investigación de Alemania; Institutos Max Planck; Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso, CINV, Iniciativa Científica Milenio y Universidad de Valparaíso.

Esta es la primera colaboración formal de los Institutos Max Planck con una institución chilena. El modelo de financiamiento es único en América Latina. Mediante esta alianza, se seleccionará a dos científicos jóvenes de excelencia, provenientes de Chile o del extranjero. Estos serán invitados a radicarse en la ciudad porteña y desde allí organizar su investigación en neurociencia, durante cinco años. Para estos fines, el CINV entregará 150 mil dólares anuales a cada investigador seleccionado, quienes van a poder disponer libremente de los recursos para ejecutar su proyecto científico.

"Los beneficiados serán completamente libres en el uso de los recursos. Cada cual verá cómo los administra y quién integra a sus equipos de trabajo. A partir del cuarto año deberán emitir un informe y, de acuerdo a los resultados, podrán recibir otros dos años de financiamiento, en forma adicional", explicó el director del Instituto Milenio CINV, Dr. Ramón Latorre. Este modelo ha sido aplicado en Alemania y Estados Unidos, asegurando generaciones de científicos de excelencia.

Semillero de futuros premios Nobel

El directivo del Centro Interdisciplinario de Neurociencia comentó que "estamos muy optimistas con esta modalidad libre de trabajo, ya que así se han desarrollado los grandes investigadores y científicos mundiales, y se han forjado Premios Nobeles. Esperamos, gracias a la Sociedad Max Planck, al apoyo de la Iniciativa Científica Milenio y la Universidad de Valparaíso, convertirnos también en el semillero de grandes científicos", acotó.

Firma de convenio

El acuerdo de cooperación, que lleva por nombre "Grupos Tandem Max Planck- CINV en Investigación en Neurociencia", se firmó en la Escuela de Derecho de la Universidad de Valparaíso, en una ceremonia encabezada por la primera autoridad universitaria, Dr. Aldo Valle; el director del Instituto Milenio CINV, Dr. Ramón Latorre; el director general del Ministerio de Educación e Investigación de Alemania, Dr. Volker Rieke; Andreas Trepte, representante del Instituto Mack Plank, y la recién nombrada Directora Ejecutiva de la Iniciativa Científica Milenio, Virginia Garretón.

En la oportunidad, el rector del UV señaló sentirse honrado de formar parte de esta iniciativa. "Junto a Max Planck, queremos que Valparaíso sea un referente de la ciencia en esta disciplina. Por eso, desde la Universidad, vamos a participar en el fortalecimiento del CINV y sus capacidades, apoyando el desarrollo e integridad de las ciencias, que es un bien que la humanidad necesita".

12) Chilean squid is used to develop drugs against pain
Web Page: Diario Publimetro
Date: February 17, 2015
Link: www.publimetro.cl
Scope: National

Utilizan jibia chilena en estudio para crear fármacos contra dolores

Doctores Francisco Bezanilla, de la U. de Chicago, y Ramón Latorre, director del Centro Interdisciplinario de Neurociencia, U. de Valparaíso, exploran funcionamiento eléctrico de estos calamares.

Generar fármacos para inhibir fuertes dolores corporales, es uno de los avances que podría ser facilitado gracias a las investigaciones de científicos chilenos los Doctores Francisco Bezanilla, profesor de la Universidad de Chicago, y Ramón Latorre, director del Centro Interdisciplinario de Neurociencia, de la Universidad de Valparaíso, CINV.

Utilizando la jibia, un calamar gigante de nuestras cosas, los investigadores están abocados a estudiar los axones de esta especie -aquellas prolongaciones de las neuronas que se conectan con el sistema nervioso- y que, debido a su gran tamaño, hacen posible comprender cómo funciona el impulso nervioso y los procesos eléctricos, comunes a todos los seres vivos.

13) **Interview with Dr. Francisco Bezanilla.**
TV Channel: TVN Señal Internacional. Programa Conectados
Date: March 3th2015
Link: http://www.audionoticias.net/videos/llambias/llambias2.htm

14) US$ 2 Million initiative is launched to investigate Neuroscience in Valparaíso
Magazine: Qué Pasa
Date: March 20, 2015
Scope: National

[Qué pasa]
En Breve

OJOS DE LA LLAVE

LA NUEVA CARA DE INTEGRAMÉDICA

Ha pasado más de un año desde la compra del 56% de Cruz Blanca Salud —ligada a la familia Said— por parte del grupo internacional British United Provident Association (BUPA), pero algunos cambios recién se empezarán a notar de ahora en adelante.
El lunes 23 de marzo llegará al país Stuart Fletcher, CEO del conglomerado internacional. Su visita no sólo contempla una serie de reuniones, sino que marcará el puntapié de un proceso de cambio de marca de los centros de Integramédica, los cuales a partir de los próximos días empezarán a asimilarse a los otros recintos que BUPA maneja en los distintos países en los que tiene presencia.
Según cuentan desde la empresa, la transición a la nueva marca será paulatina. En una primera etapa se leerá "Integramédica, parte de BUPA", en los logos que aparecen en las fachadas de los edificios en los cuales opera la red. El primer centro que cambiará su apariencia será Integramédica Manquehue, el cual está ubicado en la avenida del mismo nombre. En él se ha estado trabajando durante los últimos días para implementar la nueva imagen corporativa que Fletcher lanzará en su visita.

LANZAN FONDO DE US$ 2 MILLONES PARA INVESTIGAR NEUROCIENCIA EN VALPARAÍSO

El acuerdo, que fue firmado esta semana por el Instituto Max Planck de Alemania, el centro científico más importante del mundo; el Ministerio de Educación de ese país; el Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso y la Iniciativa Científica Milenio, tiene un objetivo: atraer a dos de las mentes jóvenes más brillantes del planeta a vivir en los cerros de Valparaíso.
Para eso, lanzaron un fondo de US$ 2 millones de dólares para dos investigadores jóvenes —150 mil dólares anuales para cada uno—, por cinco años, para que realicen investigaciones de vanguardia en neurociencia, de temática libre.
El acuerdo es la primera colaboración en Chile del Instituto Max Planck, que cuenta con 80 centros en Alemania con más de 40 mil científicos.

VOCERA DEL ANTIPOPULISMO VISITA CHILE

En septiembre de 2014, la politóloga guatemalteca Gloria Álvarez, subió al estrado del primer Parlamento Iberoamericano de la Juventud para dar un discurso antipopulista que provocó gran revuelo por sus cuestionamientos a los gobiernos latinoamericanos, y el video de su intervención se viralizó en las redes sociales, sumando más de un millón de visitas. La analista fue invitada a Chile para participar en una charla en la Universidad Adolfo Ibáñez, programada para el 22 de abril, en la que Álvarez hablará sobre el uso de las tecnologías contra el populismo. Al día siguiente, participará en la sede de la Fundación para el Progreso en una conferencia con la bloguera cubana Yoani Sánchez, quien viene a Chile invitada por la plataforma La Otra Mirada.

[En Breve || 08 - 09]

15) Agreement will enhance Neuroscience in Valparaiso
Newspaper: El Mercurio de Valparaíso
Date: March 20, 2015
Scope: Regional

Convenio permitirá potenciar el área de la neurociencia

VALPARAÍSO. *Instituto alemán y UV firmaron acuerdo por siete años.*

UV

US$2 MILLONES SE ENTRAGARÁN PARA REALIZAR LAS INVESTIGACIONES

"Para nosotros es un paso importante el acuerdo que hemos alcanzado, ya que viene a coronar el trabajo que desde hace dos décadas se realiza con el Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso". Así se refirió el director de la oficina de la sociedad Max Planck para Latinoamérica, Adreas Trepte, al convenio alcanzado por la entidad alemana con el Centro Interdisciplinario de Neurociencia (CINV).

El acuerdo permitirá destinar dos millones de dólares por un período de siete años, para investigaciones en el ámbito de la neurociencia, en donde se seleccionarán a dos científicos de excelencia quienes llegarán a Valparaíso para realizar su investigación.

"Para nosotros era importante institucionalizar la cooperación entre los institutos de ambos países. Esto será el punto inicial para los futuros proyectos de neurociencia que se quieran realizar y, de paso, potenciar el área de la neurociencia en el país. Así, muchos de los mejores científicos de Alemania, el resto del mundo y Chile, van a querer venir hasta Valparaíso a realizar sus investigaciones", expresó Tropte.

GRAN SEMILLERO

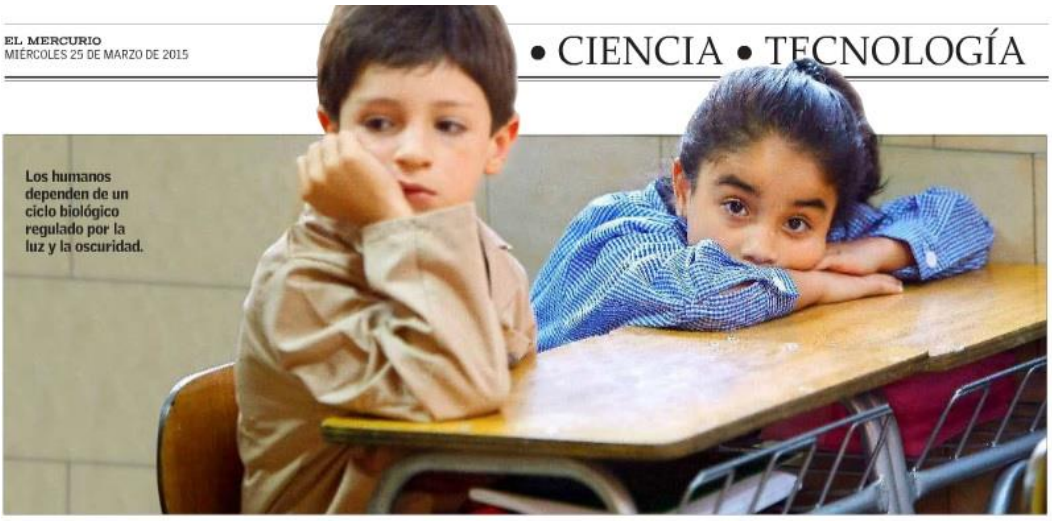
Ramón Latorre, director del Centro Interdisciplinario de Neurociencia (CINV), indicó que el convenio reafirma el buen trabajo que ha estado haciendo el instituto para posicionar a la Región como una de las líderes en Latinoamérica en materia de neurociencia.

"Estamos muy expectantes de los resultados que tendrá este acuerdo. Los otros convenios que ha realizado Max Planck ha entregado a la ciencia grandes investigadores y científicos a nivel mundial y nosotros queremos seguir ese camino. Queremos convertirnos en un semillero de grandes científicos y ser un faro de la ciencia", indicó Latorre.

Asimismo, detalló que el CINV entregará $75 millones anuales a cada investigador seleccionado para ejecutar su proyecto.

16) Getting up in the dark affects Chileans, especially in winter
Newspaper: El Mercurio
Date: March 25,2015
Scope: National

EL MERCURIO
MIÉRCOLES 25 DE MARZO DE 2015



Los humanos dependen de un ciclo biológico regulado por la luz y la oscuridad.

Consecuencias de haber optado por continuar con el horario de verano:

Levantarse sin luz afectará a los chilenos, sobre todo en invierno

Aunque es posible acostumbrarse, mañanas más oscuras suponen menos rendimiento y más riesgo de accidentes. Especialistas comentan beneficios y perjuicios de este horario constante.

M. CORDANO

Fueron truenos los que ayer iluminaron el despertar de la capital. Sin su aparición ocasional, se trató de una mañana oscura para los miles de trabajadores y escolares que comenzaron su rutina antes de las 7:48 am, hora en la que se estima que amaneció en Santiago.

El fenómeno de empezar el día cuando todavía está oscuro no será uno esporádico. Tras la decisión de dejar el área continental del país con el huso horario -3 (lo que antes llamábamos horario de verano), en invierno será común que la luz natural aparezca más tarde de lo acostumbrado, sintiéndose de mayor forma 15 días antes y después del solsticio de invierno, el 21 de junio.

"A medida que nos adentremos más en el invierno, es probable que termine amaneciendo tipo 9 am. Y eso va a tener un efecto notable en la productividad de los trabajadores, escolares y en todas las personas en general", comenta Gustavo Díaz, economista del Instituto Libertad. "Es probable que empiecen a aparecer distintos síndromes, entre ellos, la fatiga y el estrés. Eso afecta la productividad, porque aumenta el número de licencias, y por ende, el ausentismo laboral. Las empresas que trabajan con sistemas de turno, seguramente van a verse obligadas a aumentar su número, porque van a existir problemas de retención".

Aunque hay un amplio espectro de cifras y variables a considerar, Díaz se atreve a pronosticar que en un año "vamos a perder del orden de medio punto a un punto de productividad solo por efecto del cambio de hora". Esto, según aproximaciones teóricas que consideran rendimiento en intervalos con más o menos luz y datos de asistencia laboral de distintas asociaciones.

Para John Ewer, investigador del Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso, la idea de mantener un horario es buena, pero le parece poco correcta la opción de haber escogido el horario de verano por sobre el de invierno, en el que, a diferencia de lo que se espera actualmente, amanecería más temprano y oscurecería antes.

"Para nosotros, que somos animales diurnos, la luz natural es súper importante para organizar el día. Y eso se va a ver poco en invierno: las personas van a estar levantándose completamente a oscuras".

En casos extremos, el efecto puede ser muy perjudicial para quienes sufren de depresión estacional, plantea el especialista, quien en el último tiempo se ha dedicado a estudiar en torno al reloj biológico de los seres vivos.

Asimismo, dice sentirse preocupado por los efectos que esto puede tener en la seguridad: menos luz en las mañanas supone más accidentes de tránsito. Aunque a esa hora circula cerca de la mitad de los vehículos que lo hacen en el resto del día, según un estudio de la Comisión Nacional de Seguridad del Tránsito, hasta el 2013, más del 40% de los accidentes viales ocurrieron en la noche o madrugada.

Productivos de noche

"El consumo chileno de energía se asocia con levantarse no muy temprano, con quedarse con la luz prendida hasta tarde y con un máximo de consumo en la noche. A media tarde no es demasiado lo que se hace; al contrario de los países más desarrollados, donde la gente se acuesta temprano para levantarse antes", comenta —respecto a las costumbres del país— David Watts, académico del grupo de Energía de la Universidad Católica, parte de la Facultad de Ingeniería.

Aprovechar tardes

Leonardo Serra, especialista del Centro del Sueño de la Clínica Alemana, concuerda con la idea de mantener horario, pero a diferencia de Díaz, no ve mayores problemas con el horario actual.

"Hay que considerar que es una gran ventaja tener luz cuando se está en la casa. De esta forma, la gente se motiva a hacer más cosas al aire libre, y se gana en el sentido anímico; no sientes que se te acabó el día cuando llegas de vuelta del trabajo. Es una sensación psicológica agradable", indica el neurólogo.

"Si uno sigue con su vida normal, es probable que no se dé ni cuenta. Desde el punto de vista físico y orgánico, con el tiempo no es difícil adaptarse", dice. "En Chile no estamos muy acostumbrados a aprovechar la mañana, es poca la gente que madruga. En cambio, la gente sí sale en las tardes. Son cada vez más los que hacen deportes y aprovechan ese horario".

17) Japanese scientist in Valparaíso presents new methods to curb neurological diseases
Web Page: UCV Radio Valparaíso
Date: April 6, 2015
Link: www.ucvradio.cl
Scope: National

Científico japonés presentó en Valparaíso nuevos métodos para frenar enfermedades neurológicas

6 de abril de 2015

Dr. Akio Suzumura, dio a conocer estrategia para proteger a las neuronas, y ayudar a enfrentar enfermedades tales como el Parkinson, ELA, Alzheimer y Esclerosis Múltiple.

Actividad organizada por el Centro Interdisciplinario de Neurociencia, de la U. de Valparaíso, se desarrolló por primera vez en Latinoamérica.

Bloquear unas estructuras del cerebro llamadas hemicanales, es uno de los nuevos blancos terapéuticos que se estudian en el mundo, para proteger a las neuronas y frenar el desarrollo de enfermedades neurológicas como Parkinson, ELA, esclerosis múltiple y Alzheimer. En este frente trabaja el investigador japonés, Dr. Akio Suzumura, quien visitó Chile para exponer sus últimos hallazgos, en el marco del Congreso Internacional Gap Junction 2015, organizado por el Centro Interdisciplinario de Neurociencia, de la Universidad de Valparaíso, CINV.

El profesor del Instituto Nacional de Ciencias Fisiológicas de Okazaki, y editor de la revista internacional Plos One, dictó su charla en el Parque Cultural de Valparaíso, durante esta jornada científica que por primera vez se realiza en América Latina. La actividad contó con la presencia de 150 investigadores procedentes de diversos países, tales como, Canadá, Estados Unidos, Alemania, Bélgica, Reino Unido, Suiza, China, Uruguay y Chile, entre otros. Durante esta conferencia Internacional se abordaron temáticas relacionadas con diferentes patologías, como son las enfermedades neurológicas, arritmias cardíacas, sordera, cáncer y distrofias musculares.

