Annex 3
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NO signaling in retinal bipolar cells

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ABSTRACT

Nitric oxide (NO) is a neuromodulator involved in physiological and pathological processes in the retina. In the inner retina, a subgroup of amacrine cells have been shown to synthesize NO, but bipolar cells remain controversial as NO sources. This study correlates NO synthesis in dark-adapted retinas, through labeling with the NO marker DAF-FM, with neuronal nitric oxide synthase (nNOS) and inducible NOS expression, and presence of the NO receptor soluble guanylate cyclase in bipolar cells. NO containing bipolar cells were morphologically identified by dialysis of DAF fluorescent cells with intracellular dyes, or by DAF labeling followed by immunohistochemistry for nNOS and other cellular markers. DAF fluorescence was observed in all types of bipolar cells that could be identified, but the most intense DAF fluorescence was observed in bipolar cells with severed processes, supporting pathological NO signaling. Among nNOS expressing bipolar cells, type 9 was confirmed unequivocally, while types 2, 3a, 3b, 4, 5, 7, 8 and the rod bipolar cell were devoid of this enzyme. These results establish specific bipolar cell types as NO sources in the inner retina, and support the involvement of NO signaling in physiological and pathological processes in the inner retina.

Nitric oxide (NO) is a signaling molecule that exerts multiple actions throughout the central nervous system (Garthwaite, 2008). It is a product of the conversion of L-arginine to L-citrulline by the enzyme NO synthase (Knowles and Moncada, 1994). In mammals, neuronal NO synthase (nNOS) is the main isoform expressed in neurons; endothelial NOS (eNOS) is expressed in the vascular endothelium, and inducible NOS (iNOS) expression may occur in most types of cells, generally as a consequence of cellular inflammation associated with pathological processes (Blom et al., 2012; Toda and Nakanishi-Toda, 2007). NO has a half-life in the 1-s range and reacts rapidly with other free radicals and heme groups, including those of hemoglobin, resulting in pico-to low nanomolar tissue concentrations under physiological conditions (Garthwaite, 2015; Pacher et al., 2007). NO can act as a neuromodulator by two main mechanisms of action. Activation of soluble guanylate cyclase (sGC), by binding of NO to its heme group with an EC50 of about 3 nM (Rodríguez-Juárez et al., 2007), increases the synthesis of cGMP, which may stimulate protein kinase G or directly activate cyclic nucleotide-gated ion channels (Garthwaite, 2005). On the other hand, NO can modulate target proteins by reversible S-nitrosylation of specific cysteine residues, changing their structure and function in a cGMP-independent manner, but the concentration-dependence of this process remains unclear (Hess et al., 2005; Martínez-Ruiz et al., 2013).

In the inner mammalian retina, an established NO source are nNOS-expressing amacrine cells (NOACs) (Blom et al., 2012; Pang et al., 2010; Walter et al., 2014), whose processes form three more or less distinct bands in the inner plexiform layer (IPL; Fig. 1A) (Kim et al., 2000). Although multiple studies have reported nNOS expression in other retinal cell types of different species, notably in bipolar cells (BCs), a consistent pattern across vertebrates or even mammals has not yet emerged (Vielma et al., 2012). For example, nNOS expression and light-dependent NO synthesis have been shown in BCs of turtle and mouse (Giove et al., 2009b; Yu and Eldred, 2005), but the identity of the involved BC types and their consistency across individual specimen and species remain unknown.

In rat retina, over 10 types of BCs have been morphologically characterized (Euler and Wassle, 1995; Hartveit, 1997; Vielma and Schmachtenberg, 2016), but the full rat BC complement is likely

Abbreviations: BC, bipolar cell; RBC, rod bipolar cell; NO, nitric oxide; NOS, NO synthase; DAF, 4-Amino-5-methylamino-2′,7′-difluorofluorescein; IPL, inner plexiform layer; INL, inner nuclear layer; GCL, ganglion cell layer; sGC, soluble guanylate cyclase.

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NO mostly acts as a paracrine messenger between BCs.

Retinal slices incubated with DAF-FM, to detect the presence of NO and its oxidation products, showed varying degrees of fluorescence in many cell bodies of the inner nuclear and ganglion cell layers (INL and GCL), where BCs and ACs locate (Fig. 2A and B). Occasionally, cellular processes and synaptic terminals could also be distinguished by DAF labeling (Fig. 2A, center inset), but overall labeling of the IPL was subtle (Fig. 2D), in accordance with previous results obtained under dark adaptation (Blom et al., 2012; Giove et al., 2009a). Furthermore, DAF labeling patterns have been shown to differ between HEPES-buffered as opposed to bicarbonate-buffered solutions, resulting in stronger somatic staining in the latter (Tekmen-Clark and Gleason, 2013).

DAF fluorescence was weak or absent from photoreceptors and cell bodies in the outer nuclear layer, as opposed to the pattern observed in chickens (Tekmen-Clark and Gleason, 2013). Interestingly, DAF fluorescence was far more frequent in cell bodies of the INL than nNOS labeling, and co-localization was rare (Fig. 2A; right, inset). Assuming that DAF-labeling is NO-specific (Namin et al., 2013), and that DAF is taken up uniformly by BCs, NO could be the product of an nNOS splice variant undetected by our nNOS antisera (Alderton et al., 2001; Giove et al., 2009a), or of a different NOS isoform. However, eNOS is generally absent from the mature neuroretina (Blom et al., 2012; Haverkamp et al., 1999), and iNOS expression depends on cellular damage and inflammation (Toda and Nakanishi-Toda, 2007). Retinal slices labeled for iNOS showed variable degrees of staining that was most intense in the OPL, but also present in the INL and IPL, and coincided partly with DAF fluorescence patterns, supporting a role of iNOS as additional NO source in the inner retina (Fig. 2C).

In the INL, putative BC cell bodies displayed variable degrees of DAF fluorescence (Fig. 2A and B). To identify the types of BCs containing NO, we labeled strongly DAF-fluorescent BCs with dye injection through a patch pipette to allow their identification based on morphological criteria (Fig. 2D). Although the majority of BC types were identified in this way, the most frequent and most intensely DAF fluorescent cells were visibly damaged BCs with severed axons or dendrites (Fig. 2E). Because iNOS is upregulated under pathological conditions in the retina (Palamalai et al., 2006; Toda and Nakanishi-Toda, 2007), damaged cells might enter a state of cellular inflammation and express iNOS, which can generate large amounts of NO (Alderton et al., 2001).

Among the apparently intact BCs, type 8 and the RBC had the highest incidence of DAF fluorescence (Fig. 2E). The observation that these BC types do not express nNOS (Fig. 1D) suggests that they are cellular targets, but not sources, in retinal NO signaling. In the case of the RBC, the absence of nNOS expression is in line with previous studies (Walter et al., 2014). In a thorough review of the literature, we found no unequivocal evidence for nNOS expression in RBCs of rat, mouse, guinea pig and several non-mammalian vertebrates, although various studies showed nNOS expression in other or unidentified BC types (see (Vielma et al., 2012) and references therein). However, the function of RBCs as NO target has been reported previously (Snellman and Nawy, 2004). Indeed, a significant percentage of DAF fluorescent BCs also expressed sGC (Fig. 2B), supporting this interpretation. Finally, it is also possible that DAF fluorescent BCs accumulate significant amounts of NO from levels of NOS undetectable by standard immunohistochemistry, considering that DAF-FM is a signal-integrating fluorophore and therefore quite sensitive.