Mecanismo de protección
Estudios del Dr. Akio Suzumura y del CINV, han demostrado que ante ciertas enfermedades como las neurodegenerativas, existe una sobreexpresión de estos hemicanales – estructuras que actúan como conductos de comunicación entre las células y el medio extracelular-. Debido a ello, el investigador japonés ha realizado estudios en modelos in vivo, que muestran que bloqueando esas estructuras, se puede frenar la progresión de varias enfermedades neurológicas de alta incidencia en el mundo.

La investigación del Dr. Suzumura se enfoca en las microglias glías, células del cerebro relacionadas con el sistema inmune y que se activan en diversas patologías. Se ha observado que estas células gliales se comunican entre sí a través de canales y hemicanales de Uniones en Hendidura, y que al momento de activarse, generan un estado inflamatorio en el sistema nervioso, que resulta nocivo para las neuronas.

"Hay muchos desórdenes neurodegenerativos como Hungtinton, Alzheimer, Esclerosis Lateral Amiotrófica, o Parkinson, cuyo origen es diferente, pero tienen como factor común que en todos los casos se activan unas células llamadas microglías. Estas células liberan un neurotransmisor que es el glutamato, el cual en altas concentraciones resulta tóxico para la neurona. A raíz de esto encontramos una molécula que bloquea los hemicanales y la liberación de glutamato, protegiendo a la neurona. Lo hemos probado en distintos modelos animales de enfermedades humanas neurodegenerativas, constituyendo así un hallazgo importante y prometedor en el tratamiento para diferentes patologías", explicó el Dr. Akio Suzumura.

18) New method presented in Valparaíso to combat neurological diseases

Newspaper: El Mercurio de Valparaíso
Date: April 7, 2015
Scope: Regional

EL PROFESOR AKIO SUZUMURA Y EL PROFESOR JUAN CARLOS SÁEZ.

Presentan en Valparaíso nuevo método contra males neurológicos

CIENCIA. *Investigación de científico japonés cambia paradigma de análisis.*

Bloquear unas estructuras del cerebro llamadas hemicanales es uno de los nuevos blancos terapéuticos que se estudian en el mundo para proteger a las neuronas y frenar el desarrollo de enfermedades neurológicas como Parkinson, ELA, esclerosis múltiple y Alzheimer.

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"Hay muchos desórdenes neurodegenerativos como Hungtinton, Alzheimer, Esclerosis Lateral Amiotrófica o Parkinson, cuyo origen es diferente, pero tienen como factor común que se activan unas células llamadas microglías", explicó Suzumura.

Hallazgos chilenos en centro porteño

● El Dr. Juan Carlos Sáez, investigador de CINV, también tiene protagonismo en los descubrimientos sobre el cerebro. De hecho, él fue uno de los investigadores que confirmó la existencia de los hemicanales. Asimismo, descubrió que en algunas patologías el número de éstos y su tiempo de apertura aumentan más de lo requerido, fomentando con ello una excesiva entrada de calcio a la célula, lo que resultaba muy nocivo para el organismo.

19) Japanese scientist presented in Valparaíso methods to slow neurological diseases
Web Page: Soy Chile
Date: April 7, 2015
Link: www.soychile.cl
Scope: National

Científico japonés expuso en Valparaíso métodos para frenar enfermedades neurológicas

El doctor Akio Suzumura visitó el país para asistir al Congreso Internacional Gap Junction 2015, organizado por el Centro Interdisciplinario de Neurociencia, de la Universidad de Valparaíso, donde dio a conocer su estrategia para proteger a las neuronas, y ayudar a enfrentar enfermedades tales como el Parkinson, ELA, Alzheimer y Esclerosis Múltiple.

Ayer
#NEUROCIENCIA

Seguir a @soyvalparaiso

20) Tips for caring for the brain and delaying its deterioration
Newspaper: El Mercurio
Date: April 8, 2015
Scope: National

El descanso ayuda a reducir el estrés, que es un factor que daña el cerebro.

Abril estará dedicado a educar sobre este órgano:

Consejos para cuidar el cerebro y poder retrasar su deterioro

El desafío es que debido al envejecimiento de la población y al déficit de especialistas, las personas deberán hacerse responsables de su salud, adquiriendo buenos hábitos de vida.

SEBASTIÁN URBINA

El cerebro se ha transformado en el órgano más célebre de la investigación médica actual. Miles de científicos trabajan en todo el mundo para entender su funcionamiento en tiempo real, lo que ayudará a entender cómo las personas piensan, sienten, aprenden, recuerdan y se mueven.

Algo que, de paso, permitirá brindar mejores tratamientos a millones de personas con depresión, alzhéimer, esquizofrenia, epilepsia y autismo, entre otras enfermedades.

Pero lograr esto es un gran desafío, ya que la población está envejeciendo rápidamente y las enfermedades neurodegenerativas van en aumento. "Estamos viviendo mucho más, por lo que no habrá especialistas suficientes para todos. Por esta razón, cada persona debe hacerse responsable de su salud, debe aprender a cuidarse", explica el doctor Walter Feuerhake, neurólogo y director de la campaña educativa sobre el cerebro que realiza este mes la Sociedad Chilena de Neurología, Psiquiatría y Neurocirugía.

El cerebro necesita descansar para su buen funcionamiento, pero para ello es necesario que tenga un sueño de buena calidad, lo que no se consigue en cualquier ambiente.

Prevención es crucial

En Chile muere un adulto cada hora por un ataque cerebral; es decir, más de 8.700 fallecidos al año por una causa prevenible.

"La edad es lo único que no se puede modificar como factor de riesgo", dice el doctor Feuerhake.

Lo demás es parte del autocuidado, como no fumar, no abusar del alcohol, reducir el consumo de sal, de azúcar y de grasa animal, y hacer ejercicio en forma regular.

Es necesario tener en cuenta que lo habitual es que las personas por sobre los 50 años tienen varias enfermedades, como hipertensión o diabetes, que es necesario mantener bien controladas para no afectar al cerebro. "Esto permite envejecer con buena salud, porque si no, no tiene mucho sentido", advierte Claudia Cárcamo, neuróloga de la Red de Salud UC Christus.

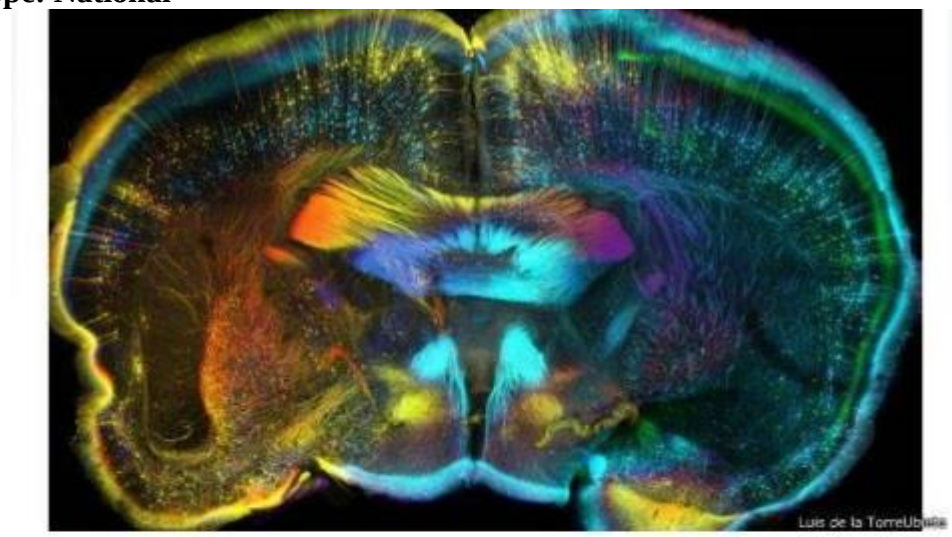
También hay que mantener activo al cerebro, agrega la doctora Soledad Matus, investigadora del Instituto Milenio de Neurociencia Biomédica (BNI). "Hay que usarlo para mantener vivas sus conexiones. Para esto es bueno leer, memorizar y hacer puzzles", explica. Tampoco hay que olvidar los momentos de esparcimiento, paseos y descanso, que permiten reducir el estrés que daña al cerebro.

Otro flanco en el que se trabaja es en la investigación que busca una cura del alzhéimer y el párkinson, según explica el doctor Ramón Latorre, director del Centro Interdisciplinario de Neurociencia de la U. de Valparaíso (CINV). "Hoy se hacen trabajos usando desde modelos animales hasta la aplicación de células madre, todos con el fin de recuperar el sistema nervioso y llegar a viejos con un cerebro que funcione bien", dice.

Y en el peor escenario, si se produce un ataque cerebral, el neurólogo Roberto Maturana, director médico de Clínica Los Coihues, aconseja una rehabilitación lo más temprana posible y por el tiempo necesario. No hay que olvidar que el cerebro es plástico durante toda su vida.

Según José Ignacio Egaña, médico del Laboratorio de Neurosistemas de la Facultad de Medicina de la U. de Chile, la investigación actual permitirá develar un gran misterio. El hecho de que simples redes de neuronas "interactúan para dar lugar a algo superior, más metafísico, como son el lenguaje, el pensamiento, el aprendizaje o la personalidad".

21) Japanese scientist in Chile presents new methods to slow neurological diseases
Web Page: Noticias TVN
Date: April 8, 2015
Link: lwww.24horas.cl
Scope: National



Científico japonés presenta en Chile nuevos métodos para frenar enfermedades neurológicas

Dr. Akio Suzumura, dio a conocer estrategia para proteger a las neuronas, y ayudar a enfrentar enfermedades tales como el Parkinson, ELA, Alzheimer y Esclerosis Múltiple.

POR 24HORAS.CL TVN

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08 abril 2015

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22) New methods to slow neurological diseases
Web Page: El Mostrador.
Date: April 8, 2015
Link: www.elmostrador.cl
Scope: National

Nuevos métodos para frenar enfermedades neurológicas

Dr. Akio Suzumura, dio a conocer estrategia para proteger a las neuronas, y ayudar a enfrentar enfermedades tales como el Parkinson, ELA, Alzheimer y Esclerosis Múltiple.

por AGENCIAS ENVIAR RECTIFICAR IMPRIMIR

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MECANISMO DE PROTECCIÓN

Estudios del Dr. Akio Suzumura y del CINV, han demostrado que ante ciertas enfermedades como las neurodegenerativas, existe una sobreexpresión de estos hemicanales – estructuras que actúan como conductos de comunicación entre las células y el medio extracelular-. Debido a ello, el investigador japonés ha realizado estudios en modelos in vivo, que muestran que bloqueando esas estructuras, se puede frenar la progresión de varias enfermedades neurológicas de alta incidencia en el mundo.

23) Japanese scientist presents advances in neurology in Congress in Valparaíso
Newspaper: El Mercurio de Valparaíso
Date: April 20, 2015
Scope: Regional

Científico japonés presentó adelantos en neurología durante congreso en Valparaíso

Fotografía: Media Training Consultores
pemoreno@mercuriovalpo.cl

Dr. Akio Suzumura, científico japonés del Instituto Nacional de Ciencias Fisiológicas de Okazaki, presentó en el Parque Cultural de Valparaíso sus investigaciones sobre nuevos métodos para frenar enfermedades neurológicas, tales como Parkinson, ELA, Alzheimer y Esclerosis Múltiple. Estos hallazgos, fueron expuestos en el marco del Congreso Internacional Gap Junction 2015, organizado por el Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso (CINV). La actividad, que por primera vez se realizó en América Latina, contó con la participación de 150 investigadores procedentes de Canadá, Estados Unidos, Alemania, Bélgica, Reino Unido, Suiza, China, Uruguay y Chile, entre otros países. cs

Dr. Akio Suzumura de Japón, y Dr. Juan Carlos Sáez, investigador Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso (CINV).

Mathieu Vinken, Universidad de Bruselas; María Dagli y Bruno Cogliati, Universidad de Sao Paulo, Brasil, y Michael Maes.

Klaus Willecke, Universidad de Bonn, Alemania, y Dr. Oliver Schmachtenberg, investigador Centro Interdisciplinario de Neurociencia, Universidad de Valparaíso CINV.

Vytas Verselis, Escuela Medicina Albert Einstein, Estados Unidos; Marwan El Sabban, Universidad de Beirut; Colin Green, Universidad de Auckland y Virgis Valiunas, Universidad de Stony Brook, Estados Unidos.

Michael Maes; Laura Walrave y Yeri Kim, Universidad de Auckland, Australia.

24) In Valparaíso two million dollars will be invested in young scientists
Newspaper: El Mercurio
Date: April 26, 2015
Scope: National

EL MERCURIO
ECONOMÍA Y NEGOCIOS

Acuerdo:

En Valparaíso se invertirán dos millones de dólares en científicos jóvenes

En la ciudad de Valparaíso se invertirán dos millones de dólares para incentivar la ciencia joven, durante un período de siete años. Esta propuesta se materializará gracias a un convenio suscrito por representantes del Ministerio de Educación e Investigación de Alemania; Institutos Max Planck; Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso, CINV, Iniciativa Científica Milenio y Universidad de Valparaíso.

Mediante esta alianza, se seleccionará a dos científicos jóvenes de excelencia, provenientes de Chile o del extranjero. Estos serán invitados a radicarse en la ciudad porteña y desde allí organizar su investigación en neurociencia, durante siete años. Para estos fines, el CINV entregará 150 mil dólares anuales a cada investigador seleccionado, quienes van a poder disponer libremente de los recursos para ejecutar su proyecto científico.

Andreas Trepte, director para América Latina de Institutos Max-Planck; Volker Rieke, director general de Ministerio de Educación e investigación de Alemania; Virginia Garretón, directora Iniciativa Científica Milenio; Aldo Valle, rector Universidad de Valparaíso, y Dr. Ramón Latorre, director Centro Interdisciplinario de la Neurociencia de Valparaíso.

El acuerdo de cooperación, que lleva por nombre "Grupos Tandem Max Planck- CINV en Investigación en Neurociencia", se firmó en la Escuela de Derecho de la Universidad de Valparaíso, en una ceremonia encabezada por la primera autoridad universitaria, Dr. Aldo Valle.

Este modelo ha sido aplicado en Alemania y Estados Unidos, asegurando generaciones de científicos de excelencia.

25) Japanese scientist presents advances in neurology during congress held in Valparaíso
Newspaper: El Mercurio
Date: April 27, 2015

Investigación científica:

Científico japonés presentó adelantos en neurología durante congreso efectuado en Valparaíso

Los doctores Akio Suzumura y Juan Carlos Sáez, durante seminario organizado por el Centro Interdisciplinario de Neurociencias, de la Universidad de Valparaíso, CINV

Dr. Akio Suzumura, científico japonés del Instituto Nacional de Ciencias Fisiológicas de Okazaki, presentó en Chile sus investigaciones sobre nuevos métodos para proteger a las neuronas y frenar enfermedades neurológicas, tales como: Parkinson, ELA, Alzheimer y Esclerosis Múltiple. Estos hallazgos, fueron expuestos en el marco del Congreso Internacional Gap Junction 2015, organizado por el Centro Interdisciplinario de Neurociencia, de la Universidad de Valparaíso, CINV.

La actividad, que por primera vez se realiza en América Latina, se efectuó en el Parque Cultural de Valparaíso y contó con la participación de 150 investigadores procedentes de Canadá, Estados Unidos, Alemania, Bélgica, Reino Unido, Suiza, China, Uruguay y Chile, entre otros países.

Durante esta conferencia se abordaron temáticas relacionadas con enfermedades neurológicas, arritmias cardíacas, sordera, cáncer y distrofias musculares.

Los estudios del Dr. Akio Suzumura y del CINV, han demostrado que ante ciertas enfermedades como las neurodegenerativas, existe una sobreexpresión de unas estructuras cerebrales llamadas hemicanales –que actúan como conductos de comunicación entre las células y el medio extracelular-. A partir de hallazgo, el investigador japonés ha realizado investigaciones en modelos in vivo, que muestran que bloqueando esas estructuras, se puede frenar la progresión de varias enfermedades neurológicas de alta incidencia en el mundo.

"Encontramos una molécula que bloquea los hemicanales y la liberación del neurotransmisor glutamato, protegiendo a la neurona. Lo hemos probado en distintos modelos animales de enfermedades humanas neurodegenerativas, constituyendo así un hallazgo importante y prometedor en el tratamiento para diferentes patologías", explicó el Dr. Suzumura.

26) Book travels through Valparaíso and shows "Entertaining" Science
Newspaper: La Estrella de Valparaíso
Date: May 11, 2015
Scope: Regional

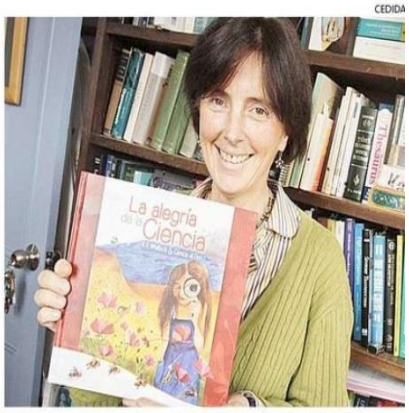
Libro recorre Valparaíso y muestra ciencia "entrete"

"La alegría de la Ciencia", escrito por la neurobióloga Kathleen Withlock

Cata, una niña porteña y muy curiosa, es la protagonista del libro "La Alegría de la Ciencia", una obra ilustrada por la artista plástica, Bettiana Castro, y escrita por Kathleen Whitlock, directora del programa "Ciencia Al Tiro" e investigadora del Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso (CINV).

La obra fue presentada en el Museo de Historia Natural de Valparaíso. La ceremonia de lanzamiento fue encabezada por el rector Aldo Valle y el director del CINV, doctor Ramón Latorre. También asistieron los decanos de las facultades de Ciencias y Medicina, Juan Kuznar y Antonio Orellana, respectivamente; la directora del museo, Loredana Rosso, junto a investigadores, académicos, estudiantes de postgrado y alumnos del programa "Ciencia al Tiro". Kathleen Whitlock destacó que el libro está orientado a niños y adultos, además intenta romper con el estereotipo que hoy existe de los científicos. "Todos piensan que ciencia es aburrida, pero en realidad es súper interesante y entretenida. Además, los 12 talleres —que están insertos en el libro— están relacionados con cosas que podemos hacer en la vida, así los niños aprenden a hacer biogás o reducir la basura para producir compost (abono orgánico). Es algo que todos podemos aprovechar", sostuvo.

CEDIDA



KATHLEEN WITHLOCK Y SU LIBRO "LA ALEGRÍA DE LA CIENCIA".

27) The Science Teacher
Magazine: Qué Pasa
Date: June 5, 2015
Scope: National

LA PROFESORA DE
ciencias

En 2008, la neurobióloga Kathleen Whitlock fundó en Valparaíso Ciencia Al Tiro, un programa de ciencia experimental para ayudar a alumnos de liceos vulnerables. Ya lanzaron su primer libro.

[Por Nicolás Alonso // Fotos: Dedvi Missene]

La casona de dos pisos, en el cerro Playa Ancha, parece un centro cultural. El portón está cubierto por un grafiti que muestra a unos niños jugando con pájaros. Tras la fachada verde, en la sala principal, hay otro: una mapuche, un español y una mestiza tocan música, y un pájaro se posa sobre el chelo. Es lunes, y la estadounidense Kathleen Whitlock (51), neurobióloga del Centro Interdisciplinario de Neurociencia de Valparaíso, también chelista y artista plástica, observa esa imagen. Ella la ideó, como toda la casona, que compró quemada en 2012 y reinauguró, con vitrales en las ventanas, al año siguiente. Y bajo los vitrales situó los microscopios, y en ellos los embriones de pez cebra, las trozos de cerebro, las muestras. Todas las cosas que transforman este centro cultural en lo que en realidad es: un raro y hermoso centro científico.

Abre la puerta de una sala y queda claro: a la izquierda hay una jaula de un erizo, conectada a una máquina que mide su actividad nocturna. A la derecha, los microscopios con em-

28) Valparaiso: launching of educational book on "The Joy of Science"
Newspaper: El Mercurio
Date: June 6, 2015
Scope: National

· Vida Empresarial ·

EL MERCURIO

B 15

Ceremonia:

En Valparaíso lanzaron libro educativo sobre "La Alegría de la Ciencia"

En una ceremonia encabezada por el Dr. Ramón Latorre, director del Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso, CINV, y Aldo Valle, rector de la U. de Valparaíso, se realizó el lanzamiento del libro La Alegría de la Ciencia, elaborado por la Dra. Kathleen Whitlock, investigadora del CINV.

La presentación de este material artístico y educativo se desarrolló en el Museo de Historia Natural de Valparaíso y contó con la presencia de Loredena Rosso, directora del museo; científicos, autoridades y estudiantes de la escuela Pacífico, de la ciudad porteña.

Juan Carlos García, gerente Centro Interdisciplinario Neurociencia, Universidad de Valparaíso, CINV; Dr. Ramón Latorre, director Centro Interdisciplinario Neurociencia, Universidad de Valparaíso, CINV; Dra. Kathleen Whitlock, investigadora CINV y autora del libro; Loredana Rosso, directora Museo Historia Natural de Valparaíso, y Aldo Valle, rector Universidad de Valparaíso.

CIENCIA Y ARTE

La obra, que cuenta con doce capítulos y coloridas ilustraciones, tiene como protagonista a Cata, personaje de ficción que recorre la geografía del país y de Valparaíso —ciudad en la que vive—, fabricando guateros mágicos para los pescadores o un motor simple con imanes, entre otras tareas, que buscan enseñar y transmitir la idea de que "la ciencia es entretenida y está en todas partes", según comenta su autora.

Las acuarelas son de Bettiana Castro y las ilustraciones de Albert "Alex" Rojas, ambos, artistas porteños que rescatan nuestro patrimonio, a través de paisajes chilenos, como las Torres del Paine, San Pedro de Atacama , el Volcán Llaima —en Región de La Araucanía—, y principalmente, las calles del puerto.

Durante el lanzamiento, Ramón Latorre destacó la importancia de este proyecto, que combina ciencia y arte, como un ejemplo vital para mejorar el modelo educativo chileno.

"Hay una falta de educación cariñosa en Chile, que enseñe cómo acercarse al descubrimiento, y a ver la naturaleza directamente. Por tanto, ejemplos como este libro son los que pueden contribuir a mejorar nuestro país. Si los niños aprenden a descubrir la verdad desde pequeños, probablemente no tendremos los problemas que hoy existen"

Por su parte, el rector Aldo Valle felicitó a la Dra. Whitlock y a los científicos de CINV. "Contamos con un centro de neurociencia, cuyo equipo de científicos agrega un sentido cívico y social que nos llena de orgullo como universidad. Este libro es una iniciativa que celebramos, pues hace una contribución edificante".

29) **Interview with Dr. John Ewer on the change in time schedule.**
TV Channel: Megavisión. Noticias Ahora Noticias, edición Matinal
Date: June 12, 2015
Link: www.audionoticias.net/videos/llambias/llambias2.htm
Scope: National

30) On schedules and other Demons
Newspaper: La Segunda
Date: June 17, 2015
Scope: National

Migración como oportunidad

Felipe Alessandri V.
Concejal de Santiago

A mediados del siglo pasado la población migrante era mayoritariamente europea, algunas familias árabes y algo de orientales. En medio siglo aquello mutó y hoy la migración tiene un fuerte acento sudamericano. Comunas como Santiago, han recibido parte importante de la población de extranjeros que ha llegado al país, considerándolos en todos los planes municipales.

No por nada, la colonia peruana residente celebra sus distintas festividades en nuestras calles y parques e incluso a un sector de calle Catedral le llaman la pequeña Lima. Los hijos de migrantes acuden a nuestras escuelas y consultorios, siendo el Municipio capitalino un promotor de la inclusión. Generándose por lo general, una considerable acogida a los extranjeros en nuestra comuna, lo que es extrapolable a todo el territorio nacional.

No obstante aquello, Chile cuenta con la legislación migratoria más antigua de América Latina, y que a estas alturas resulta anacrónica. El Decreto Ley 1095, de 1975, que nos rige fue concebido bajo términos restrictivos, se caracteriza por el proteccionismo y la rigidez, probablemente inspirado en la situación regional y nacional que se vivía al momento de su dictación. La migración, por esos años, se miraba con cierto recelo y se percibía por algunos sectores como una amenaza; lo que hoy resulta insostenible, añejo e injustificado.

"Chile cuenta con la legislación migratoria más antigua de América Latina, que resulta anacrónica".

El fenómeno migratorio abordado integralmente y bien conducido, puede ser tremendamente positivo para el país. Desde aspectos muy diversos, tales como el aumento de la natalidad, el emprendimiento y como elemento para solucionar las deficiencias propias del mercado laboral, entre otras. Un avance sustancial fue el Proyecto de Ley de Migraciones, ingresado a tramitación bajo la administración Piñera. Sorprende eso sí, que a quince meses de iniciado el actual Gobierno, no se hayan visto avances concretos en la materia.

Así las cosas, surge la imperiosa necesidad, tal como la actual Presidenta comprometió en su campaña presidencial, de señalar que: "(...) evaluaremos modificaciones a la legislación migratoria que cambie el enfoque actual, basado en una perspectiva de seguridad y de gestión de mano de obra inmigrante, por una perspectiva de inclusión, integración regional y un enfoque de derechos que aseguren la inserción efectiva de esta población al país y que permita una coordinación dinámica, cooperadora y eficiente de todos los entes públicos relacionados con la política migratoria"; de avanzar en el tema y ponernos al día en materia migratoria; ya que lejos de ser una amenaza, la migración es una oportunidad.

Testigos del compromiso ético

María Isabel Muñoz
Unión Social de Empresarios Cristianos

Mucho se ha hablado de la imperiosa necesidad de recomponer las confianzas, mal endémico y transversal, que afecta a varios sectores de nuestra sociedad, incluida la empresa, que enfrenta el desafío de revertir esta tendencia, reorientando con un enfoque moderno y sostenible aquellos aspectos clásicos de administración y el viejo estilo de liderazgo en el desarrollo de negocios, con eminente sesgo economicista.

Para transitar de un primer estado de asombro e indignación al compromiso real, el país requerirá, de parte de nuestra clase empresarial, un renovado compromiso por un actuar coherente. Consistencia entre lo que se dice y se hace, como condición para recuperar la credibilidad y construir mejor reputación. Esto implica apuntar a lo esencial, a los valores y convicciones, desarrollar una nueva competencia: la pasión por el compromiso ético, sin perder de vista la huella del sentido humano y de la transcendencia de lo que hacemos, para evitar zonas opacas y conflictos éticos, para decidir en conciencia por sobre la conveniencia, cualquiera sea el eslabón de la creación de valor o segmento de industria, si se tiene en cuenta que un objetivo fundamental de las empresas de hoy y del mañana, será ser percibidos y reconocidos como sustentables, en una carretera que se desplaza a enorme velocidad, en un ambiente de híper-competencia, híper-transparencia y en plena era del comportamiento.

"El país requerirá, de parte de nuestra clase empresarial, un renovado compromiso por un actuar coherente".

Y esta aclamación y expectativa recibida desde distintos ejes, comenzando por el virtuoso y firme llamado del Papa Francisco, nos invita a acoger con humildad los valores fundamentales del recambio, recobrando fuerza la idea de articular una sociedad de testigos y no sólo de maestros, para hablar e interactuar desde la consistencia de cómo decidimos vivir nuestras vidas y la de nuestro entorno.

Tal vez no sea casualidad, que de la mano de la globalización y la hiperconexión, seamos "testigos y espectadores" de la proliferación de múltiples escándalos, casos de abusos, malas prácticas y trasgresiones graves a la ética y al sentido común, en principios que rigen actualmente como estándar del s. XXI para nuestros países y mercados -donde el mundo desarrollado ha acordado y establecido un amplio marco de consenso- pues son otros los tiempos y desafíos los que enfrentamos, en un espacio cada día más horizontal, con una nueva arquitectura del poder, donde pesa mucho más el impacto y fuerza de los hechos que la retórica de 1.000 palabras. Desde este enfoque, el desafío de hacer empresa, crear valor y aportar al desarrollo, implica de manera irrefutable integrar el valor de la solidaridad, la verdad y el bien común ¡Porque las palabras mueven, pero el ejemplo arrastra!

John Ewer
Centro Interdisciplinario de Neurociencia de Valparaíso
U. Valparaíso

De horarios y otros demonios

A raíz del reportaje al Ministro de Energía, Máximo Pacheco de ayer, respecto de la decisión del Gobierno de mantener el horario y elegir el de verano, la primera decisión me parece acertada.

Existe evidencia que el cambio de hora que se realiza en la primavera tiene consecuencias nefastas para muchos, ya que en la mañana obliga a personas a funcionar una hora antes de lo normal, periodo durante el cual su estado de alerta es menor que antes del cambio de hora. Este adelanto horario puede por lo tanto aumentar la probabilidad de accidentes y también tiene consecuencias para la salud. Interesantemente, en algunas personas este llamado "jet-lag social" persiste por varias semanas; ello no es aparente simplemente porque nuestras vidas están regidas por relojes y horarios, pero es fácil de demostrar.

La segunda decisión, de mantener el horario de verano, me parece por lo contrario muy poco acertada. Nuestros patrones de sueño y vigilia son regulados por nuestro reloj biológico, y el estímulo que es de lejos el más importante en fijar cuando comienza el dia es la luz solar. Utilizar el horario de verano significa que durante las mañanas invernales los habitantes de Chile tienen que funcionar mucho antes de lo dictado por su reloj biológico, con las consecuencias ya descritas. Esta situación es especialmente crítica para los niños: ellos naturalmente se despiertan mas tarde que los adultos (también duermen más, pero ese es otro tema). Es muy probable que no estén muy alertas durante las primeras horas de colegio, lo cual naturalmente impactaría en cuánto pueden aprender.

Cabe señalar además que el horario que le corresponde a Chile es en realidad de +1 hora con respecto al horario de invierno, o sea que el horario actual está desfasado en dos horas, no una. Por ultimo, es interesante que entre los 150 países que eligen no cambiar su horario, Chile es el único que decidió quedarse con el horario de verano. Si se quiere adoptar esta solución radical, debe demostrarse que este horario efectivamente es el mejor para nuestro país. Y basado en lo expuesto, este análisis debe incluir no solo el tema energético, sino los costos en salud, accidentes, y en cambios en el rendimiento escolar y en el trabajo.

31) **Studio interview with Dr. John Ewer, on the change of time schedule.**
TV Channel: Megavisión. Noticias Ahora Noticias, edición Mediodía
Date: June 19, 2015
Link: www.audionoticias.net/videos/llambias/llambias2.htm
Scope: National

32) **Interview with Dr. Ramón Latorre on the brain.**
TV Channel: TVN Señal Internacional. Programa Conectados
Date: June 19, 2015
Link: http://youtu.be/zXHldxr7WwU
Scope: National

33) The construction of the Severín building in the "Barrio Puerto", where 130 scientists will be housed, will start in October.
Newspaper: El Mercurio
Date: June 22, 2015
Scope: National

El nuevo recinto no implica solo recuperar un edificio en ruinas. Radicará en un barrio deteriorado la investigación de frontera y la vida diaria de sus protagonistas.

Centro tendrá tecnología pionera en sector fundacional, hoy degradado

Valparaíso: En octubre parten obras en edificio Severín para que 130 científicos se instalen en barrio Puerto

Ocuparán dependencias del nuevo centro interdisciplinario de neurociencia, que se levantará sobre las ruinas del recinto, que resultó afectado por un incendio.

MAURICIO SILVA

Tras la iglesia de La Matriz, donde nació Valparaíso, hay un lugar que en la Colonia fue convento; al nacer la República, sede del primer Congreso Bicameral chileno, batallón cívico en el siglo XIX, y 3ª Comisaría del Puerto en el siglo XX.

Desde hace décadas, en consonancia con el resto del barrio Puerto, se encuentra en decadencia, y en la actualidad solo hay ruinas. Pero, a partir de mediados de 2017, se llenará de académicos y estudiantes que explorarán las fronteras de la ciencia.

Eso, según el cronograma fijado por el Ministerio de Obras Públicas (MOP), que el 22 de mayo convocó a la licitación para construir, desde los calcinados muros del edificio Severín, destruido por un incendio en 2004, la sede del Centro Interdisciplinario de Neurociencia de la U. de Valparaíso (Cinv).

La empresa que se adjudique el proyecto, que tiene un presupuesto de $4.974 millones, deberá iniciar los trabajos en octubre y tendrá 540 días para concluir el edificio, que pasará a llamarse Abate Molina, en honor al primer científico chileno, el jesuita del siglo XVIII que también vivió en el lugar.

PLAN
El párroco de la iglesia de La Matriz, Gonzalo Bravo, espera que los proyectos en el barrio incluyan a los vecinos.

El centro científico aprovechará la energía solar y la reutilización del agua. Dispondrá de jardines y cafetería en su terraza y un auditorio para 200 personas donde tendrán lugar tertulias de neurociencia sobre diversos temas, tales como la conciencia, los sueños y el dolor. Albergará a 130 personas vinculadas al Cinv (entre científicos, estudiantes de pre y posgrado y técnicos), hoy desperdigados en diferentes dependencias de la U. de Valparaíso.

"Uno es visto como un científico loco. Costó que creyeran la idea de erigir algo así en un barrio deteriorado. Nadie pensaba que se pudiera recuperar", dice el Premio Nacional Ramón Latorre, que tardó siete años en conseguir el financiamiento de la Subdere, el MOP y la universidad.

Destaca que el impacto irá más allá de recuperar rasgos de la centenaria arquitectura ecléctica porteña, instalando complejos laboratorios tras una fachada de balcones voladizos y torreón. Los científicos del Cinv, algunos reclutados junto a la sociedad Max Planck, arrendarán habitaciones, comprarán en el comercio y comentarán con dependientes sus proyectos biotecnológicos.