On the other hand, DAF fluorescence was observed in some nNOS-positive BCs (Fig. 2A). This is to be expected, since both BCs and ACs are differentially depolarized under light or dark adaptation in retinal ON and OFF pathways. Previous studies have shown NO synthesis in dark-adapted retinas, although light stimulation, especially with flickering light, appeared to increase NO levels (Blom et al., 2012). Still, we would have expected to find a significant number of DAF fluorescent BC type 9 cells compared to other, nNOS-negative BC types. Certainly, the quantitative relationship between nNOS and iNOS expression versus NO synthesis remains to be established for retinal BCs and neurons in general. Together, the results presented here suggest that NO signaling in the inner retina is more complex than previously thought, involving specific BC types as additional nNOS-positive NO sources, while iNOS expression and high levels of NO are generally associated with cellular damage.

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ORIGINAL ARTICLE

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Amphetamine treatment affects the extra-hypothalamic vasopressinergic system in a sex- and nucleus-dependent manner

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The lateral septum (LS), a brain structure implicated in addictive behaviours, regulates the activation of dopaminergic neurones in the ventral tegmental area. Vasopressinergic projections from the extended amygdala to the LS, which are sexually dimorphic, could be responsible for the vulnerability to addiction in a sex-dependent manner. The present study aimed to investigate the modulatory effects of amphetamine (AMPH) on the expression of vasopressin (AVP) in the vasopressinergic extra-hypothalamic system in sensitised male and female rats. Adult male and female Sprague-Dawley rats underwent an AMPH-locomotor sensitisation protocol. Acute AMPH increased AVP mRNA expression in the medial amygdala (MeA), whereas AMPH-induced sensitisation increased AVP mRNA expression in the bed nucleus of the stria terminalis (BNST) only in females. Interestingly, the increase in AVP expression in BNST was higher in oestrus females compared to dioestrus females and acute AMPH resulted in a decrease in AVP levels in the LS, only in males. Thus, there are complex and region-specific interactions between AMPH and the extra-hypothalamic vasopressinergic system in the brain, underlying possible alterations in different behaviours caused by acute and chronic AMPH exposure.

KEYWORDS
amphetamine, extended amygdala, lateral septum, male and female rats, vasopressin

1 | INTRODUCTION

Drug addiction is a chronic brain disease characterised by a loss of control in drug consumption and negative emotional states such as dysphoria and anxiety, both being exacerbated during withdrawal.[1] Drugs of abuse activate the reward system via different mechanisms, ultimately resulting in the increase of dopamine release in the nucleus accumbens.[2,3] The pathophysiology of addiction is based on molecular, genetic and physiological neuroadaptations that could mediate the development and maintenance of long-term drug addictive behaviours.[4] At the neurobiological level, one of the main dopaminergic nucleus in the reward system is the ventral tegmental area (VTA)[5] that projects to the nucleus accumbens, prefrontal cortex[4] and other limbic areas, such as the lateral septum (LS).[6] The VTA GABAergic interneurones regulate the VTA dopaminergic neurones via an inhibitory tone.[7,8] In addition, extrinsic GABAergic regulation from the LS has also been described.[9]

The involvement of the LS in reward-related processes has been documented in an early study by Olds and Milner,[10] who reported that animals experience a greater reward when the septal area is self-stimulated. The LS is considered a relay station for neural information from and into different brain areas[11] through its GABAergic projections.[12] Similar to the VTA, the LS has intrinsic GABAergic regulation.[9,12,13] Some nuclei connected to the LS are the hippocampus (regulating memory),

with changes in LS AVP content. This may suggest an increase in AVP release and/or peptide degradation in the LS, which may feedback to subsequently produce an increase in AVP transcription. Also, the AVP increase in AMPH-AMPH females could be related to the acquisition of sensitised behaviour because a lack of behavioural sensitisation to cocaine is observed in vasopressin-deficient Brattleboro rats.[47] On the other hand, it is well known that extra-hypothalamic vasopressin system is involved in social behaviours such as social interaction, sexual behaviour and aggression; all of these could be altered by drugs of abuse.[24] In recent years, AMPH treatment has been shown to impair pair bonding and partner preference formation[48] by altering oxytocin and AVP systems. Also, AVP is related to anxiety and depression states[24] and drugs of abuse produce and alter the stress response and consequent anxiety behaviour.[49] Therefore, the alterations that we observed in this system may result in more anxious behaviour in females after AMPH treatment. Little is known about sex differences in behaviours that are altered following chronic drug use, although future experiments are needed to address this question and may help to explain why females are more vulnerable to relapse than males.

In summary, the extra-hypothalamic vasopressinergic system is susceptible to changes according to treatment duration, doses and the withdrawal period following drug abuse. The results of the present study suggest the existence of complex and region-specific interactions between AMPH and the extra-hypothalamic vasopressinergic system in the brain that underlie the different behaviours caused by acute and chronic AMPH exposure.

4.4 | AVP levels in LS and AVP mRNA expression in MeA and BNST according to the stage of the oestrous cycle

The results of the present study show that AVP expression in sensitised females varies depending on the stage of the oestrous cycle. Sensitised females in oestrus, when progesterone levels are minimal, have greater levels of AVP mRNA in the BNST than females in dioestrus, when progesterone levels peak.[50] In this sense, progesterone was shown to inhibit BNST and MeA vasopressin neurones that project to the LS and lateral habenula.[45] However, these differences do not occur in the MeA, probably because of the presence of different types and/or quantity of sex hormone receptors. Therefore, we conclude that the effect of AMPH on AVP levels according to the stage of the oestrous cycle is dependent on the brain nucleus.

5 | CONCLUSIONS

In the present study, we have shown that an acute AMPH injection in males decreased AVP content in the LS. On the other hand, AMPH-induced behavioural sensitisation elicits a significant increase in AVP expression in the BNST of females. Interestingly, this increase in AVP is higher when females are at the oestrus stage. Because the extra-hypothalamic vasopressinergic system regulates social and anxiety behaviours that could be altered by drugs of abuse, further studies are necessary to claridy the implications of the alterations observed in the vasopressinergic system induced by AMPH.

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$$J = \frac{Dz U_m}{a} \frac{c_{in} e^{zU_m} - c_{ex}}{e^{zU_m} - 1}. \quad \text{(A8)}$$

Introducing the partition coefficient, β, relating the membrane and solution concentrations, $c = \beta c'$, and defining the permeability coefficient P as $P = D\beta / a$.

$$J = Pz U_m \frac{c_{in} e^{zU_m} - c_{ex}}{e^{zU_m} - 1}. \quad \text{(A9)}$$

The electric current density, I, is zF times the mass flow, J:

$$I = Pz^2 F U_m \frac{c_{in} e^{zU_m} - c_{ex}}{e^{zU_m} - 1}. \quad \text{(A10)}$$

Expanding the reduced potential, we get the electric current density in terms of membrane potential, V_m, and ion concentrations (Eq. 1):

$$I = \frac{Pz^2F^2}{RT} V_m \frac{c_{in} e^{zFV_m/RT} - c_{ex}}{e^{zFV_m/RT} - 1}. \quad \text{(A11)}$$

For the condition $I = 0$ and from Eq. A10, we can define a Nernst reduced potential, U_{Nernst}. $U_{Nernst} = c_e / c_{in}$.