La inversión apunta al corazón y, al mismo tiempo, zona más degradada del sitio declarado Patrimonio de la Humanidad, proceso que se aceleró en 2007 tras la explosión en calle Serrano. Otrora el principal sector comercial de Chile, cuando Valparaíso era el emporio del Pacífico Sur, las señoriales casonas del barrio Puerto están subdivididas y habitadas por jubilados y cesantes.

El alcalde Jorge Castro dice que se está gestando "la mayor inversión en 50 años" en el barrio Puerto.

34) Interview with Dr. John Ewer about the change in schedule.
TV Channel: Canal 13. Noticias Teletrace, edición central
Date: June 22, 2015
Link: https://youtu.be/-Bmu-7WL_NY
Scope: National

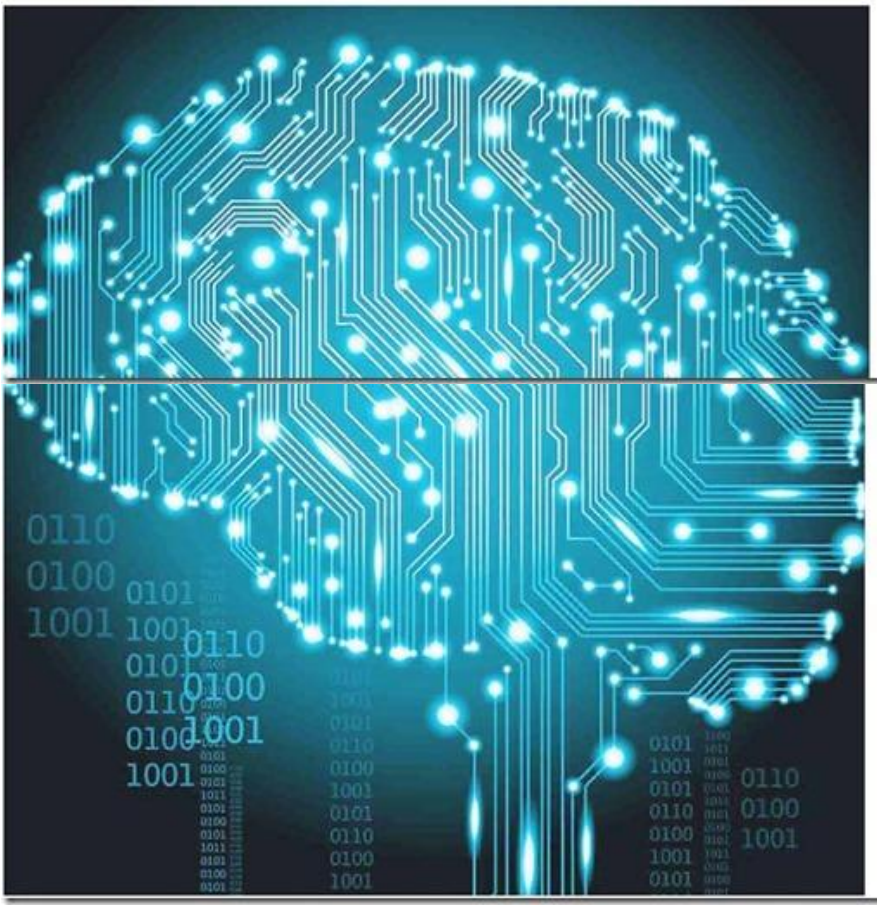
35) Interview on stage, Dr. John Ewer on controversy over new time schedule
TV Channel: TVN. Canal 24 Horas, Noticias
Date: June 23, 2015
Link: https://youtu.be/VCYAPwTXUdA
Scope: National

36) Construction of Valparaíso Science Building to begin in October
Newspaper: El Mercurio de Valparaíso, Special Edition "Centros de Estudio en Investigación de Tecnología y Ciencia"
Date: June 27, 2015
Scope: Regional

Futuro científico regional

Edificio de la Ciencia porteño comenzará su construcción en octubre

Obra impulsada por el Centro Interdisciplinario de Neurociencia de la U. de Valparaíso, busca posicionar a la ciudad como referente de la investigación en Latinoamérica. Futuro inmueble Abate Molina, ubicado en Barrio La Matriz, acogerá a más de 150 científicos nacionales y extranjeros. Edificación considera aportes del CORE, Universidad de Valparaíso y Ministerio de Obras Públicas. El proyecto considera una inversión de 5 mil millones de pesos.



El futuro edificio de la ciencia en Valparaíso, llamado Abate Molina (en honor al primer científico chileno, el jesuita del siglo XVIII que también vivió en el lugar), es un proyecto arquitectónico cada día más cercano a su creación, luego de que el Ministerio de Obras Públicas convocara a fines de mayo a una licitación para construir este inmueble, que contará con una inversión de 5 mil millones de pesos. Esta obra, situada en el barrio La Matriz, nacerá por iniciativa del Centro Interdisciplinario de Neurociencia de la U. de Valparaíso, entidad que busca posicionar a la ciudad puerto en la referencia de la investigación científica latinoamericana.

El edificio, ex sede del primer Congreso en Chile, se levantará con aportes del CORE ($2.500 millones), la Universidad de Valparaíso ($1.500 millones) y Ministerio de Obras Públicas ($1.000 millones). El inicio de obras comenzará el próximo mes de octubre.

El inmueble acogerá a más de 150 investigadores nacionales y extranjeros y estará abierto a la comunidad a través de actividades científicas y culturales. Su materialización, subrayan en el CINV, espera contribuir a la revitalización económica del barrio fundacional de la ciudad puerto.

"El nuevo edificio constituirá un faro de la ciencia para Chile, acogiendo a investigadores, estudiantes y también a la comunidad. Queremos hacer de este espacio abierto al conocimiento uno de los centros de investigación en neurociencia de excelencia a nivel mundial", subraya Ramón Latorre, Premio Nacional de Ciencias y director del CINV.

37) Cuban Biophysicist discovered how asthma develops
Newspaper: El Mercurio de Valparaíso
Date: July 7, 2015
Scope: Regional

EL DOCTOR CARLOS GONZÁLEZ SALIÓ A LOS 16 AÑOS DE CUBA.

Biofísico cubano descubrió cómo se desarrolla el asma

CIENCIA. *Trabaja en el CINV de la Universidad de Valparaíso.*

El doctor Carlos González, científico cubano de 49 años de edad, quien llegó a nuestro país y, especialmente a Valparaíso, motivado por el "gran desarrollo de la biofísica", descubrió cómo se desarrolla el asma, desorden inflamatorio crónico que afecta, aproximadamente, a un 10% de la población mundial, generando episodios de dificultad respiratoria, tos y ruido torácicos agudos, entre otras manifestaciones.

El investigador es integrante del Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso (CINV) y profesor titular de dicha institución, dedicada a investigar el funcionamiento del sistema nervioso, desde las proteínas que captan señales del exterior, hasta el comportamiento de redes neuronales. Dicho centro es dirigido por el Dr. Ramón Latorre, Premio Nacional de Ciencias.

10%
de la población padece de esta afección respiratoria que se gatilla principalmente de noche o en la madrugada.

1965
salió el doctor González de Cuba a Rusia y desde el principio lo preocupó el asma como enfermedad debido a que la padece.

vedette del asma es el canal de protones" que por tantos años ha estado explorando. "Esta enfermedad es un tema viejo para la humanidad, pero a la vez, bastante nuevo a nivel molecular y tiene mucha relación con el sistema inmune.

El Dr. González logró indagar en estos mecanismos y conocer el funcionamiento de proteínas claves en esta enfermedad, cuyos resultados han sido publicados en revistas de alto impacto científico, como Nature. Esto permitirá, en un futuro, establecer medicamentos y terapias paliativas contra el asma.

El biofísico ha peregrinado por diversos países desde su salida a los 16 años de Cuba rumbo a Rusia (Unión Soviética, en ese entonces). De ahí que muchos de sus colegas lo llaman "el patiperro de la ciencia".

El CINV tiene un enfoque multidisciplinario, reuniendo a biofísicos, fisiólogos, neurobiólogos, expertos en genómica, bioinformática y modelación molecular. Asimismo, se preocupa de la formación de futuros neurobiólogos.

LA "VEDETTE" DEL ASMA

El investigador es asmático desde los tres meses de edad, situación que lo impulsó a explorar esta enfermedad desde la biofísica, algo inédito para esta disciplina. El objetivo era entender su comportamiento desde un nivel molecular.

El asma, originada particularmente por contaminación ambiental y factores alérgicos, suele gatillarse con más frecuencia durante la noche y en las madrugadas. Para el científico, conocer en profundidad cómo funcionan ciertas estructuras del organismo, llamadas canales iónicos, es la base para determinar el desarrollo de fármacos.

Para el investigador la "gran

"ES UN APORTE"

"Los biofísicos nos dedicamos a investigar las estructuras de las moléculas, como las proteínas. Pero si tú logras conocer la interioridad de estas proteínas, cómo se activan o reaccionan, puedes ayudar al diseño de mejores terapias y medicamentos para contrarrestar el problema. En ese sentido, este trabajo sin duda es un aporte".

González señala que éste es el rol de la ciencia de base, "ya que sin un conocimiento profundo del ser humano es imposible encontrar respuesta a nuestras enfermedades y el diseño de medicamentos como parte de la investigación aplicada".

Agrega que jamás se debe menospreciar la ciencia básica, que es aquella que entrega todas las coordenadas. Asimismo, declara que si no se dedican esfuerzos y recursos al desarrollo de las teorías, no se pueden generar ni mejorar, las estrategias terapéuticas y fármacos. C3

38) The biology of schedules
Newspaper: El Mercurio de Valparaíso
Date: July 9,2015
Scope: National

ESPACIOABIERTO

Biología de los horarios

John Ewer Lothian
Centro Interdisciplinario de Neurociencia U. de Valparaíso

VIVIMOS EN un mundo de despertadores y de luz artificial, y olvidamos que la luz diaria del sol es la señal que fija cuando nos despertamos. Y aunque pensamos que podemos organizar nuestros horarios (y los de otros) a nuestra conveniencia, nuestro despertar está regido por nuestro reloj biológico. Así, el horario de la luz del sol instruye a nuestro reloj interno y determina cuando nos despertaremos. Tan preciso es este control que en ausencia del despertador, nos despertamos cuatro minutos más tarde por cada grado de longitud que nos desplazamos hacia el oeste (aproximadamente 100 Km), que es exactamente el tiempo que tarda el sol en recorrer esta distancia al amanecer.

La influencia del reloj biológico es evidente en días libres, cuando la mayoría nos despertamos una a dos horas más tarde. Esto significa que todos los días en que usamos un despertador quedamos con un déficit de sueño de una a dos horas. Este "jet-lag social" tiene consecuencias a largo plazo sobre nuestra salud: por ejemplo, personas con tendencia a aumentar de peso son más propensas a ser obesas; otros podrían desarrollar diabetes o hipertensión.

Cuanto mayor el déficit de sueño, mayor el impacto. Así, los que trabajan de noche (por ejemplo, nocheros) son los más afectados; pero el déficit de sueño también impacta al resto de la población, contribuyendo al aumento en la incidencia de estas y otras enfermedades.

Estos desajustes también afectan nuestro desempeño. El grupo más impactado son los adolescentes, quienes a esa edad se despiertan naturalmente más tarde. Pero los niños también aprenden menos en el colegio durante las primeras horas del día y los adultos hacemos menos bien nuestro trabajo. Por ello, atrasar el horario de algunas actividades tendría un efecto positivo sobre el aprendizaje y el desempeño de muchos, medida que están tomando algunos países europeos atrasando la hora del inicio de clases, de exámenes, etc.

Con estos antecedentes se hace evidente el problema causado por la decisión que ha tomado Chile de mantener el horario de verano durante todo el año. En un día invernal nuestro reloj interno nos despierta más tarde, y por lo tanto durante un día iniciado por un despertador nuestro déficit de sueño es ahora aún mayor, exacerbando los problemas de salud y desempeño antes mencionados.

En resumen, causar desajustes entre nuestro reloj biológico y el horario impuesto tiene consecuencias negativas documentadas sobre la salud y la productividad, y es importante que el gobierno abandone cuanto antes el horario actual. Y si quisiera hacer un experimento interesante podría cambiar el horario en dos horas en la otra dirección. Este sería el mejor horario para nuestra localización geográfica, ya que maximizaría el ajuste entre el reloj social y el biológico.

Apelar a la situación de países de latitudes extremas como los países nórdicos, que en el invierno tienen menos horas de sol que la mayoría de los chilenos, no viene al caso. Ellos han vivido por siglos con esta situación, condicionada por su ubicación geográfica, y buscan, al igual que nosotros, pero con mayores dificultades, adecuar lo más posible su horario a su reloj biológico.

39) Venezuelan Biophysicist signs agreement with Valparaíso Scientists
Newspaper: El Mercurio de Valparaíso
Date: July 23, 2015
Scope: Regional

Biofísico venezolano sella alianza con científicos de Valparaíso

CIENCIA. *Buscan identificar drogas que ayudan a remediar arritmias.*

El Dr. Luis Cuello, científico venezolano, autor de investigación sobre seis fármacos que permiten inhibir y enfrentar las enfermedades cardiacas, llegó a Chile para asistir -como relator invitado- a la Primera Jornada Invernal de Neurociencia, a realizarse mañana, en la Facultad de Ciencias de la Universidad de Valparaíso.

La actividad, dirigida a estudiantes de pregrado e interesados en perfeccionar sus estudios en neurociencia, busca generar espacios de interacción con alumnos y conocer los últimos avances en este ámbito científico. En la oportunidad, el Dr. Cuello se reunirá con estudiantes de postgrado, abordando aspectos teóricos de canales iónicos y estructuras de proteínas de membrana, así como temas prácticos sobre técnicas de avanzada. "Esperamos que ésta sea una experiencia de mucha interacción y diálogo con los estudiantes. Ellos también tendrán la oportunidad de presentar sus trabajos", señala el investigador.

RESOLVER PREGUNTAS

Para los directores de CINV contar con la presencia del biofísico venezolano y conocer sus técnicas de investigación abre las puertas a "resolver nuevas preguntas científicas y plantear los desafíos futuro de este centro".

CINV

DOCTOR LUIS CUELLO.

Conocer la forma y funcionamiento de pequeñas moléculas del ser humano, llamadas canales de potasio, es un elemento clave de la ciencia para estudiar nuevos fármacos contra enfermedades cardíacas.

El doctor Cuello, biofísico venezolano y profesor de Texas Tech University Health Sciences Center (TTUHSC), en colaboración con el Centro Interdisciplinario de Neurociencia, de la Universidad de Valparaíso, CINV, comenzará a analizar seis nuevas drogas contra males cardíacos, entre ellos, las arritmias.

"Contribuir a corregir arritmias y otros problemas cardíacos es de gran importancia, ya que estas patologías afectan a la población mundial cada vez con más frecuencia. Y por ello, esta alianza con CINV, forjada hace varios años, constituye un bello esfuerzo multilateral. En este caso particular, nuestro objetivo es realizar una caracterización sistemática de cómo funcionan estos fármacos", señala el Dr. Cuello.

40) Scientists from 15 countries compete in Chilean neuroscience contest.
Newspaper: El Mercurio

Date: August 17, 2015
Scope: National

Investigación:

Científicos de 15 países compiten en concurso chileno de neurociencia

Investigadores residentes en 15 países respondieron a la convocatoria del concurso internacional organizado por la alianza de cooperación entre el Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso (CINV) y el instituto Max Planck de Alemania, y que permitirá que dos científicos jóvenes desarrollen neurociencia de impacto global en la zona patrimonial de la ciudad puerto, por los próximos cinco años.

Los postulantes son 36 científicos, de 33 a 44 años de edad, provenientes de cuatro continentes. El 50% de los concursantes son chilenos que, en su mayoría, están cursando programas de doctorado y posdoctorado en diferentes países del mundo. Una comisión evaluadora formada por académicos del CINV, Max Planck y Universidad de Valparaíso realizará una preselección para convocar a diez finalistas a un simposio que tendrá lugar los próximos 19 y 20 de noviembre. Allí se elegirán los dos ganadores, quienes recibirán un total de US$ 1.500.000 para realizar investigaciones por los próximos cinco años.

Autoridades del instituto alemán Max Planck y de la Universidad de Valparaíso, durante visita al terreno donde se construirá el edificio de la ciencia, en barrio la Matriz de Valparaíso.

"Sus postulaciones reflejan el interés de científicos jóvenes de gran nivel, de todas partes del mundo, por hacer ciencia desde Chile y en particular desde Valparaíso. Esto demuestra que con los incentivos adecuados, como libertad para la creatividad y recursos suficientes para desarrollar su trabajo, se pueden generar cambios. Nuestro anhelo es convertirnos en un semillero de científicos de nivel mundial", dijo Ramón Latorre, premio Nacional de Ciencias y director del CINV.

Los estudiantes comenzarán su labor en 2016, bajo una modalidad de trabajo que les dará libertad para administrar recursos y conformar equipos. El programa contempla evaluaciones internacionales para garantizar excelencia y viajes anuales a Alemania para recibir apoyo directo por parte de especialistas del Max Planck.

41) Minister of National Assets granted land for site where science building in Valparaíso will be built.
Newspaper: El Mercurio

Date: August 3, 2015
Scope: National

Ceremonia:

Ministro de Bienes Nacionales entregó concesión de terrenos para construir edificio de la ciencia en Valparaíso

Rector Aldo Valle recibe formalmente los terrenos para la construcción del futuro edificio de la ciencia. A la izquierda, el Dr. Ramón Latorre.

El ministro de Bienes Nacional, Víctor Osorio, hizo entrega de la concesión de terrenos en que el Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso, CINV, construirá el nuevo edificio de la ciencia, llamado Abate Molina.

La ceremonia contó con la presencia del intendente de la Región de Valparaíso, Ricardo Bravo; el gobernador provincial, Omar Jara; el rector de la U. de Valparaíso, Aldo Valle; la directora regional de Cultura, Nélida Pozo, y el director de CINV, Dr. Ramón Latorre.

El inmueble, ex sede del primer Congreso en Chile, rescatará la fachada del antiguo Edificio Severín y contará con una inversión de 5 mil millones de pesos. En su interior acogerá a más de 150 investigadores nacionales y extranjeros, permaneciendo abierto a la comunidad en torno a la actividad científica y cultural. El Dr. Latorre, premio nacional de Ciencias, explica que con esta iniciativa se busca recuperar un espacio patrimonial, revitalizar la ciudad puerto y "posicionar a Valparaíso en la referencia de la investigación científica latinoamericana".

42) Cuban biophysicist in Chile discovered how asthma develops
Web Page: Diario Financiero – Portafolio de Salud
Date: August 7, 2015
Link: www.df.cl

Scope: National

ACTUALIDAD 28/07/2015

Biofísico cubano descubrió en Chile cómo se desarrolla el asma

Artículo | Comentarios (0)

Compartir

(28/07/2015).- El Doctor Carlos González, científico cubano que llegó a nuestro país motivado por el "gran desarrollo de la biofísica", descubrió cómo se desarrolla el asma, desorden inflamatorio crónico que afecta aproximadamente al 10% de la población mundial, generando episodios de dificultad respiratoria, tos y ruido torácicos agudos, entre otras manifestaciones.

El asma, originada particularmente por contaminación ambiental y factores alérgicos, suele gatillarse con más frecuencia durante la noche y en las madrugadas.

Noticias Relacionadas

1. Se espera pronta aprobación de terapia para frenar distrofia muscular de Duchenne
2. Detectan barreras para aumentar diagnóstico de VIH/SIDA en Chile
3. Ingesta de aspirina reduciría hasta en 50% la posibilidad de padecer cáncer de estomago

Para el científico del Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso (CINV), conocer en profundidad cómo funcionan ciertas estructuras del organismo, llamadas canales iónicos, es la base para determinar el desarrollo de fármacos. El objetivo del científico era poder entender el comportamiento del asma desde un nivel molecular.

El Dr. González logró indagar en estos mecanismos y conocer el funcionamiento de proteínas claves en esta enfermedad, cuyos resultados han sido publicados en revistas de alto impacto científico –como Nature–, lo que permitirá, en un futuro, establecer medicamentos y terapias paliativas contra el asma.

"Los biofísicos nos dedicamos a investigar las estructuras de las moléculas, como las proteínas. Pero si tú logras conocer la interioridad de estas proteínas, cómo se activan o reaccionan, puedes ayudar al diseño de mejores terapias y medicamentos para contrarrestar el problema. La gran vedette del asma es el canal de protones".

43) Drugs against heart diseases are studied at the the University of Valparaíso.
Web Page: Diario La Nación
Date: August 10, 2015
Link: www.lanacion.cl
Scope: National

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EN UNIVERSIDAD DE VALPARAÍSO ESTUDIARÁN FÁRMACOS CONTRA MALES CARDÍACOS

Investigación que se realizará en el Centro Interdisciplinario de Neurociencia de la casa de estudios será dirigida por el biofísico venezolano y profesor de de Texas Tech University Health Sciences Center, Luis Cuello. Los 6 medicamentos analizados ya han sido aprobados para otros usos por la Food and Drugs Administration norteamericana, FDA, hecho que imprime optimismo en los investigadores, puesto que garantiza la seguridad de su aplicación en seres humanos.

Viernes 7 de agosto de 2015 | por La Nación Publicado por: Patricia Schüller G. + Sigue a La Nación en Facebook y Twitter

Seis nuevas drogas contra **males cardíacos**, entre ellos las **arritmias**, comenzarán a ser analizadas en el Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso **(CINV)**. Investigación será dirigida por **Luis G. Cuello, biofísico venezolano y profesor de Texas Tech University Health Sciences Center** (TTUHSC).

Los medicamentos que serán estudiados, ya han sido aprobados para otros usos por la **Food and Drugs Administration norteamericana, FDA**, hecho que imprime optimismo en los investigadores, puesto que garantiza la seguridad de su aplicación en seres humanos.

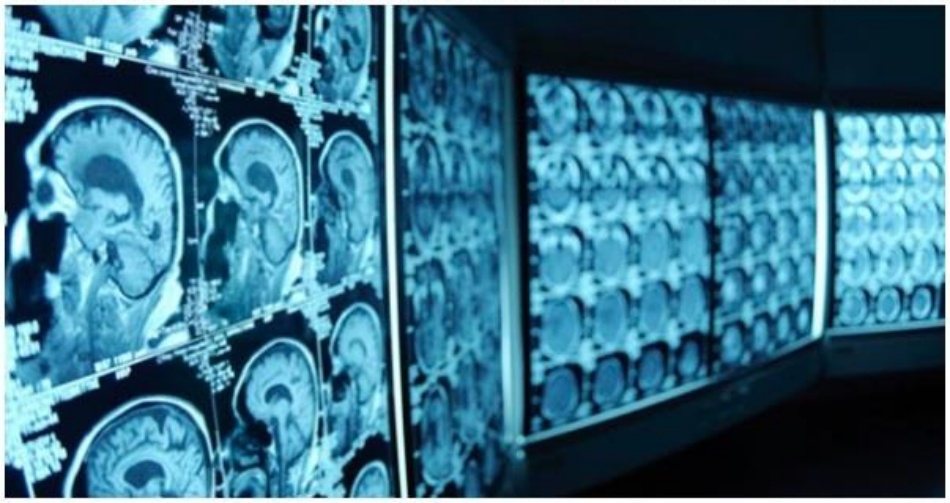
Estos fármacos fueron descubiertos por **Cuello, junto al doctor Guillermo Altenberg**, ambos profesores del departamento de Fisiología Molecular en la TTUHSC .

Por medio de técnicas como la cristalografía, que permite obtener imágenes de moléculas para luego "generar películas de alta resolución", Cuello busca ayudar a la generación de tratamientos más específicos y que no generen efectos adversos, labores que realizará con apoyo del doctor **Carlos González, del CINV.**

"Contribuir a corregir arritmias y otros problemas cardíacos es de gran importancia, ya que estas patologías afectan a la población mundial cada vez con más frecuencia. Y por ello, esta alianza con CINV, forjada hace varios años, constituye un bello esfuerzo multilateral. En este caso particular, **nuestro objetivo es realizar una caracterización sistemática de cómo funcionan estos fármacos**", explica Cuello.

44) Chilean neurosciences contest in Valparaíso seeks to become hotbed of word-class scientists
Web Page: El Mostrador.
Date: August 14, 2015
Link: www.elmostrador.cl
Scope: National

Concurso chileno de neurociencias en Valparaíso busca convertirse en semillero de científicos de nivel mundial



Un total de 36 postulantes presentaron sus proyectos y solo diez llegarán al gran evento final del 19 y 20 de noviembre. Certamen contempla la elección de dos ganadores. Cada uno recibirá 750 mil dólares para realizar sus investigaciones desde la ciudad puerto. Convocatoria fue realizada por Centro Interdisciplinario CINV de Valparaíso e instituto alemán Max Planck.

Investigadores residentes en 15 países, respondieron a la convocatoria del concurso internacional organizado por la alianza de cooperación entre el Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso (CINV) y el instituto Max Planck de Alemania, y que permitirá que dos científicos jóvenes desarrollen neurociencia de impacto global en la zona patrimonial de la ciudad puerto, por los próximos cinco años.

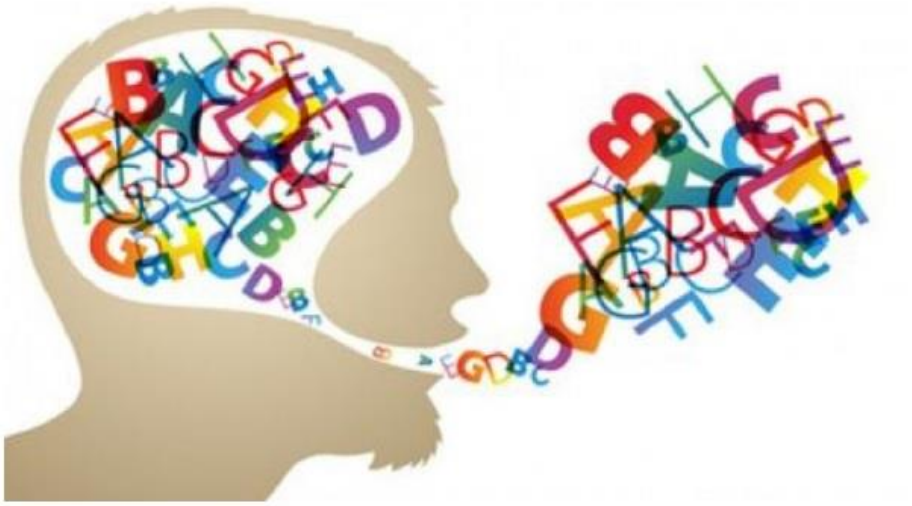
45) Studio interview with Dr. Ramón Latorre
Radio: Duna. Programa Aire Fresco conducido por Polo Ramírez y Francisco Aravena
Date: August 20, 2015
Link: http://www.audionoticias.net/videos/llambias/llambiasradio.htm
Scope: National

46) Start of Tertulias Porteñas Cycle with "What do we know about language?"
Extension Center of the National Council for Culture and Arts, August 27th
Web Page: El Mostrador.
Date: August 24, 2015
Link: www.elmostrador.cl
Scope: National

Cultura

Parte Ciclo de las Tertulias Porteñas con "¿Qué Sabemos del Lenguaje?" en Centro de Extensión del Consejo Nacional de la Cultura y las Artes, 27 de agosto

por CULTURA+CIUDAD, EL MAGAZINE DE EL MOSTRADOR | 24 agosto 2015



El dramaturgo nacional Marco Antonio de la Parra conducirá el nuevo ciclo de las Tertulias Porteñas que comenzará el próximo 27 de agosto en el edificio del Consejo Nacional de la Cultura y las Artes en Valparaíso. Se trata de la tercera versión del encuentro organizado por el Instituto Milenio Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso, y que reúne a científicos, artistas e intelectuales nacionales para analizar fenómenos de la vida cotidiana desde distintas perspectivas y disciplinas.

47) New diagnostic tools and cutting-edge molecular techniques in Valparaíso
Magazine: Portafolio de Salud, Diario Financiero
Date: August 27, 2015
Scope: National

Nuevas herramientas de diagnóstico y técnicas moleculares de vanguardia en Valparaíso

El desarrollo de un dispositivo no invasivo para medir la presión y monitoreo continuo de la hipertensión. Genómica de enfermedades de trastornos de la ansiedad, como la esquizofrenia y otras patologías. Un proyecto de diagnóstico temprano de Parkinson. La búsqueda de marcadores biológicos tempranos. Evaluación de la metabolómica para diagnósticos precisos en cáncer. Son algunas de las líneas de trabajo que tendrá el Centro Interdisciplinario para la Innovación en Salud (CIIS), un centro de medicina de precisión que se creará en la V Región, al alero de la Facultad de Medicina de la U. de Valparaíso (UV) y cuyo eje será la generación de nuevas herramientas de diagnóstico y técnicas moleculares de vanguardia para cáncer, enfermedades neurodegenerativas e infertilidad, buscando su inserción en las redes de salud hospitalarias y públicas de la región. Con una inversión de más de US$ 3 millones que provienen del Ministerio de Educación y la UV, se prevé que el CIIS –iniciativa de esta universidad y del Centro Interdisciplinario de Neurociencia (CINV) de esa misma casa de estudios– se inaugure oficialmente en marzo de 2016 con el objetivo, en una primera fase, de desarrollar ciencia y tecnología aplicada, relacionada al diagnóstico y terapia de enfermedades, con utilidad regional. Es decir, que pueda ser transferible a nivel nacional y, a futuro, al resto del mundo.

"En los próximos tres o cinco años, el CIIS debería ser un centro de referencia para Chile y la región. Esperamos que con el trabajo conjunto con las facultades de la UV, el CINV e instituciones tecnológicas nacionales y extranjeras asociadas al proyecto –como el instituto alemán Max Planck–, se puedan generar conocimientos de vanguardia", sostiene el director del CIIS, Dr. Hugo Peña-Cortés. La idea es complementar los avances científicos que logre el centro, con el trabajo de médicos y profesionales de la salud de la V Región, para implementar servicios y productos de aplicación clínica. "Realizaremos todos los esfuerzos para crear los conocimientos que permitan desarrollar alternativas tecnológicas de un costo tal que puedan ser asequibles masivamente, posibilitando hacer una medicina de precisión aplicable al sistema de salud nacional", añade.

ENFERMEDADES BAJO EL MICROSCOPIO

El estudio de moléculas pequeñas (metabolómica) para el diagnóstico preciso de cáncer a través de marcadores que generan estos productos metabólicos en organismos enfermos, será una de las líneas de investigación principales del CIIS. Se trata de un perfil bioquímico de alta precisión basado en la espectroscopia de masa, una técnica que permite identificar cantidades muy bajas de moléculas, explica el Dr. Alan Neely, director ejecutivo del proyecto financiado por el Mineduc y subdirector del CINV.

El Dr. Peña-Cortés, que estará a cargo de esta área, trabajó con el Instituto Max Planck en esta disciplina, obteniendo muestras de distintos tipos de neoplasias y estableciendo protocolos de muestra.

"En el CIIS, partiremos con el área de cáncer colorrectal, pues ya se cuenta con un estudio prospectivo con 800 muestras de sangre de pacientes de la V Región, que ha permitido establecer la efectividad de la metabolómica para identificar biomarcadores de esta enfermedad. Ese proceso debe seguir y buscar marcadores para diferentes tipos de tumores", detalla el Dr. Neely.

El subdirector del CINV explica que la aproximación médica normal en diferentes patologías, como la hipertensión, por ejemplo, es usar tratamientos de acuerdo con una estadística médica global, pero la medicina de precisión que desarrollarán posibilita elegir *a priori* la terapia más apropiada. En ese sentido, añade que dentro del grupo del nuevo centro hay médicos especialistas en estadísticas, "lo que nos podrá ayudar a ver cuántos subtipos de enfermedades y pacientes existen. En Parkinson o Alzheimer, hay formas genéticas diferentes, pero no se sabe cuántas son las variantes. La idea es que podamos comprender mejor eso", comenta.

48) MOP calls for bids to build Science building in Valparaíso
Magazine: Portafolio de Salud, Diario Financieo
Date: August 27, 2015
Scope: National

MOP LLAMA A LICITACIÓN PARA CONSTRUIR EDIFICIO DE LA CIENCIA EN VALPARAÍSO

El Ministerio de Obras Públicas (MOP) convocó a licitación para construir el edificio de la ciencia Abate Molina en el barrio La Matriz (Valparaíso). La obra es iniciativa del Centro Interdisciplinario de Neurociencia de la U. de Valparaíso (CINV), entidad que busca posicionar a la ciudad como referente de la investigación científica latinoamericana, y contará con una inversión de $ 5.000 millones aportados por el CORE, la casa universitaria y el MOP.

El nuevo edificio contempla la construcción de un moderno centro de investigación, que mantendrá la fachada del inmueble donde sesionó el primer Congreso bicameral de Chile, en el siglo XIX. Su reconstrucción busca devolver la vida a un espacio en abandono, destruido prácticamente en su totalidad por un incendio en 2004.

Una vez recuperado, tendrá una sala de reuniones, WiFi gratuito para todo el entorno y una dotación cercana a las 150 personas, entre académicos, estudiantes y personal administrativo. En su interior existirán instalaciones de alta tecnología, laboratorios para investigación neurocientífica y espacios que permitirán acoger actividades abiertas a la comunidad, como exposiciones de arte. Se espera que el Centro reciba cada año a más de dos mil visitas de especialistas ligados a la investigación científica, explicó el gerente del CINV, Juan Carlos García.