We introduce the Nernst reduced potential as

$$I = Pz^2 F U_m c_{ex} \frac{e^{z(U_m - U_{Nernst})} - 1}{e^{zU_m} - 1} \quad \text{(A12)}$$

for $c_{in} \approx c_{ex}$, $U_{Nersnt} \approx 0$ and for $U_m \approx U_{Nernst}$. The approximation $e^x - 1 = x$ is accurate for $x \to 0$, so the current density becomes a liner function of voltage:

$$\begin{aligned} &I = Pz^2 F c_{ex} (U_m - U_{Nernst}) \\ &\text{or} \\ &I = \frac{Pz^2F^2 c_{ex}}{RT} (V_m - V_{Nernst}), \end{aligned} \quad \text{(A13)}$$

which is the widely used linear form, a variation of Ohm's law

$$I = G(V_m - V_{Nernst}), \quad \text{(A14)}$$

where the conductance, G, is

$$G = \frac{Pz^2F^2 c_{ex}}{RT}. \quad \text{(A15)}$$

Appendix B

There is no need for the assumption that the field is constant within the membrane for the case of univalent cations alone (e.g., Na^+ and K^+ in the case of a cation- or anion-selective membrane), as long as the ions are of the same valence.

From Eq. A4, we get

$$J e^{zU} dx = -D d(c e^{zU}). \quad \text{(B1)}$$

We prepare for integration

$$J \int_0^a e^{zU} dx = -D \int_0^a d(c e^{zU}). \quad \text{(B2)}$$

We integrate the right side of Eq. B2 using the boundary conditions defined in Appendix A.

$$J \int_0^a e^{zU} dx = -D(c'_{ext} - c'_{in} e^{zU_m}), \quad \text{(B3)}$$

$$J = -\frac{D(c'_{ext} - c'_{in} e^{zU_m})}{\int_0^a e^{zU} dx}. \quad \text{(B4)}$$

Kramers (1940) first derived Eq. B4. Making zero the sum of the flows of sodium and potassium, the integral on the common denominator in Eq. B4 disappears as well as the constants to get P from D, so we can write

$$\begin{aligned} 0 &= J_{Na} + J_K = \\ &P_{Na}([Na]_{ex} - [Na]_{in} e^{U_m}) + P_K([K]_{ex} - [K]_{in} e^{U_m}). \end{aligned} \quad \text{(B5)}$$

Finally, we solve for the zero-current potential V_m:

$$e^{U_m} = \frac{P_{Na}[Na]_{ex} + P_K[K]_{ex}}{P_{Na}[Na]_{in} + P_K[K]_{in}}, \quad \text{(B6)}$$

$$V_m = \frac{RT}{F} ln \frac{P_{Na}[Na]_{ex} + P_K[K]_{ex}}{P_{Na}[Na]_{in} + P_K[K]_{in}}. \quad \text{(B7)}$$

Thus, we recover the GHK equation for sodium and potassium without using the constant-field assumption.

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Editorial

Molecular and Cellular Mechanisms of Synaptopathies

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Synapses, contact points between neurons, are essential elements supporting the ability of neurons to communicate and to transmit relevant information to each other. They play an integral role in brain development and wiring neurons into neural circuits, for example, those related to our behavior. Therefore, alterations affecting the integrity and/or functionality of synapses can lead to synaptic pathologies or synaptopathies. For instance, many neurological disorders including Alzheimer's disease, Down syndrome, epilepsy, and Parkinson's disease and neurodevelopmental disorders such as autism spectrum disorders, intellectual disability, and fragile X syndrome have consistently been reported to exhibit abnormalities in synaptic composition, morphology, and function. This special issue discusses various aspects of the molecular interactions that underlie synaptic protein networks and the complex signaling pathways that are activated by them, knowledge that is crucial to understand the cellular and molecular mechanisms involved in different synaptopathies. Synapses comprise a presynaptic compartment, consisting of the axon terminal and their protein machinery implicated in the release of neurotransmitters. Upon exocytosis of presynaptic vesicles, neurotransmitters spill out into the extracellular space called the "synaptic cleft" and diffuse to reach a postsynaptic compartment, composed of the protein machinery that receives and transduces the neurotransmitter-induced signals [1]. Most synaptopathies directly or indirectly affect the molecular repertoire of synaptic proteins.

V. I. Torres et al. provide a comprehensive review describing specific pre- and postsynaptic proteins that are involved in the physiopathology of various synaptopathies and how deficits in these molecules contribute to different synaptopathic mechanisms. V. I. Torres et al. catalogue the different presynaptic and postsynaptic proteins that to date have been implicated in neuropsychiatric, neurodevelopmental, and neurodegenerative disorders. A more specific review by G. Rudenko focusses on synaptic adhesion molecules (SAMs), many of which are now implicated in neuropsychiatric, neurodevelopmental, and neurodegenerative diseases as well. SAMs tether to the pre- or postsynaptic membranes extending their extracellular domains into the synaptic cleft where they coordinate protein interaction networks. A much larger diversity of SAMs and their protein interactions exists than has previously been appreciated. In

addition, SAMs drive more complex functions than purely the adhesion of presynaptic and postsynaptic membranes. Furthermore, SAMs are under dynamic control through a variety of mechanisms, enabling them to play a key role in plasticity at synapses. Thus, considering the crucial role that SAMs play in synapse development, they may yield novel therapeutic targets which can be exploited to ameliorate certain synaptopathies.

Several articles in this special issue focus on specific synaptic proteins in detail. S. Biggi et al. present evidence that c-Jun N-terminal kinase (JNK), acting presynaptically, may have an important functional role in synapses. JNK is part of a signaling pathway strongly activated by NMDA stimulation and involved in synaptic plasticity. It is noteworthy that until now, most studies have been focused on the postsynaptic mechanism of JNK action, and less is known about JNK presynaptic localization and its physiological role at this site. S. Biggi et al. demonstrate that activation of presynaptic NMDA receptors leads to the activation of JNK which modulates neurotransmitter release through a direct interaction with SNARE proteins. These findings are relevant considering that JNK activity had been associated with not only neurodegenerative disorders like Alzheimer's, Huntington's, and Parkinson's disease but also psychiatric disorders and intellectual disabilities. F. Longhena et al. review recent evidence indicating that α-synuclein possesses a novel "prion-like" behavior, whereby the protein can spread transsynaptically to trigger the aggregation of α-synuclein in neighboring neurons, a phenomenon observed in Parkinson's disease animal models and patients [2–5]. Monomeric, oligomeric, and fibrillary α-synuclein forms accumulate at the synaptic terminal due to their high affinity for vesicular membranes and can be released in a process that is thought to be mediated mainly by exosomal vesicles [6, 7]. Once released, α-synuclein could affect endogenous α-synuclein on recipient neurons, thereby affecting their function, and/or could interact with membrane lipids leading to both pre- and postsynaptic alterations and hence synaptic failure. The elucidation of this novel mechanism of α-synuclein transmission can shed lights on the contribution of α-synuclein spreading to Parkinson's disease related synaptopathy. E. Pérez-Palma et al. introduce new evidence relating to Wnt/β-catenin signaling and the expression of novel Wnt/β-catenin target genes. In addition to genes involved in neural precursors, forebrain development, and stem cell differentiation, E. Pérez-Palma et al. also identified a significant number of genes with transcription factor activity. The genes modulated by Wnt/β-catenin signaling are involved in biological processes such as neuronal structure and activity, and that are affected in synaptopathies. B. K. Unda et al. provide evidence that the secreted neurotrophic factor neuregulin-1 (NRG1) and its receptor ErbB4 signal through the multifunctional scaffold protein, disrupted in schizophrenia 1 (DISC1). Together, they regulate the development of cortical inhibitory interneurons and thus also impact the proper balance between excitatory and inhibitory transmissions. Since the genes encoding these proteins have been identified independently as risk factors for schizophrenia, a complete understanding of how these proteins interact to regulate the development of cortical inhibitory neuron morphology and synapse formation may provide insights into how these processes progress into synaptopathies.