Portafolio**SALUD** *Agosto, 2015*

49) Science, art and emotions are complemented
Web Page: El Mostrador.
Date: October 13, 2015
Link: www.elmostrador.cl
Scope: National

El evento es organizado por Centro Interdisciplinario de Neurociencias de Valparaíso

Cultura

La ciencia, el arte y las emociones se co
Valparaíso en las Tertulias Porteñas

El capítulo titulado "¿Qué Sabemos de las Emociones?" reunirá a la neuróloga Andrea Slachevsky, al director teatral Elías Cohen, y al literato Ernesto Pfeiffer. "Las tertulias son un ejemplo de acercamiento de la gran cultura a la gente", dijo el moderador del conversatorio, el dramaturgo Marco Antonio de la Parra.

La segunda entrega del ciclo 2015 de las Tertulias Porteñas –conversatorio que conecta la ciencia, la cultura y el arte y que es organizado por el Instituto Milenio Centro Interdisciplinario de Neurociencia de Valparaíso– tendrá lugar el próximo jueves 15 de octubre en el Centro de Extensión del Consejo Nacional de la Cultura y las Artes (Centex) de la ciudad puerto.

El capítulo reunirá a la neuróloga Andrea Slachevsky, al director teatral Elías Cohen y al literato Ernesto Pfeiffer en torno a la pregunta "¿Qué sabemos de las emociones?". Moderados por el dramaturgo Marco Antonio de la Parra, los expositores compartirán con el público su particular aproximación intelectual y disciplinaria ante el fenómeno.

"A las emociones les debemos las decisiones, la personalidad, la vida misma. Desde que Darwin describió las emociones básicas se ha progresado enormemente en ubicarlas en el cerebro y convertirlas en base de la mente. Nuestro 'corazón' las porta y nos comunican y nos sirven de orientación en el mundo", afirmó De la Parra, conductor del ciclo esta temporada.

Según escritor, el encuentro "es una oportunidad muy estimulante y única de acercar la neurociencia al gran público. Esperamos un diálogo abierto y fluido. Los invitados son de lujo y el público porteño también. Son únicas, son un pequeño lujo, ojalá se repitieran en otros sitios, ojalá las copiaran. Son un ejemplo de acercamiento de la gran cultura a la gente".

50) Chilean scientist creates mathematical model to predict human behavior during disasters and zombie attacks
Web Page: El Mostrador.
Date: October 15, 2015
Link: www.elmostrador.cl
Scope: National

Modelo recrea escenarios virtuales, inspirados en ciudades chilenas

Cultura

Científico chileno crea modelo matemático para predecir comportamiento humano ante catástrofes y ataques zombie

Simulación de terremoto del 2010 y la dispersión de enfermedades infecciosas, entre otros eventos, son investigados por el Dr. Tomás Pérez–Acle, científico del Centro Interdisciplinario de Neurociencia, de la Universidad de Valparaíso, CINV, y de Fundación Ciencia para la Vida. A mayor entrega de información a los individuos, mejor es el manejo del pánico y la toma de decisiones correctas, asegura investigador.

Científicos chilenos han desarrollado una aplicación computacional que permite estudiar el comportamiento de poblaciones humanas, frente a catástrofes naturales y otros eventos críticos tales como: terremotos, enfermedades infecciosas y actos terroristas.

Los estudios son dirigidos por el Dr. Tomas Perez-Acle, investigador del Instituto Milenio Centro Interdisciplinario de Neurociencia -de la Universidad de Valparaíso-; y director del laboratorio de Biología Computacional de la Fundación Ciencia para la Vida.

La dispersión de un rumor o un virus contagioso, el pánico, y las respuestas positivas o negativas de las personas tras a una situación de emergencia -como fue el terremoto de Concepción en 2010-, han sido puestos a prueba en este modelo virtual que recuerda a la *Matrix*, y para el cual, se requiere la potencia de supercomputadores con altísima capacidad de procesamiento. Junto a esta tecnología, el científico cuenta con un equipo de trabajo transdisciplinario, integrado por otros seis profesionales, entre los que destacan sociólogos, biólogos, físicos, matemáticos e ingenieros en computación.

51) Dra. Karen Castillo: Ionic Channels as therapeutic targets
Magazine: Portafolio de Salud, Diario Financieo
Date: October 21, 2015
Scope: National

Dra. Karen Castillo

Canales iónicos como blancos terapéuticos

"Es en las primeras etapas educativas cuando los cimientos que construyen el futuro del país deben ser los más sólidos. Esto, *per se*, genera mayor participación y un capital humano fortalecido"

Hace un par de años que la Dra. Karen Castillo llegó a trabajar como investigadora del Centro Interdisciplinario de la U. de Valparaíso (CINV) con la intención de estudiar biofísica, un campo donde se aplican principios de la física para entender fenómenos biológicos y que a nivel mundial es dominado mayoritariamente por hombres.

En esta área del conocimiento, añade esta ingeniera en Biotecnología Molecular, se busca comprender los fenómenos relacionados con el balance en la excitabilidad celular y con el papel que juegan los canales iónicos –proteínas residentes en las membranas celulares– en la detección de estímulos de diversa naturaleza que, como consecuencia, lleva a la apertura de poros contenidos en su estructura, selectivos al paso de iones, y que modifican el equilibrio eléctrico de la célula.

Este complejo proceso, "permite modular procesos biológicos en el cerebro, el corazón y los músculos. Los canales iónicos están distribuidos en prácticamente todo tipo de tejidos en los organismos vivos; ahí detectan, integran y traducen en cambios eléctricos las fluctuaciones ambientales y así nuestro cuerpo es capaz de detectar frío, calor, dolor y un sinnúmero de sustancias, permitiéndonos adaptarnos a los cambios del medio", explica la Dra. Castillo.

Para cumplir todas estas labores, acota, los canales iónicos están capacitados con diversas clases de sensores, dependiendo del contexto celular en donde se desarrolla su actividad. En el laboratorio de Sensores Moleculares del CINV, donde trabaja actualmente, estudia los canales BK –llamados así por su alta conductancia a potasio–, que ajustan sus funciones a las necesidades fisiológicas particulares de un determinado tejido o tipo celular y, mediante herramientas moleculares y electrofisiológicas, analiza la influencia de diversos factores sobre la activación de los sensores moleculares del canal, y los elementos moleculares implicados en esta regulación.

La importancia de esta investigación, dice, es que existen varias condiciones patológicas relacionadas con el funcionamiento defectuoso de estos canales, como diabetes, asma, epilepsia, neurodegeneración y cáncer. "Si podemos establecer cómo ocurre el funcionamiento normal de estos canales y cómo su actividad es modificada por diversos estímulos, podríamos llegar a comprender cómo se desregulan en ciertas condiciones patológicas, y proponer mecanismos para reparar este malfuncionamiento, ya sea ajustando el rango de respuesta de sus sensores o la apertura de sus poros", detalla.

APLICACIÓN BIOMÉDICA

A la Dra. Castillo la motiva llevar el conocimiento generado en el laboratorio hacia la investigación aplicada en áreas biomédicas. Como los canales iónicos están involucrados en eventos patológicos, dice, éstos pueden ser blancos terapéuticos para el desarrollo de compuestos naturales o drogas que permitan modular su función.

Por ejemplo, en el laboratorio están estudiando las bases moleculares del funcionamiento de los canales de protones (Hv), que se activan por cambios en el pH de las células y que están involucrados en la compensación eléctrica durante la respuesta inmune.

"Participo en proyectos para ampliar el impacto de la investigación en áreas de aplicación en medicina y desarrollo tecnológico", dice la doctorada en Biología Celular, Molecular y Neurociencias, con entrenamiento postdoctoral en ciencias biomédicas (bases celulares y moleculares de la neurodegeneración) y biofísica de los canales iónicos.

52) Scientists creates application that predicts how people behave during disasters
Newspaper: El Mercurio de Valparaíso
Date: October 21, 2015
Scope: Regional

Crean aplicación que predice cómo se comporta la gente en catástrofes

CINV

DR. TOMÁS PÉREZ-ACLE.

● Científicos chilenos, uno de ellos con trabajo en Valparaíso, han desarrollado una aplicación computacional que permite estudiar el comportamiento de poblaciones humanas frente a catástrofes naturales y otros eventos críticos tales como terremotos, enfermedades infecciosas y actos terroristas. Los estudios son dirigidos por el Dr. Tomas Pérez-Acle, investigador del Instituto Milenio Centro Interdisciplinario de Neurociencia -de la Universidad de Valparaíso- y director del laboratorio de Biología Computacional de la Fundación Ciencia para la Vida, quien destaca sus aplicaciones.

53) Life in an equation
Magazine: Qué Pasa
Date: October 23, 2015
Scope: National

› CIENCIAS

LA VIDA EN UNA ecuación

El equipo del Laboratorio de Biología Computacional de la Fundación Ciencia & Vida busca resolver una pregunta utópica: qué es la vida. Para eso, escriben modelos matemáticos que van desde mecanismos celulares a eventos poblacionales. Detrás de todo está el biólogo Tomás Pérez-Acle, un niño pobre que pasó de estacionar autos a refugiarse en un mundo de ciencia ficción.

[Por Nicolás Alonso // Ilustración: Vicente Reinamontes]

Lo vio esa tarde. Pudo ser 1978 o después, en algún punto entre los siete y los diez años. En una tarde como cualquier otra de una niñez poco celebrada, que se había consumido mientras estacionaba autos en Bellavista, recolectaba botellas y esquivaba la brutalidad de su padre, un alcohólico que los mudaba de villa en villa por Santiago, y que acabaría por abandonarlos y quitarse la vida. Lo vio esa tarde: en una bóveda vidriada, en el trabajo de su madre, que los sacaba adelante a él y a sus dos hermanos digitando en una empresa del Estado, se distinguía una máquina enorme, llena de cintas, que emitía ruidos que no se parecían a nada que hubiera oído o visto jamás.

Lo que vio esa tarde Tomás Pérez-Acle, el doctor en Biotecnología que tres décadas después montaría en la Universidad de Chile el único laboratorio de supercomputadores del país, y que fundaría un laboratorio para la Fundación Ciencia & Vida, donde se unirían cerebros brillantes en física, en bioquímica, en sociología, en computación y en matemáticas detrás de una misma utopía, modelar la vida humana en un computador, fue justamente eso: un computador.

Entonces no entendió lo que veía, pero volvió a sentir esa emoción una vez más, frente a otro gran cúmulo de inteligencia encriptada: la biblioteca de su abuelo materno. Entre un evento y otro pasaron unos años, quizás los peores de su vida, que terminaron cuando su padre desapareció y él llegó, con su madre y sus hermanos, a la casona de su abuelo en Bellavista, un palestino que había llegado a los cinco años a Chile, y aunque apenas podía escribir, era amante de la literatura. De un género sobre todo: la ciencia ficción.

Entonces los libros fueron el refugio: primero *Crónicas Marcianas*, de Ray Bradbury, y luego *Fundación*, de Isaac Asimov; *Dune*, de Frank Herbert, y el resto de los clásicos que los fines de semana, si no estaba estacio-

54) President of Göttingen University visited Valparaíso scientists
Newspaper: El Mercurio
Date: October 28, 2015
Scope: National

Investigación:

Rectora de Universidad de Göttingen visitó a científicos de Valparaíso

Con el fin de sellar acuerdos de cooperación científica entre Alemania y Chile, la rectora de la Universidad alemana de Göttingen, Urlike Beisiegel, se reunió con el rector de la Universidad de Valparaíso, doctor Aldo Valle, y el director del Instituto Milenio Centro Interdisciplinario de Neurociencia de Valparaíso, CINV, doctor Ramón Latorre.

El objetivo de esta actividad fue definir las posibilidades de desarrollar alianzas futuras entre ambas casas de estudio, en materia científica, para implementar cursos, magísteres y doctorados en neurociencia y biofísica computacional.

Los directores del CINV e investigadores de Valparaíso informaron también a la delegación germana las características del futuro edificio de la ciencia Abate Juan Ignacio Molina, cuya construcción se desarrollará próximamente en el sector patrimonial del Puerto, a un costado de la Iglesia La Matriz.

Juan Carlos García, gerente general Instituto Milenio Centro Interdisciplinario de Neurociencia, Universidad de Valparaíso, CINV; Vania Carvalho, asistente rectoría Universidad de Göttingen, Alemania; Aldo Valle, rector Universidad de Valparaíso; Urlike Beisiegel, rectora Universidad de Göttingen, Alemania; doctor Ramón Latorre, director Centro Interdisciplinario de Neurociencia, Universidad de Valparaíso, CINV, y Alejandro Rodríguez, director de Vínculos y Cooperación Internacional, Universidad de Valparaíso.

Durante su visita, la doctora Ulrike Beisiegel también se reunió con representantes diplomáticos y con el director de Vínculos y Relaciones Internacionales de la Universidad de Valparaíso, Alejandro Rodríguez, a fin de materializar posibles áreas de intercambio entre ambas instituciones, a partir del próximo año académico.

55) Resignation of Brieva to Conicyt Presidency annoys Chilean scientists
Newspaper: La Tercera
Date: October 30, 2015
Scope: National

74 LA TERCERA Viernes 30 de octubre de 2015

Sociedad
Tendencias

"Es engorroso, porque, ¿quién escuchó sus fundamentaciones del presupuesto? Aparentemente nadie"
Jorge Babul
Consejo Sociedades Científicas

"Francisco Brieva era la persona indicada. El es un creador, podría haberlo hecho muy bien en Conicyt".
Ramón Latorre
Premio Nac. de Cs. Naturales

Renuncia de Brieva a Conicyt desata la molestia de científicos chilenos

► Afirman que su partida demuestra que al Estado no le importa el desarrollo de la ciencia.

► Acusan que falta de presupuesto y excesiva burocracia impidieron su continuidad.

C. Yáñez / C. Espinoza

Tres presidentes en cuatro años, muestra que algo no funciona bien al interior del organismo encargado del desarrollo científico y tecnológico del país. Ayer, se confirmó la renuncia de Francisco Brieva, presidente de la Comisión Nacional de Investigación Científica y Tecnológica (Conicyt).

El ingeniero alcanzó a estar apenas 13 meses en el cargo y pese a que llegó con todo el ímpetu de mejorar la situación, renunció el miércoles (**ver entrevista pág. 75**).

El cese no fue sorpresa en el mundo científico, sino una muestra más de la poca importancia que la ciencia tiene para el Estado.

Ramón Latorre, director del Instituto Milenio Centro Interdisciplinario de Neurociencia de la U. de Valparaíso y Premio Nacional de Ciencias Naturales cree que existe un innegable "problema de institucionalidad y de funcionamiento".

"Francisco Brieva es un hombre extremadamente preparado, gran científico. Las medidas que tomó, las conversaciones que sostuvimos con él, decían que era la persona indicada. El es un creador, podría haberlo hecho muy bien", dice.

Fernando Valiente, coordinador nacional del Movimiento Ciudadano Más Ciencia para Chile, dice que no hay institucionalidad que permita, a quien está en el cargo, poder "pelear por el presupuesto", porque hay muchos con quienes se tiene que enfrentar más arriba, entre ellos el Ministerio de Educación (del que depende Conicyt) y de Hacienda.

"Es engorroso, porque, ¿quién escuchó al presidente de Conicyt? ¿Quién escuchó sus fundamentaciones del presupuesto? Aparentemente nadie", dice Jorge Babul, presidente del Consejo de Sociedades Científicas.

El académico de la U. de Chile recuerda que tras la salida de José Miguel Aguilera (septiembre de 2013), Conicyt estuvo casi un año sin presidente y eso fue muy serio para la institución y la comunidad científica. Según él, los cambios que Brieva trató de hacer se toparon con burocracia. "La renuncia nos pilla en un momento muy malo para la ciencia, tampoco se nos ha hecho caso con una propuesta de ministerio y yo lo veo como muy preocupante. No tener presidente, un presupuesto que no aumente como debería y no salgamos del último lugar de la Ocde", dice Babul.

Para Katia Soto, directora de Fundación Más Ciencia, la renuncia de los dos últimos presidentes, habla de la falta de interés del gobierno. "Había una señal. Se iba a tomar en serio y queda en punto muerto. Si no hay avance, quienes están en estos puestos ven la falta de voluntad del gobierno por avanzar, ni ministerio ni presupuesto", explica.

La directora del Instituto Milenio para la Investigación en Depresión y Personalidad (Midap), Mariana Krause, también cree que el escenario actual, demuestra la poca preocupación del Estado. "Lo conocí bien en el trabajo de la comisión. El está por la creación de un ministerio", dice Krause.

Andrés Couve, director Instituto Milenio Neurociencia Biomédica (BNI) dice que si "el mandante -gobierno- no está a la altura y no comparte el trabajo desequilibrante que realiza Brieva, el desafío para él, desaparece. No es una caja pagadora de proyectos. Si Conicyt no cambia su estructura y quiere seguir siendo eso, está bien. Pero para eso, no necesita a alguien del calibre de Brieva", señala Couve.

Desde el Centro para la Comunicación de las Ciencias de la U. Andrés Bello, Gabriel León, dice que el actual es escenario es triste para toda la ciencia. "Lo más lamentable es que a pocos les extraña su renuncia. Chile tiene problemas que se pueden resolver con ciencia y tecnología, pero al Estado no le preocupa", se queja.

El representante de los trabajadores de Conicyt, Arturo Pino, cree que Brieva se vio impedido de manejar algunas situaciones relacionadas con cambios a la orgánica y estructura de la institución. "Cuando llegó, los trabajadores vimos mucho entusiasmo en él, decía que teníamos que hacer de Conicyt un lugar donde diera gusto llegar a trabajar. Pero en el tiempo el entusiasmo se fue apagando por las trabas burocráticas, porque al final, no puedes solucionar el problema", dice.

La Tercera intentó comunicarse con Conicyt sin éxito. ●

►► Andrés Couve, en el Instituto de Ciencias Biomédicas de la U. de Chile.

FOTO: AGENCIA UNO.

CRONOLOGÍA

1967 Ley 16.746 crea Conicyt, destinada a asesorar al Presidente en ciencia.

1971 Se define que la Comisión será dirigida por un consejo multidisciplinario y transversal.

1973 Tras el golpe de Estado deja de funcionar y en 1974 se elimina el consejo.

1981 Se crea el Fondecyt, que financia proyectos de ciencia básica. Diez años después, el Fondef, para investigación aplicada.

2009 Mineduc pide renuncia a VivianHeyl por escándalo en la asignación de las Becas Chile.

2013 Por primera vez, un presidente de Conicyt, José Miguel Aguilera, renuncia.

Las críticas a la carencia de un plan y de institucionalidad

Chile invierte hoy en torno al 0,38% de su producto interno bruto (PIB) en ciencia, tecnología e innovación, cifra que jamás ha superado el 0,4%, mientras sus pares de la Ocde, promedian 2,4%.

El bajo presupuesto ha sido una de las críticas de la comunidad científica, no obstante, ningún gobierno ha definido hacer algo concreto por cambiar la situación.

Durante los periodos de Sebastián Piñera y el actual de Michelle Bachelet, se crearon comisiones para analizar la situación y, ambas recomendaron la creación de un ministerio, sin embargo, tras la entrega del último informe la presidenta no dio fechas y sólo anunció el traspaso de la Iniciativa Científica Milenio desde Economía a Conicyt.

Entre las críticas al sistema, que ambas comisiones han mencionado, está su fragmentación. Las agencias que gestionan recursos para investigación (como Conicyt o Corfo) están dispersas en varios ministerios y, en algunos casos ni siquiera tienen coordinación, no se toman decisiones en torno a objetivos comunes y la gestión de recursos, por lo mismo, es ineficiente.

Conicyt depende del Mineduc, que está centrado en llevar a cabo una reforma y, por lo mismo, esta tiene la prioridad de sus recursos.

Ex presidentes de Conicyt, como José Miguel Aguilera y el mismo Brieva, durante su periodo, han criticado además, la poca agilidad de la administración estatal. "Es frustrante", señaló Brieva el mes pasado. "Es extremadamente lenta, tiene una lógica de asignación y control de los recursos que cuesta entender que uno vaya a salir ganador después de un cierto proceso. Es muy lento, el país avanza a un ritmo que es incompatible". ●

56) Scientific research is endanged by the "ignorance of the Chilean governments"
Web Page: El Mostrador
Date: November 10, 2015
Link: www.elmostrador.cl
Scope: National

Cultura

Las investigaciones científicas que están en peligro por la "ignorancia de los gobiernos de Chile"

Una vacuna contra el virus sincicial, un tratamiento contra la diabetes y un instituto especialista en la biodiversidad se cuentan entre los afectados. Los científicos presionan por la creación de un Ministerio de la Ciencia y la repatriación de doctorados en el exterior, entre otras medidas. El jueves realizarán una protesta frente a La Moneda.

745 Compartir
77 Twittear
Compartir
Compartir
Imprimir
Enviar por mail
Rectificar

Varios productos científicos se encuentran en peligro por la falta de inversión del Estado en el área, una situación que fue denunciada por una carta abierta que la comunidad publicó el domingo en diversos medios de comunicación, bajo el nombre "Nuestros gobiernos han elegido la ignorancia".

Entre los afectados concretos se encuentran una vacuna contra el virus respiratorio sincicial y un tratamiento contra la diabetes tipo I, en manos del Instituto Milenio de Inmunología e Inmunoterapia (IMII), que agrupa a investigadores de la Pontificia Universidad Católica de Chile (PUC).

La vacuna contra un mal que afecta especialmente a los bebés –cada año deben ser hospitalizados unos 5.000 niños en Chile– es un antídoto que cruzó con éxito todas las pruebas preclínicas y que ya cuenta con patente en China y Estados Unidos. Sin embargo, para seguir adelante con ensayos clínicos se requiere una importante suma de recursos.

En cuanto al tratamiento contra la diabetes, actualmente se espera iniciar estudios clínicos en Francia, para lo cual también necesita financiamiento, cercano a un millón de dólares. Según uno de los últimos estudios disponibles, este mal aumentó solo entre el año 2000 y el 2004 de un 5,44% a un 8,33%.

57) Studio interview with Dr. Tomás Pérez-Acle
Radio: Duna. Programa Aire Fresco conducido por Polo Ramírez y Francisco Aravena
Date: November 12, 2015
Link: http://we.tl/P2buYdJw42
Scope: National

58) Thousands of researchers protest outside La Moneda asking for for greater support for science
Newspaper: El Mercurio
Date: November 13, 2015
Scope: National

Entregaron cartas dirigidas a la Presidenta Bachelet:

Miles de investigadores protestaron frente a La Moneda por mayor apoyo para la ciencia

Las manifestaciones se repitieron a lo largo de Chile bajo la consigna "Rebelión científica". Junto con mejoras laborales, abogaron por más recursos y un ministerio del ramo.

ALEXIS IBARRA O.

Ayer, la Plaza de la Constitución se tiñó de blanco. Eran los delantales de los miles de científicos que llegaron a mediodía a protestar para que se mejoren las condiciones de la ciencia en Chile. La gran mayoría eran jóvenes, pero también se divisaban varios científicos de renombre.

"El cobre no es eterno; y cuando se acabe, tendremos un Chile pobre e ignorante", rezaba uno de los carteles. La ordenada manifestación fue acompañada con gritos y hasta una pequeña batucada. Su objetivo, el cual lograron, era entregar tres cartas a la Presidenta (de distintas instituciones) para luego protestar frente a Conicyt, una cuadra más allá.

La convocatoria la hizo la organización Ciencia con Contrato, que aboga por mejoras en las condiciones laborales de los investigadores, mucho de los cuales trabajan con sueldos bajos y boleteando por muchos años. "La precariedad del trabajo en la ciencia incumbe a profesionales con distintos grados académicos y de disciplinas variadas. Yo misma llevo trabajando nueve años y nunca he sido contratada, a pesar de que tenemos que cumplir un horario y hay una relación de dependencia como cualquier trabajador", dice Natalia Muñoz, quien lidera Ciencia con Contrato.

El problema, según Muñoz, es que las universidades que se adjudican fondos concursables no contratan a los investigadores que ejercen como ayudantes. Esto, ya que Conicyt, en las bases de fondos concursables, permite tener trabajadores a honorarios. "Mientras esa reglamentación no cambie, nadie va a hacer nada para que mejoren las condiciones laborales de miles de científicos", dice.

La convocatoria inicial a la protesta se amplió tras los últimos acontecimientos desencadenados por la renuncia de Francisco Brieva a la presidencia de Conicyt, y que evidenció problemas serios en el aparato científico, como la falta de recursos. Por eso, la manifestación fue masiva —los organizadores calculan más de 2 mil asistentes—, y las consignas y los discursos apelaban a que el Estado valore la importancia de la ciencia como motor del desarrollo.

Con pitos y hasta una pequeña batucada, los científicos se manifestaron frente a La Moneda. Los organizadores calculan que asistieron más de 2 mil personas.

Una esperanza

Un grupo de científicos fue convocado el miércoles por la Comisión de Ciencia y Tecnología de la Cámara Baja. "Para nuestro asombro, la comisión aprobó votar en contra de la partida presupuestaria y solicitar el 20% de aumento de los fondos para Fondecyt, como también recursos para atraer a los científicos que están en el extranjero. Además, sacarán una declaración en la que se solicitará al Gobierno la creación de un Ministerio de Ciencia y Tecnología", dijo Tomás Perez-Acle, del Centro Interdisciplinario de Ciencia de Valparaíso.

En todo el país

En Valparaíso, Coquimbo, Valdivia, Concepción y en otras ciudades, los científicos también se manifestaron. La consigna en los carteles y las redes sociales fue "Rebelión científica".

En Santiago, representantes de las Sociedades Científicas, la Fundación Más Ciencia y la Asociación Nacional de Investigadores de Posgrado (Anip) tomaron un megáfono y se dirigieron a la masa. El más aplaudido fue Jorge Babul, presidente de las Sociedades Científicas. "Una de las cosas que nos pidió la Presidenta es que entusiasmemos a los jóvenes... y ¿en qué estamos? Los doctorados nacionales se quieren ir, y los que están afuera se quieren quedar afuera... Eso no puede ser. Eso se llama estupidez", dijo entre vítores.

Y agregó la importancia de crear un Ministerio de Ciencia y Tecnología. "No es posible que a los ministros de Educación, que duran en promedio un año, tengamos que enseñarles cada vez la importancia de la ciencia", aclaró.

Katia Soto, de la Fundación Más Ciencia, fue dura con la comunidad científica, y señaló que los investigadores *seniors* han hecho por mucho tiempo la vista gorda del problema. "Mientras recibieran recursos para hacer investigación... no les importó qué iba a pasar con la comunidad", aclaró.

En tanto, Matías Pérez, de Anip, reclamó por la falta de transparencia: "¿Hasta cuándo los investigadores tenemos que soportar que los concursos emanados por Conicyt tengan nombre y apellido? No puede ser que los mismos investigadores sean juez y parte en los procesos de adjudicación de proyectos", dijo.

En medio del tumulto estaba Cecilia Hidalgo, Premio Nacional de Ciencias, quien apoyaba la manifestación. "¿Cuánto tiempo podemos exportar materias primas sin valor agregado? Desde las frutas sin insecticidas hasta el vino de mejor calidad requieren de conocimiento, y ese conocimiento tenemos que generarlo a través de la ciencia", declaró.

Los científicos también se reunieron en Valparaíso para abogar por más recursos para fondos concursables y mejores condiciones laborales.

59) "Molecular cuisine" demonstrated by scientist cook.
Newspaper: El Mercurio
Date: November 29, 2015
Scope: National

A 14 VIDA • CIENCIA • TECNOLOGÍA EL MERCURIO DOMINGO 29 DE NOVIEMBRE DE 2015

Investigador estuvo en Chile invitado por el Centro de Neurociencia de Valparaíso:

La cocina molecular explicada por un científico con las manos en la masa

Chris Chipot, biofísico y compañero de experimentos de Ferran Adrià, revela cómo se obtienen sabores y texturas con un poco de jugo, una pizca de gelificante y un baño de calcio.

"De la ciencia de materiales a la cocina de vanguardia" se llamó la charla que Chris Chipot dictó en el restaurante El Internado, de Valparaíso.

PAULA LEIGHTON N.

Tome nota. Para que la yema de los huevos duros no quede con ese desagradable color verde grisáceo, pinche la cáscara con un alfiler antes de ponerlos a hervir. Y si quiere un merengue que no se baje o licue, eche unas gotas de limón a las claras al batir. Van a subir como la espuma, pero firmes y consistentes.

No son trucos de abuelita. Es pura ciencia. Pinchar la cáscara permite que el sulfuro de la yema escape en vez de quedar atrapado dentro del huevo, mientras que el limón desnaturaliza la proteína de la clara. "Así se forma un merengue estable", explica el francés Chris Chipot a una audiencia reunida en el restaurante El Internado, de Valparaíso, para escuchar su charla "De la ciencia de materiales a la cocina de vanguardia".

Chipot no es chef. "El 90% de su tiempo lo gasta en ciencia, pero debe pasar la mayor parte de sus vacaciones cocinando con los mejores chefs de Francia y Barcelona", datea Ramón Latorre, director del Instituto Milenio Centro Interdisciplinario de Neurociencia de Valparaíso (CINV), donde el investigador francés estuvo de visita.

Chipot es biofísico teórico, director de investigación en el Centro Nacional de Investigación Científica (CNRS) de Francia y codirector del Laboratorio Internacional Asociado del CNRS y la U. de Illinois.

De ahí que su cocina tenga varias cucharadas de ciencia y una pizca de "ingredientes naturales que habitualmente no se encuentran en la cocina", dice.

Se refiere a extractos de alga, pectina, hidrocolides y otros elementos que se usan para espesar, formar geles, encapsular moléculas de líquido o crear emulsiones perfectas.

Ciencia en la mesa

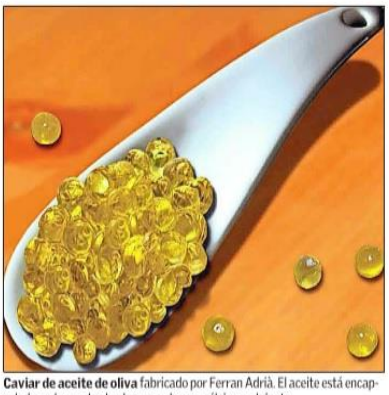
"En pocas palabras, la cocina de vanguardia o gastronomía molecular está en la confluencia de ciencia y cocina", resume Chipot, describiendo la técnica que tiene al célebre chef catalán Ferran Adrià como su más eximio exponente. Ambos han cocinado y experimentado juntos.

Mientras revela los trucos de la cocina molecular "para jugar con las texturas y optimizar la sensación en la boca", el francés prepara unos huevos fritos falsos. La clara está hecha con leche de coco y colapez, y para hacer la yema —que en realidad es jugo de naranja espesado con alginato de sodio— recurre a la icónica técnica de la esferificación, ampliamente usada ("y abusada", añade Chipot) por Adrià en El Bulli.

Con un pequeño cucharón sumerge una porción del jugo espeso en una solución de gluconolactato de calcio. Así se obtiene una esfera cuya superficie gelatinosa encapsula el líquido que se desee.

Esta vez la yema —como pasa muchas veces con los verdaderos huevos fritos— quedó un poco aplastada. Pero sí se lució con su "deconstrucción de la ensalada caprese", donde dos bolitas de tomate confitado compartían plato con un caviar verde brillante de albahaca y una esfera de mozzarella blanca contrastaba con el negro del vinagre balsámico.

"Lo bueno, dice Chipot, es que esta es una cocina sin secretos". Además de generar emociones gustativas, es absolutamente precisa en temperatura, mililitros y gramos. "Eso permite reproducirla cada vez y que sea infalible. Y si no resulta, te permite entender qué pasó, en qué etapa falló", concluye, poseído por su lado 90% científico.



Caviar de aceite de oliva fabricado por Ferran Adrià. El aceite está encapsulado en las perlas hechas con cloruro cálcico y alginato.

Revisitar las recetas clásicas para generar algo inesperado y atraer la vista son dos principios de la cocina molecular, que el biofísico Chris Chipot aplicó en su deconstrucción de ensalada la caprese.

60) French Biophysicist reveals secrets of the "spheronization" of molecular cuisine
Web Page: El Mostrador
Date: December 2, 2015
Link: www.elmostrador.cl
Scope: National

Cultura

Biofísico francés revela secretos de la "esfericación" de la cocina molecular

Invitado por el Centro Interdisciplinario de Neurociencias de Valparaíso, el científico Chris Chipot compartió en las Tertulias Porteñas y realizó una charla en el restaurante El Internado de Valparaíso donde reveló el proceso de "esfericación" que hizo tan famoso al chef catalán Ferrán Adriá.

Dicen que en la cocina clásica abundan los secretos; en la *nouvelle cuisine* sobran las sorpresas, como las cuentas obscenas; y en la cocina de Vanguardia rebosa la ciencia.

Dicen que en la cocina clásica abundan los secretos; en la *nouvelle cuisine* sobran las sorpresas, como las cuentas obscenas; y en la cocina de Vanguardia rebosa la ciencia.

Chris Chipot, biofísico francés, y uno de los más activos defensores de la cocina molecular, racional o de vanguardia, hace ya algunos años logró conciliar dos de sus más importantes intereses: la gastronomía y la curiosidad científica.

61) Chemistry on the Menu
Magazine: Qué Pasa
Date: December 7, 2015
Scope: National

› CIENCIAS

QUÍMICA A LA CARTA

La semana pasada, el biofísico francés Chris Chipot fue invitado por el Centro Interdisciplinario de Neurociencia de Valparaíso a enseñar cómo hacer una esferificación, la técnica gastronómica que describió el año pasado junto a Ferran Adrià, y que publicaron en un extraño *paper* científico-culinario.