Further, in this special issue, two papers focus on the use of animal models to yield a wealth of information about the underlying mechanisms of synaptopathies. Epilepsy is a neuropsychiatric condition characterized by an abnormal and excessive neural activity leading to a predisposition to recurrent unprovoked seizures affecting both excitatory and inhibitory synapses, synaptic plasticity, and behavior [8]. M. Lenz et al. use a pilocarpine-induced status epilepticus animal model to study synaptopodin, an actin-binding protein expressed in cortical neurons that is involved in the modulation of synaptic plasticity and spine remodeling [9, 10]. They propose that synaptopodin is a valuable diagnostic marker to detect alterations in the ability of neurons to undergo synaptic plasticity such as can be observed in seizure-induced synaptopathies; M. Lenz et al. provide further support for the role of synaptopodin in the ability of hippocampal neurons to exhibit synaptic plasticity, demonstrating that intraperitoneal pilocarpine injection reduces synaptopodin levels and affects the induction of long-term potentiation (LTP). M. I. Herrera et al. review the literature linking perinatal asphyxia and synaptic dysfunction obtained from an experimental model of perinatal asphyxia. Perinatal asphyxia is an obstetric complication resulting to abnormal brain development in term and preterm neonates; if affected babies survive, they develop neurological disorders such as epilepsy, cerebral palsy, mental retardation, attention-deficit disorder, and schizophrenia. M. I. Herrera et al. present evidence that perinatal asphyxia induces a series of modifications in synaptic composition, structure, and function leading to alterations in synapses and their function.

Lastly, focusing on the role of gene-environment interaction in mental disorders, S. Pfaender et al. provide evidence that zinc signaling plays a role in proliferation and neuronal differentiation of stem cells. They show that zinc deficiency during brain development impairs neurogenesis and modulates the expression of synaptic proteins, primary mechanisms influencing brain function.

In summary, articles published in this special issue highlight the very complex nature of synaptopathies, the participation of a very diverse portfolio of synaptic proteins linked to synaptopathies, and emphasize the importance of understanding these proteins to identify potential novel targets which might benefit the development of therapies for neuropsychiatric, neurodevelopment, and neurodegenerative diseases.

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ION CHANNELS, RECEPTORS AND TRANSPORTERS

The inhibition of voltage-gated H^+ channel (HVCN1) induces acidification of leukemic Jurkat T cells promoting cell death by apoptosis

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Abstract Cellular energetic deregulation is widely known to produce an overproduction of acidic species in cancer cells. This acid overload must be counterbalanced with a high rate of H^+ extrusion to maintain cell viability. In this sense, many H^+ transporters have been reported to be crucial for cell survival and proposed as antineoplastic target. By the way, voltage-gated proton channels (Hv1) mediate highly selective H^+ outward currents, capable to compensate acid burden in brief periods of time. This structure is canonically described acting as NADPH oxidase counterbalance in reactive oxygen species production. In this work, we show, for the first time in a oncohematologic cell line, that inhibition of Hv1 channels by Zn^{2+} and the more selective blocker 2-(6-chloro-1H-benzimidazol-2-yl)guanidine (ClGBI) progressively decreases intracellular pH in resting conditions. This acidification is evident minutes after blockade and progresses under prolonged exposure (2, 17, and 48 h), and we firstly demonstrate that this is followed by cell death through apoptosis (annexin V binding). Altogether, these results contribute strong evidence that this channel might be a new therapeutic target in cancer.

Keywords HVCN1 · Voltage-gated proton channel · Intracellular pH · Apoptosis · Leukemia · Cancer

Introduction

Voltage-gated H^+ channels (Hv1), encoded by the *hvcn1* gene, mediate highly selective H^+ outward currents [42], avoiding cell acidification and depolarization. This relevant homeostatic function of Hv1 channels is essential for a variety of immune cells such as neutrophils [33, 46, 57], eosinophils [9, 21, 39], basophiles, B lymphocytes [6] as well as spermatozoa [34], osteoclasts [44], and myocardial fibroblasts [7]. The intracellular pH (pH_i), which typically ranges in a narrow window (7.2–7.4), is finely controlled for cell survival since the vast majority of cell machinery has a well-defined pH for optimal activity (i.e., enzyme activity). Indeed, intracellular acidification is an early key event leading to cell death by different apoptotic stimuli [31, 36, 37]. Particularly, in cancer cells, it has been widely demonstrated that, regardless of the amount of oxygen available, a metabolic deregulation promotes the use of the less efficient glycolytic pathway increasing acidic species concentration [29, 56]. In the absence of compensatory mechanisms, tumor cell survival would be compromised. Thus, cell structures capable of reducing the acid load in tumor cells give them an advantage to escape from the acidification-related cell death. Hv1 channels are excellent candidates that can contribute to this process. The activity of Hv1 channels allows a quick compensation of acidic cell

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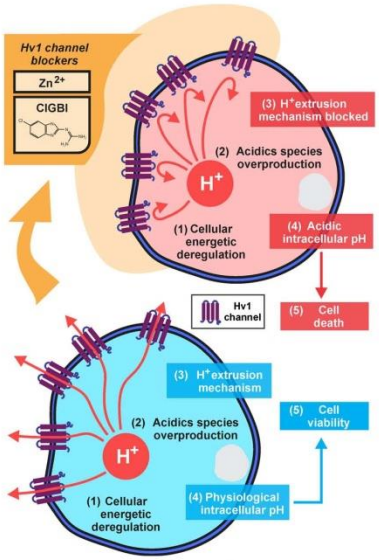

Fig. 6 The inhibition of voltage-gated H^+ channel induces intracellular acidification promoting cell death by apoptosis in the cell line studied. Depiction representing the hypothesis of Hv1 channel role in the context of neoplastic cells: Jurkat T lymphocytes, as other neoplastic cells, obtain energy via glycolytic pathways producing a high amount of acidic species that must be removed to the extracellular milieu. Blocking H^+ extrusion structures, mainly Hv1 channel, produces intracellular acidification that further derives in apoptosis

Ion channels are also frequently implicated in the control of proliferation, apoptosis, and cell migration of cancer cells, and the leukemic cells are not the exception. Recently, Arcangeli et al. reviewed the experimental and preclinical evidence that have ion channels as biological target in leukemia treatment [1]. However, up to date, no review of the field has mentioned Hv1 as a channel to be considered in apoptosis. In the same line, another feature to be further evaluated is if Hv1 blockade derives in membrane depolarization, another event coherent with apoptosis development [4, 43]. The use of new drugs or endogenous modulators that could selectively inhibit the channel may be useful not only in leukemia cells but also in solid tumors; recent works have shown that Hv1 downregulation or inhibition decreases the migratory and invasive abilities of highly metastatic colorectal [64], human breast [62, 63], and glioma [65] tumor cell, as well as impairing proliferation. Latest reports from the field reveal that Hv1 is also functionally expressed in human glioblastoma multiforme cells to which Zn^{2+} treatment induces cell death (PI staining), notwithstanding that significant intracellular acidification is only seen under extracellular Na^+ deprivation [49].

Our work presents evidences suggesting that Hv1 inhibition might be a new and promising therapeutic target in leukemia treatment. It is worthwhile to note that KO mice lacking Hv1 channel are not associated with immunosuppression [48, 52], although decreased ROS production. Indeed, Clapham et al. challenged $Hv1^{-/-}$ mice against *Staphylococcus aureus* intraperitoneal injection, *Pseudomonas aeurginosa*, and *Burkholderia cepacia* nasal inoculation and were unable to see a significant impairment on bacterial clearance in vivo compared with *w/w* mice [48]. These facts let us speculate that Hv1 pharmacological inhibition might be a safe strategy in oncohematological diseases in contrast to classical antineoplastic drugs. This work, among others, may contribute to keeping this potent structure in mind.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Structural determinants of 5′,6′-epoxyeicosatrienoic acid binding to and activation of TRPV4 channel

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TRPV4 cation channel activation by cytochrome P450-mediated derivatives of arachidonic acid (AA), epoxyeicosatrienoic acids (EETs), constitute a major mechanisms of endothelium-derived vasodilatation. Besides, TRPV4 mechano/osmosensitivity depends on phospholipase A_2 (PLA_2) activation and subsequent production of AA and EETs. However, the lack of evidence for a direct interaction of EETs with TRPV4 together with claims of EET-independent mechanical activation of TRPV4 has cast doubts on the validity of this mechanism. We now report: 1) The identification of an EET-binding pocket that specifically mediates TRPV4 activation by 5′,6′-EET, AA and hypotonic cell swelling, thereby suggesting that all these stimuli shared a common structural target within the TRPV4 channel; and 2) A structural insight into the gating of TRPV4 by a natural agonist (5′,6′-EET) in which K535 plays a crucial role, as mutant TRPV4-K535A losses binding of and gating by EET, without affecting GSK1016790A, 4α-phorbol 12,13-didecanoate and heat mediated channel activation. Together, our data demonstrates that the mechano- and osmotransducing messenger EET gates TRPV4 by a direct action on a site formed by residues from the S2-S3 linker, S4 and S4-S5 linker.

The transient receptor potential vanilloid 4 (TRPV4) is a widely expressed nonselective cation channel that shows a polymodal gating behavior[1,2]. TRPV4 is activated by physical stimuli such as hypotonicity[3–5], mechanical forces[6–8], moderate heat[9–11] or UVB radiation[12], and by both natural (epoxyeicosatrienoic acids, EETs[13,14] and bisandrographolide[15]) and synthetic agonists (e.g., 4α-phorbol 12,13-didecanoate (4α-PDD)[16] and GSK1016790A[17]). Due to this gating promiscuity, TRPV4 participates in multiple physiological processes, including cellular[5,18] and systemic volume homeostasis[19,20], endothelial function and angiogenesis[14,21–23], epithelial hydroelectrolyte transport[24], nociception[25], bladder voiding[26], ciliary beat frequency regulation[8,27], innate immunity[28], matrix stiffness[29], cartilage maintenance and chondroprotection[30,31], and bone development[32].

Intracellular lipid metabolites are important modulators of TRPV4 gating: Phosphatidylinositol 4,5-bisphosphate (PIP_2) binding to a stretch of positive charges within the N-tail of each cannel subunit is required for TRPV4 activation by hypotonicity and heat[11] while EETs derived from AA promote TRPV4 opening[13]. EETs also appear to act as messengers that mediate TRPV4 activation in response to either hypoosmotic shock[33] or mechanical stimulation[8,34]. In this regard, PLA_2 is activated by hypotonic and mechanical stimulation[35,36] but no direct measurements of EETs have been reported in response to these stimuli. Besides, EETs constitute a major type of endothelium-derived hyperpolarizing factors that promote vascular relaxation through two plausible mechanisms involving TRPV4. First, EETs induce TRPV4-mediated Ca^{2+} influx into smooth muscle cells

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Author Contributions

F.G.-N., J.M.F.-F. and M.A.V. designed research; A.B.-E., M.I.-S., R.V.S., F.R.-M., P.D.-M., S.A.S., J.C.-G., A.P.-M. and J.M. F.-F. performed research; A.B.-E., M.I.-S., R.V.S., F.G.-N., J.M.F.-F. and M.A.V. analyzed data; and J.M.F.-F. and M.A.V. wrote the paper. All authors collaborated in paper edition.

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Short communication

Short Communication

The Chilean Recluse Spider (Araneae: Sicariidae) Displays Behavioral Responses to Conspecific Odors, but Not to Several General Odorants

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Abstract

Spiders of the family Sicariidae pose a serious threat to affected populations, and *Loxosceles laeta* (Nicolet) is considered the most venomous species. Development of nontoxic olfaction-based spider repellents or traps is hindered by a current lack of knowledge regarding olfactory system function in arachnids. In the present study, general plant odorants and conspecific odors were tested for behavioral responses in *L. laeta*. Although general odorants triggered neither attraction nor aversion, conspecific odor of the opposite sex caused aversion in females, and attraction in males. These results support the presence of a specific olfactory system for the detection of conspecifics in *L. laeta*, but suggest the absence of a broadly tuned system for general odorant detection in this species.

Key words: arachnid, sicariid, olfaction, chemosensation, pheromone

Over 100 species of the genus *Loxosceles*, originally native to the Americas, East India, Mediterranean Europe, and Africa, are now distributed worldwide, except for the Antarctic (Nentwig 2013). Of these, several species have become synanthropic, notably *Loxosceles reclusa* Gertsch & Mulaik, *Loxosceles intermedia* Mello-Leitão, and *Loxosceles laeta* (Nicolet, 1849), creating a public health problem owing to their ability to cause severe dermonecrotic lesions with occasionally fatal complications (Binford and Wells 2003). The latter species stands out within its genus because its venom, containing among others, hyaluronidases, proteases, allergenic factors, and phospholipase D, is comparatively potent, causing a higher percentage of fatalities than other *Loxosceles* species (Gremski et al. 2014). Owing to its relevance for public health in countries where it is endemic, the biology of this species has become a focus of investigation in recent years (Vetter and Rust 2008, 2010; Canals et al. 2015a,b).

As for other spiders, the presence of an olfactory sense in *L. laeta*, able to convey chemical information regarding potential prey, predators, conspecifics, or the environment in general, remains uncertain, although it has been proposed early (Dahl 1885). Knowledge about olfactory capabilities, preferences, and aversions of *Loxosceles* spiders could in principle be employed to create specific and nontoxic repellants or traps, but a persistent lack of fundamental science hinders the development of such products. This study reveals the presence of an olfactory sense detecting conspecifics in *L. laeta*, but individual plant-derived odorants, presumably without biological meaning to *L. laeta* and therefore considered general odorants (Touhara and Vosshall 2009), failed to generate any attractive or repulsive responses in this species.

Materials and Methods

All experiments were performed on adult male and female specimens of *L. laeta*, obtained from the houses of members of the University of Valparaiso in Valparaiso, Chile. Spiders were staged by size according to Canals and Solis (2014), and male sexual maturity was confirmed by visual inspection of the pedipalps. The average body mass of the specimens was 121 ± 24 mg for males and 108 ± 28 mg for females. Spiders were kept individually in aerated 100-ml cup vials under a photoperiod of 12:12 (L:D) h at 20 °C and 80% relative humidity for up to 3 mo, with water but without food. Two different mazes, with two and four arms, respectively, were designed and printed using T-Glase plastic (Taulman, Saint Peters, MO) on a commercial 3D-printer. The mazes were covered with glass and mounted in a dark Faraday chamber under infrared illumination. A membrane pump connected by teflon tubing was used to deliver odorants with a constant flow of air from impregnated (2-µl) filter papers, inserted in 10-ml glass vials connected to the ends of

Results and Discussion

Most animals use combinatorial odor detection with fairly unspecific receptors to analyze a potentially large variety of general odorants, and highly sensitive complementary systems with high affinity receptors for the detection of a limited number of specific odorants, or pheromones (Buck 2004). The present study set out to obtain behavioral evidence for general and specific, in this case conspecific, odor detection by *L. laeta*. *L. laeta* is a significant biological risk factor for public health in the Americas, and anecdotical information suggests that certain odoriferous products, e.g., containing peppermint, limonene, or citrus, can have a repellent and toxic action on this and other arachnids (Junker et al. 2011). However, experiments with five synthetic odorants commonly used in olfactory research, caused no statistically significant behavioral response patterns, notably neither attraction nor aversion ($H = 2.36$, $df = 3$, $P = 0.50$; Fig. 2A, and $H = 2.37$, $df = 3$, $P = 0.50$; Fig. 2B), and consequently do not constitute evidence for their detection by a putative olfactory system in *L. laeta*.