[Por Nicolás Alonso // Fotos: José Miguel Méndez]

Chris Chipot va a decir toda la mañana que lo que está cocinando es una ensalada *caprese*, y hasta cierto punto será cierto. Durante cuatro horas, encerrado en la cocina del restaurante El Internado, en Cerro Alegre, el biofísico, director de investigación del Centro Nacional de Investigación de Francia y codirector de su laboratorio internacional junto a la Universidad de Illinois, va a tomar los tomates, la albahaca y el queso parmesano que compró el día anterior en el Mercado de Valparaíso, y va a intentar transformarlos en eso, en una ensalada *caprese*. En lo que él entiende por una.

—En una deconstrucción —dirá, haciendo una mueca de misterio impostado.

Sin ningún delantal ni gorro que pudieran quitarle el aspecto de intruso que tiene dentro una cocina —de Ph.D. en Química tratando de hacer una ensalada—, el hombre que trabajó codo a codo con el más famoso de los cocineros moleculares, el catalán Ferran Adrià, y que publicó junto a él un *paper* extrañísimo en el que le dieron sustento científico a sus platos, ahora repetirá esos procesos con instrumentos de cocina que no llamarían la atención en un laboratorio: jeringas, balanzas de precisión decimal, batidoras de acero cromado. Con sus dedos largos filtrará la albahaca hasta quitarle la clorofila, la licuará y la mezclará con un polvo blanco, el alginato de sodio, un compuesto químico extraído de algas que le dará la consistencia de un gel, y que al entrar en contacto con un baño de calcio formará esferas sólidas por fuera y líquidas por dentro. Esa será la magia.

—Lo que hacemos es encajar un líquido dentro de un sólido. La idea subyacente es que se organizan las moléculas del gel dentro de una red tridimensional, que encarcela a las moléculas de agua.

Ese proceso, que se llama "esferificación" y es una de las bases de la comida que sirven en elBulli y en otros

62) Second season of "The mysteries of the brain" deals with topics that span from social neuroscience to post-partum depression
Web Page: El Mostrador. Neuronews
Date: May 7, 2015
Link: www.elmostrador.cl
Scope: National

Ciudad

Los artículos saldrán todos los lunes a partir del próximo 11 de mayo

Segunda temporada de "Los misterios del cerebro" abordará desde la neurociencia social hasta la depresión post-partum

Vuelve el espacio que nació de la alianza entre **El Mostrador Cultura+Ciudad** y el Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso (CINV) con el objetivo de acercar la ciencia a la población y dar preeminencia a los estudios e investigaciones del campo. Para su segunda temporada, el espectro temático será más amplio y abordará publicaciones más desconocidas como la neurociencia forense y el estrés durante la lactancia.

63) Neuroscientists discover that traumatic experiences can be inherited by sperm
Web Page: El Mostrador. Neuronews
Date: May 20th2015
Link: www.elmostrador.cl
Scope: National

Segunda temporada del Convenio con el Centro Interdisciplinario de Neurociencias de Valparaíso

Neurocientíficos descubren que las experiencias traumáticas pueden heredarse por el esperma

Un investigación científica de la Universidad de Zürich encabezada por la Dra. Katharina Gapp concluyó que un tipo de ARNs era el responsable de la transmisión transgeneracional de las experiencias traumáticas durante la infancia y que, específicamente, era heredado por los padres a través de la esperma. En términos clínicos, los antecedentes planteados resultan relevantes, ya que la identificación de varios ARNs pequeños como mediadores de estos efectos pueden utilizarse como marcadores moleculares del estrés traumático para el uso potencial en el diagnóstico de predisposición a estrés.

64) **Welcome to The Matrix!: Scientists achieve direct transfer of information from one brain to another via the internet**
Web Page: El Mostrador. Neuronews
Date: June 2, 2015
Link: www.elmostrador.cl

Scope: National

Tercera publicación del convenio con el Centro Interdisciplinario de Neurociencias de Valparaiso

¡Bienvenido a La Matrix!: Científicos logran traspasar información directa de un cerebro a otro mediante internet

¿Se imaginan tener la posibilidad de conectarse a un computador y descargar directamente en tu cerebro el programa titulado "Pelé" y en unas pocas horas terminar jugando fútbol como "El Rey"? Bueno, todo esto podría ser posible algún día, no necesariamente lejano. Científicos de la U. de Washington, en Estados Unidos, lograron transmitir de forma exitosa información entre dos cerebros humanos mediante una interfase computacional, sin necesidad de algún tipo de lenguaje oral o escrito.

por JESÚS OLIVARES DUBART ENVIAR RECTIFICAR IMPRIM

65) Scientists conclude that financial worries diminish cognitive abilities
Web Page: El Mostrador. Neuronews
Date: June 17, 2015
Link: www.elmostrador.cl
Scope: National

66) Neuroscience study reveals that temperament is transmitted through breast milk
Web Page: El Mostrador. Neuronews
Date: June 24, 2015
Link: www.elmostrador.cl
Scope: National

Estudio en neurociencias arroja que el temperamento es transmisible por la leche materna

La leche materna no sólo nos permite una adecuada nutrición y el desarrollo del sistema inmune, sino que tiene otro tipo de características que podrían determinar incluso nuestra personalidad y, en efecto, el tipo de relaciones sociales que establecemos.

67) Scientists discover that sun exposure helps prevent cognitive decline
Web Page: El Mostrador. Neuronews
Date: July 3, 2015
Link: www.elmostrador.cl
Scope: National

Nueva publicación del convenio con el Centro Interdisciplinarios de Neurociencias de Valparaíso (CINV)

Cultura

Científicos descubren que exposición al sol ayuda a evitar el deterioro cognitivo

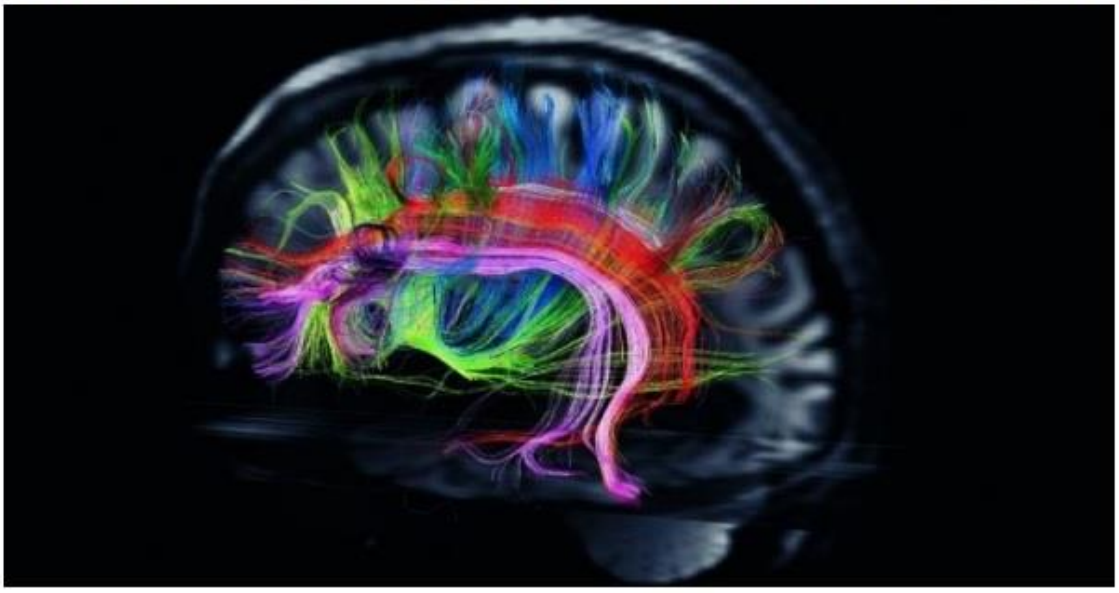
por MARCIA ARRIAGADA / CINV | 2 julio 2015

Si bien la radiación ultravioleta es el principal responsable del cáncer a la piel en nuestro país, también produce la activación de la vitamina D. Investigadores de la U. de Kentucky concluyeron que esta tendría una función importante en estructuras cerebrales como el hipocampo, mejorando la salud mental durante el envejecimiento.

68) Neuroscience concludes that economic status influences the structural development of the brain
Web Page: El Mostrador. Neuronews
Date: August 5, 2015
Link: www.elmostrador.cl

Scope: National

Neurociencia concluye que estatus económico influye en el desarrollo estructural del cerebro



Estudios han encontrado relaciones entre la inteligencia y parámetros estructurales como el grosor de la corteza cerebral. Por ejemplo, a la edad de 10 años, los niños más inteligentes tienen cortezas más delgadas. Estos estudios sugieren que si el estatus socioeconómico modela algunas habilidades cognitivas, entonces también habría un correlato entre el estatus socioeconómico y la estructura del cerebro.

Estudios revelan que el nivel socioeconómico familiar y la educación de los padres están correlacionados con la estructura del cerebro en regiones críticas para el desarrollo del lenguaje, funciones ejecutivas y memoria.

69) Post-partum depression: The first cause of disability in adult Chilean women
Web Page: El Mostrador. Neuronews
Date: September 16, 2015
Link: www.elmostrador.cl
Scope: National

Convenio con Centro Interdisciplinario de Neurociencias de Valparaíso

Cultura

Depresión Post-parto: El primer motivo de discapacidad en mujeres adultas chilenas

por MACARENA GÁRATE PÉREZ/CINV | 16 septiembre 2015

La depresión post-parto afecta a toda la familia y se asocia con peleas maritales, incapacidad de la madre para hacer cosas que antes realizaba sola (por ejemplo, ir de compras o incluso trabajar), y afectando principalmente la interacción materno infantil, presentando conductas tales como hostilidad y desapego. Por otra parte, estudios han demostrado que la depresión post-parto tiene efectos en el desarrollo cognitivo y emocional de los niños durante la infancia y la niñez tardía.

La depresión es un trastorno neuropsiquiátrico de gran impacto social y económico, el cual se caracteriza principalmente por dos síntomas; la anhedonia, o incapacidad de sentir placer y el ánimo deprimido, dentro de los cuales podemos describir síntomas como tristeza constante, decaimiento, irritabilidad, sensación de malestar y frustración.

70) On the trail of the human race: Scientists date to 2.8 million years the oldest fossil of the genus Homo
Web Page: El Mostrador. Neuronews
Date: September 24, 2015
Link: www.elmostrador.cl
Scope: National

Artículo producido en el Convenio con el Centro Interdisciplinario de Neurociencias de Valparaíso

Cultura

Tras la pista del linaje humano: Científicos datan en 2.8 millones de años el fósil más antiguo del género homo

El registro más temprano del género Homo -el género humano- corresponde a una mandíbula con dientes que data entre 2.75 a 2.8 millones de años. El espécimen presenta una combinación de rasgos, algunos de ellos pertenecientes al género Homo y otros al género Australopihecus, del que se cree, surgió el género humano.

Durante décadas, científicos han buscado fósiles que documenten las fases más tempranas del género *Homo*, pero los especímenes recuperados entre 3 y 2.5 millones de años atrás (un periodo crítico en la evolución hacia el género humano) han sido escasos y mal preservados. Como resultado de esto, ha existido poco acuerdo en la época de origen del linaje que finalmente dio paso a los humanos modernos. Con 2.8 millones de años de antigüedad, el hallazgo publicado por el Dr. Brian Villmoare y colaboradores, en *Revista Science*, entrega pistas que apuntan a cambios en la mandíbula y dentadura en el género *Homo* tan solo 200.000 años después de la última aparición de *Australopithecus afarensis*, del cual habrían surgido los humanos y que desapareció hace 3 millones de años, aproximadamente.

71) Neural networks created by neuroscientists bring us closer to the big jump to "life" of Artificial Intelligence
Web Page: El Mostrador. Neuronews
Date: October 7, 2015
Link: www.elmostrador.cl
Scope: National

Convenio con el Centro Interdisciplinario de Neurociencias de Valparaíso (CINV)

Cultura

Redes neuronales creadas por neurocientíficos acercan el gran salto a la "vida" de la Inteligencia Artificial

Expertos han desarrollado un nuevo método para que una Inteligencia Artificial aprenda a jugar un videojuego sólo a partir de información visual, entendiendo por si misma las reglas y la forma de jugar, llegando a superar el desempeño de un humano profesional. Los avances que antes era parte de la ciencia ficción, hoy están a punto de ser parte de la vida diaria.

Una de las preguntas más trascendentales de la humanidad, y que ha eludido respuesta durante siglos, es entender cómo funciona la inteligencia. Una forma de solucionar este problema ha sido construir la llamada inteligencia artificial: si tenemos algo construido por nosotros mismos, sabremos exactamente cómo funciona; así, si logramos construir una inteligencia comparable a la de un ser humano, estaremos más cerca de entender cómo funciona nuestra inteligencia.

Pero ¿por qué conformarse con alcanzar el nivel humano? Muchos sueñan con que podremos llegar a construir inteligencias que sean capaces de superar a los humanos. El primer paso para que este sueño se hiciera realidad, o más bien uno de sus primeros éxitos, fue la famosa *Deep Blue* desarrollada en los años 90, un enorme computador que logró derrotar en ajedrez a Garry Kasparov, lo que sería la primera vez que un computador le ganaba a un campeón mundial. Sin embargo, esta máquina tenía un par de importantes limitaciones: sólo sabía jugar ajedrez, y además había sido programada para ello, no aprendió a jugar por si misma.

Es precisamente el aprendizaje una de las claves para definir la inteligencia. Existen muchas formas de definirla, pero en este caso nos quedaremos con la siguiente: Una inteligencia es un sistema que puede obtener información del ambiente y en base a eso aprender a responder con un comportamiento acorde a cada situación. En este sentido, los videojuegos son un interesante método para poner a competir a un humano contra una máquina, principalmente porque implican realizar acciones complejas frente a distintos escenarios.

Esto no tiene nada que ver con las veces que usted perdió contra "la computadora", ya sea que los fantasmas lo hayan atrapado en el Pacman o los goles que el Messi virtual le haya hecho en el FIFA. En esos casos, el sistema está programado para actuar de cierta forma, siempre se comportará igual, nunca realizará una acción que no esté en su programa ni mejorará su desempeño con el tiempo.

72) Neuroscience: Plastic Containers associated with failures in working memory
Web Page: El Mostrador. Neuronews
Date: October 15, 2015
Link: www.elmostrador.cl
Scope: National

Convenio con Centro Interdisciplinario de Neurociencias de Valparaíso (CINV)

Cultura

Neurociencia: Envases plásticos incidirían en fallas de la memoria de trabajo

La exposición del organismo a altos niveles de BPA ha sido ampliamente relacionada con disfunciones a nivel endocrino y digestivo. Sin embargo, se han realizado estudios que sugieren que este compuesto también podría producir problemas de aprendizaje y de memoria e interferir en la formación de sinapsis, o comunicación entre las neuronas de nuestro cerebro, las que tienen un rol importante en la funciones cognitivas.

¿Alguna vez te has preguntado qué sucede si constantemente reutilizas las botellas plásticas para tomar agua?, o ¿De qué están hechos los recipientes en que conservamos los alimentos?

La mayoría de los recipientes que tenemos a nuestro alcance para conservar alimentos o líquidos están hechos de plástico o de metal. Botellas de agua, biberones, latas de comida y de bebidas, están elaborados de materiales que tienen como materia prima un compuesto llamado bisphenol A (BPA).

El BPA es un compuesto orgánico que se utiliza hace más de 50 años en diferentes tipos de industrias, como en la producción de equipamiento deportivo, dispositivos médicos y dentales, sellantes dentales, anteojos orgánicos, CD-DVD, electrodomésticos y algunos tipos de boletas y facturas.

La exposición del organismo a altos niveles de BPA ha sido ampliamente relacionada con disfunciones a nivel endocrino y digestivo. Sin embargo, se han realizado estudios que sugieren que este compuesto también podría producir problemas de aprendizaje y de memoria e interferir en la formación de sinapsis, o comunicación entre las neuronas de nuestro cerebro, las que tienen un rol importante en la funciones cognitivas.

John D. Elsworth, junto a un grupo de científicos de la Universidad de Yale, New Haven, se preguntaron si la exposición continua a BPA produce alteraciones morfológicas de las neuronas que forman parte de la corteza prefrontal y del hipocampo, regiones de nuestro cerebro que regulan funciones cognitivas, como la atención y la memoria, respectivamente.

73) Science: Menopausal individuals act as "repositories of ecological knowledge"
Web Page: El Mostrador. Neuronews
Date: November 6, 2015
Link: www.elmostrador.cl
Scope: National

Convenio con el Centro Interdisciplinario de Neurociencias de Valparaíso

Cultura

Ciencia: Seres menopáusicos actúan como "repositorios de conocimiento ecológico"

En base a un estudio con una población de Orcas, un equipo de científicos de las universidades de Exeter y York, liderados por el doctor Darren Croft, publicó un artículo en la revista Current Biology, en el que proponen que las hembras en un estado post reproductivo son capaces de transmitir conocimientos importantes a la población, que les permiten sobrevivir en tiempos difíciles.