Regarding conspecific odor detection, when offered the choice between odor from a live mature conspecific of the opposite sex or blank controls, females showed no preference in the four-arm maze ($H = 0.50$, $df = 3$, $P = 0.91$; Fig. 2C), but significantly preferred the blank arm over the male-odor arm in the two-arm maze ($W = -39$, $df = 1$, $P = 0.024$; Fig. 2E). On the other hand, males displayed a strong preference for maze arms exposed to female odor, in both the four-arm ($H = 21$, $df = 3$, $P = 0.0001$; Fig. 2D) and two-arm maze experiments ($W = 49$, $df = 1$, $P = 0.014$; Fig. 2F). In the absence of any other, visual or tactile, clue as to the odor source, olfactory detection of conspecific odors, possibly containing pheromones, is the most likely trigger of these behavioral responses. The differences between males and females are in agreement with the generally more active role of male spiders in courtship behavior, compared with the mostly passive and expectant role of females (Foelix 2011).

Most arachnids have sensory hair-like structures called sensilla on their legs or leg-like appendices (Brownell 1998). Sensilla chemoreceptors are concentrated on the distal segments of feet and palps. They are S-shaped hairs with distal pores and were thought to operate mainly as contact chemoreceptors (Foelix and Chu-Wang 1973). Electrophysiological recordings from chemosensory sensilla in *Cupiennius salei* Keyserling (Ctenidae) revealed responses to direct stimulation of the pore with putative pheromones, and also to superfusion of the pore with different odors (Barth 2002), supporting these sensilla as candidate olfactory organs in arachnids (Foelix 2011). However, crucial elements of any olfactory system, such as receptors, pathways, and central processing units, are only beginning to be investigated in spiders (Nentwig 2013). On the other hand, behavioral evidence has shown in a variety of species that arachnids have the ability to detect specific odors, relevant for the identification of potential prey or conspecifics. Specific odorant detection, including potential pheromones, has been shown among others in members of the families Pholcidae, Araneidae, Lyniphiidae, Agenelidae, and Ctenidae (Schulz 2013). *Cupiennius salei* is certainly the most studied species, in which a characteristic male courtship response to contact with silk woven by a female has been described (Tichy et al. 2001). More recently, olfactory capabilities were evaluated with an olfactometer in four species of the genus *Portia* (Salticidae), revealing olfactory identification of potential mates by males, but not by females (Jackson and Cross 2011). Food odors were also shown to be detected and analyzed by *C. salei*, which has been reported to use its olfactory sense to judge the sensitivity of potential prey to its venom, allowing economization of this valuable resource (Hostettler and Nentwig 2006). Further examples for the olfactory detection of specific food and prey were found in *Zodarion rubidum* (Canestrini) (Zodariidae) and *Evarcha culicivora* Wesolowska & Jackson (Salticidae) (Cross and Jackson 2010, Cárdenas et al. 2012). The aforementioned examples suggest the presence of olfactory systems for the detection of a limited number of special, biologically relevant odorants in arachnids, but their capability for the detection of general odorants, as present in most vertebrates and many insects (Kaupp 2010), among others the honey bee and *Drosophila melanogaster* Meigen, remains doubtful. Accordingly, the present study, the first to investigate distance chemoreception in a species of the family Sicariidae, found no evidence for the detection to several general odorants, but olfactory responses to conspecifics were readily obtained. These data are in line with a prior study showing that acetone extract of conspecific silk was repellent to *L. laeta* (Vetter and Rust 2010).

In conclusion, our results support the interpretation that spiders possess, apart from contact chemoreceptors, the capability for pheromone and specific odor detection, but lack universal combinatorial-type olfactory systems for general odorant detection. Therefore, odorant-based spider repellents are unlikely to be effective for sicariid spiders, and specific traps might require application of pheromones, which have yet to be identified.

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ORIGINAL ARTICLE

Knockdown of Myo-Inositol Transporter SMIT1 Normalizes Cholinergic and Glutamatergic Function in an Immortalized Cell Line Established from the Cerebral Cortex of a Trisomy 16 Fetal Mouse, an Animal Model of Human Trisomy 21 (Down Syndrome)

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Abstract The Na^+/myo-inositol cotransporter (SMIT1) is overexpressed in human Down syndrome (DS) and in trisomy 16 fetal mice (Ts16), an animal model of the human condition. SMIT1 overexpression determines increased levels of intracellular myo-inositol, a precursor of phophoinositide synthesis. SMIT1 is overexpressed in CTb cells, an immortalized cell line established from the cerebral cortex of a Ts16 mouse fetus. CTb cells exhibit impaired cytosolic Ca^{2+} signals in response to glutamatergic and cholinergic stimuli (increased amplitude and delayed time-dependent kinetics in the decay post-stimulation), compared to our CNh cell line, derived from the cerebral cortex of a euploid animal. Considering the role of myo-inositol in intracellular signaling, we normalized SMIT1 expression in CTb cells using specific mRNA antisenses. Forty-eight hours post-transfection, SMIT1 levels in CTb cells reached values comparable to those of CNh cells. At this time, decay kinetics of Ca^{2+} signals induced by either glutamate, nicotine, or muscarine were accelerated in transfected CTb cells, to values similar to those of CNh cells. The amplitude of glutamate-induced cytosolic Ca^{2+} signals in CTb cells was also normalized. The results suggest that SMIT1 overexpression contributes to abnormal cholinergic and glutamatergic Ca^{2+} signals in the trisomic condition, and knockdown of DS-related genes in our Ts16-derived cell line could constitute a relevant tool to study DS-related neuronal dysfunction.

Keywords Down syndrome · SMIT1 · Myo-inositol · Calcium · Glutamate · Cholinergic

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Introduction

Down syndrome (DS) in humans results from the trisomy of autosome 21. The condition constitutes the major genetic cause of mental retardation to survive birth. This condition determines an increased incidence of congenital malformations, muscle hypotonia, and an early onset of Alzheimer's disease neuropathology (Epstein 1986). Such abnormalities are a consequence of gene overdose determined by the extra copy of chromosome 21. Human chromosome 21 was the second human autosome to be fully sequenced (Hattori et al. 2000), an analysis which identified 127 genes and 98 new genes, which may potentially be overexpressed in DS. The syndrome requires as a minimum the presence of a portion located at the end of the long arm of chromosome 21 (namely, from bands 21q22.1 to qter), referred to as the DS critical region. However, in spite of the accumulated knowledge to date, the consequences of the overexpression of

were normalized by the knockdown of SMIT1. However, we have previously demonstrated that stimulation with the aforementioned agonist results in Ca^{2+} signal increases that are dependent on extracellular Ca^{2+} (Cárdenas et al. 1999, Rojas et al. 2008), as removal of external Ca^{2+} results in the abolishment of the response. Hence, it is more likely that the effects of SMIT1 knockdown in this parameter are linked to other impairments in Ca^{2+} homeostasis, such as cell Ca^{2+} buffering and/or altered membrane receptor kinetics.

Recent evidence also links the overexpression of SMIT1 to regulation of the KCNQ2/3 K^+ channels, key players in membrane excitability (Dai et al. 2016). These authors demonstrated that total PI levels were unchanged in cells overexpressing the transporter. However, a reciprocal regulation has been reported in the brain between KCNQ1 and SMIT1, possibly related to the formation channel-transporter complexes (Abbott et al., 2014). Although such regulation is yet to be confirmed for KCNQ2/3, it is tempting to speculate that excess SMIT1 could be affecting K^+ channel activity and in turn neuronal excitability and hence partly explain our findings reported herein.

Our current results pose SMIT1 and the mechanisms that it influences as an interesting target from the pharmacological point of view. SMIT1 could result in increased intracellular myo-inositol concentration. In this regard, treatment with lithium has been shown to reduce myo-inositol levels in the brain of Ts65Dn animals (Huang et al. 2000) and, by the same token, rescue synaptic plasticity in the same animal model (Contestabile et al. 2013). Testing the effects of lithium in our cell models, and exploring its effects on the impairments in Ca^{2+} homeostasis described herein, may provide further mechanistic evidence of DS-related alterations, a possibility well worth exploring.

Finally, it is important to consider that several genes are overexpressed in the trisomic condition, and a number of them cooperatively contribute to the cellular alterations observed in this condition. In this regard, in a previous communication, we reported that the knockdown of the amyloid precursor protein, which is also overexpressed in the human and murine trisomic condition, restores normal $[Ca^{2+}]_c$ homeostasis in CTb cells (Rojas et al. 2008). Indeed, amyloid precursor protein knockdown reduced resting $[Ca^{2+}]_c$ and accelerated decay kinetics of $[Ca^{2+}]_c$ responses induced by either depolarization with high extracellular K^+, application of glutamatergic agonists, nicotine, or ionomycin. It seems, therefore, that excess amyloid precursor protein contributes to a general mechanism implicated in the deregulation of $[Ca^{2+}]_c$ homeostasis, whereas in light of our present results, the effect of SMIT1 knockdown on $[Ca^{2+}]_c$ signals seems to be more specific. However, independent of the underlying mechanisms, the overexpression of both SMIT1 and APP converges in common alterations related with $[Ca^{2+}]_c$ responses and homeostasis. Therefore, a potential therapy for DS should consider more than one target gene.

Conclusions

SMIT1 overexpression contributes to the altered Ca^{2+} signals observed in response to cholinergic and glutamatergic receptor activation in the cell line CTb, a cellular model of the DS. Then, the normalization of SMIT1 overexpression could constitute a relevant therapeutic target for treatment of the DS-related neuronal dysfunction, as well as the signaling pathways that are disturbed by the overexpressed transporter. Also, the cell membrane localization of the transporter and the neurotransmitter receptors studied herein provides even better access to potential modulating drugs.

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Physical Biology

TOPICAL REVIEW

Thermally activated TRP channels: molecular sensors for temperature detection

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Abstract

Temperature sensing is one of the oldest capabilities of living organisms, and is essential for sustaining life, because failure to avoid extreme noxious temperatures can result in tissue damage or death. A subset of members of the transient receptor potential (TRP) ion channel family is finely tuned to detect temperatures ranging from extreme cold to noxious heat, giving rise to thermoTRP channels. Structural and functional experiments have shown that thermoTRP channels are allosteric proteins, containing different domains that sense changes in temperature, among other stimuli, triggering pore opening. Although temperature-dependence is well characterized in thermoTRP channels, the molecular nature of temperature-sensing elements remains unknown. Importantly, thermoTRP channels are involved in pain sensation, related to pathological conditions. Here, we provide an overview of thermoTRP channel activation. We also discuss the structural and functional evidence supporting the existence of an intrinsic temperature sensor in this class of channels, and we explore the basic thermodynamic principles for channel activation. Finally, we give a view of their role in painful pathophysiological conditions.

Introduction

Thermosensation is the ability to estimate temperature. The molecular basis of this response lies in the activation of thermally gated ion channels, expressed in the membrane of sensory neurons from the trigeminal (TG) and dorsal root ganglia (DRG), which innervate the face and body, respectively. The expression of thermal receptors is also found in keratinocytes and the brain, where they play a role in thermoregulation [1]. Most temperature-sensitive ion channels belong to the transient receptor potential (TRP) ion channel superfamily. A hallmark of TRP ion channels is that they behave, in most systems, as physical and chemical sensors. Among the stimuli they can sense are temperature, voltage, pressure, ligands and osmolarity [2]. In these polymodal receptors, stimuli are detected by a sensing domain allosterically coupled to the catalysis site (ion conduction), which is the channel pore. TRP channels are grouped into seven subfamilies, with thermoTRP channels being found in the TRPA, TRPV, TRPC and TRPM subsets. The modular architecture of thermoTRP is a common feature of several ion channels, such as voltage-gated Ca^{2+} and voltage-gated K^+ channels. In these channels, voltage or calcium sensing domains control the channel opening probability due to their coupling with the channel pore [3–6].

ThermoTRP channel activity is extremely temperature-dependent, which is reflected in the high values of Q_{10} (>6) and enthalpy change (>40 kcal mol^{-1}) associated with channel gating. The structures of several thermoTRP channel members have recently been resolved, confirming the modular nature of these channels, providing a structural framework for interpreting decades of experimental work, and helping reveal the gating mechanisms. However, despite major progress in structural biology, the molecular determinants and the mechanisms by which the temperature sensor accomplishes its function remain unknown. Temperature sensing is related to pain sensation in several pathological conditions, making thermoTRP channels a molecular target for pain relief therapies. Thus, the search for a temperature sensor in thermally gated TRP channels is the goal for both ion channel biophysicists and pain therapy researchers. Below, we provide an overview of

channels have been identified as thermal transducers in different species, where its deficiency has been shown to impair thermosensation *in vivo*. ThermoTRP channel gating—particularly temperature mediated gating—has been extensively characterized, but the molecular mechanisms underlying activation-by-temperature remain obscure. The accumulation of functional and structural data obtained during the last 30 years has led researchers to propose several plausible mechanisms accounting for thermal gating in thermoTRP channels, including changes in the heat capacity, enthalpy changes during activation and allosteric coupling between sensing domains. To date, there is no consensus regarding which mechanism accounts for the large amount of thermal sensitivity in thermoTRP channels, and more experimental evidence is required. As temperature and pain are intimately linked, future functional understanding of this molecular sensor may provide novel tools and strategies for pain relief.

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CHAPTER SEVEN

A Molecular Reporter for Monitoring Autophagic Flux in Nervous System In Vivo

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Contents

Abstract

The relevance of autophagy in neuronal health has been extensively reported in a plethora of conditions affecting the nervous system, such as neurodegenerative diseases, cancer, diabetes, and tissue injury, where altered autophagic activity may contribute to the pathological process. Autophagy is a dynamic pathway involving the formation of a membrane surrounding and enclosing cargoes that are delivered to lysosomal compartments for degradation. Cargoes can include large protein aggregates,

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inhibition. Moreover, our method enables to measure autophagosome dynamics in vivo in the context of the 3D structure of the brain in real time by using multiphoton microscopy and study single-particle features by superresolution microscopy. In summary, the use of ICV injection of the molecular reporter AAV2/2_mCherry-GFP-LC3 represents a valuable tool in the advancement toward defining the contribution of autophagy to diverse physiological and pathological conditions affecting the nervous system.

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ORIGINAL RESEARCH
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Comparative Genomics Analysis of a New *Exiguobacterium* Strain from Salar de Huasco Reveals a Repertoire of Stress-Related Genes and Arsenic Resistance

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The Atacama Desert hosts diverse ecosystems including salt flats and shallow Andean lakes. Several heavy metals are found in the Atacama Desert, and microorganisms growing in this environment show varying levels of resistance/tolerance to copper, tellurium, and arsenic, among others. Herein, we report the genome sequence and comparative genomic analysis of a new *Exiguobacterium* strain, sp. SH31, isolated from an altiplanic shallow athalassohaline lake. *Exiguobacterium* sp. SH31 belongs to the phylogenetic Group II and its closest relative is *Exiguobacterium* sp. S17, isolated from the Argentinian Altiplano (95% average nucleotide identity). Strain SH31 encodes a wide repertoire of proteins required for cadmium, copper, mercury, tellurium, chromium, and arsenic resistance. Of the 34 *Exiguobacterium* genomes that were inspected, only isolates SH31 and S17 encode the arsenic efflux pump Acr3. Strain SH31 was able to grow in up to 10 mM arsenite and 100 mM arsenate, indicating that it is arsenic resistant. Further, expression of the *ars* operon and *acr3* was strongly induced in response to both toxics, suggesting that the arsenic efflux pump Acr3 mediates arsenic resistance in *Exiguobacterium* sp. SH31.

Keywords: *Exiguobacterium*, polyextremophile, stress, comparative genomics, Chilean Altiplano

INTRODUCTION

Extremophiles are microorganisms from all three domains of life (Bacteria, Archaea and Eukarya) that grow in the most hostile environments found on Earth, where they must withstand conditions including extreme pH, temperature, salinity, pressure, UV radiation, and the presence of heavy metals or toxic compounds. In some cases, extremophiles face more than one extreme condition

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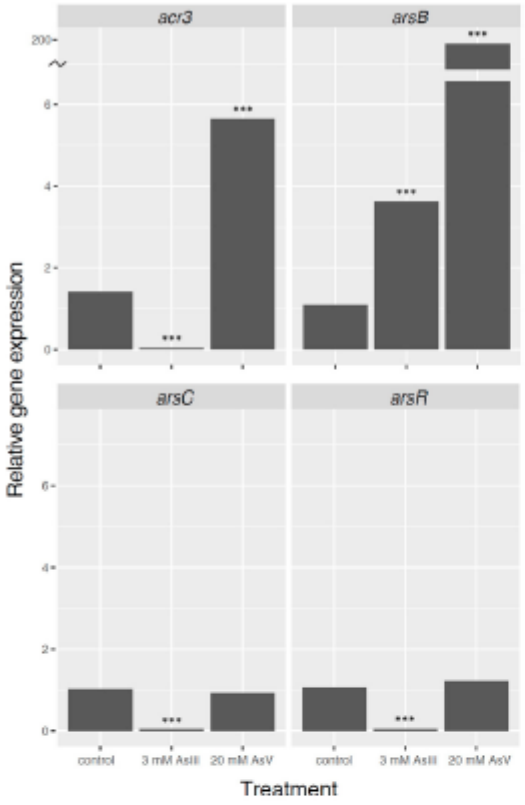

FIGURE 5 | Expression of genes required for arsenic resistance in *Exiguobacterium* sp. SH31 in response to As(III) and As(V) exposure. Expression of the 16S rRNA gene was used as a normalizer. ***$p < 0.001$.

experienced the duplication of gene families involved in stress response.

Some *Exiguobacterium* strains are resistant to NaCl and heavy metals such as Pb, Cu, and Hg (Petrova et al., 2002; Bian et al., 2011). In particular, *Exiguobacterium* isolated from permafrost sediments in Russia and Canada that contained 0.001 – 2.9% of mercury carried the *mer* operon (Petrova et al., 2002). Our results show that all of the *Exiguobacterium* strains included in the analyses have the *merA* gene, but that only 6 strains had additional genes of the operon (Supplementary Table S4). The *mer* operon can be located in the chromosome, plasmids, or transposons, and has been studied as a mechanism of horizontal dissemination that confers a broad spectrum of mercury resistance among *Bacillus* and related species (Bogdanova et al., 2001). Other strains such as ZM-2, isolated from agricultural soils irrigated with tannery effluents in India, resist up to 12.4 mM of potassium chromate (Alam and Malik, 2008), and some isolates tolerate up to 1.5 mM and reduce 0.75 mM of Cr(VI) (Sarangi and Krishnan, 2008). Additionally, some *Exiguobacterium* isolates are able to reduce Cr(VI) anaerobically in a large range of temperatures, pH and salt concentrations (Pattanapipitpaisal et al., 2002; Okeke, 2008). Our comparative genomics approach confirms that all 34 *Exiguobacterium* strains possess the genes encoding proteins required for chromate reduction and efflux, namely *chrR* and *srpC*, respectively, which could be responsible for chromate resistance in this genus.

Exiguobacterium sp. SH31 is more closely related to the arsenic resistant strain S17 (Belfiore et al., 2013). The high arsenic resistance of strain S17 has been explained by an increased number of genes encoding proteins required to detoxify this toxic compound and by the presence of the *acr3* gene, which confers increased arsenite and arsenate resistance (10 and 150 mM, respectively; Belfiore et al., 2013; Ordoñez et al., 2015). In agreement, strain SH31 is also arsenic resistant, which could be explained by the presence of several proteins required for its detoxification, including Acr3.

In the post-genomic era, high-throughput technologies have enabled researchers with the power to interrogate genomes from the dark matter of the microbial tree of life (Wu et al., 2009). Yet, the traditional use of morphology, phenotype, biochemical traits, and single-gene inferences to classify microorganisms implies that our current understanding of what constitutes a species or genus is imperfect (Hugenholtz et al., 2016). Herein, we show that using an extreme cut-off of 75% ANI in a relatively small dataset yielded as many as six clusters in an otherwise unified genus. While comparative genomics studies of polyextremohiles are needed to understand their distribution, evolutionary history, and biotechnological potential, thorough sampling designs, metagenomics-based studies, and functional assays such as those based on metabolomics, transcriptomics, and proteomics, will enable researchers to develop a systems level understanding of the patterns and processes leading to molecular adaptation.

AUTHOR CONTRIBUTIONS

JF, PA, CD, and FR performed field work, processed samples and sequenced data; JC-S, EC-N, and CPS conceived and designed the study; JC-S, FR, and CS performed the experiments; JC-S, SV, DA, and EC-N analyzed data; RQ, FM, EC-N, CD, FR, and CPS contributed with reagents/materials/analysis tools; JC-S, CP-E, EC-N, FR, and CPS wrote the paper. All authors read and approved the final manuscript.

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frontiers in Cellular Neuroscience

REVIEW
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Connexin-Dependent Neuroglial Networking as a New Therapeutic Target

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Astrocytes and neurons dynamically interact during physiological processes, and it is now widely accepted that they are both organized in plastic and tightly regulated networks. Astrocytes are connected through connexin-based gap junction channels, with brain region specificities, and those networks modulate neuronal activities, such as those involved in sleep-wake cycle, cognitive, or sensory functions. Additionally, astrocyte domains have been involved in neurogenesis and neuronal differentiation during development; they participate in the "tripartite synapse" with both pre-synaptic and post-synaptic neurons by tuning down or up neuronal activities through the control of neuronal synaptic strength. Connexin-based hemichannels are also involved in those regulations of neuronal activities, however, this feature will not be considered in the present review. Furthermore, neuronal processes, transmitting electrical signals to chemical synapses, stringently control astroglial connexin expression, and channel functions. Long-range energy trafficking toward neurons through connexin-coupled astrocytes and plasticity of those networks are hence largely dependent on neuronal activity. Such reciprocal interactions between neurons and astrocyte networks involve neurotransmitters, cytokines, endogenous lipids, and peptides released by neurons but also other brain cell types, including microglial and endothelial cells. Over the past 10 years, knowledge about neuroglial interactions has widened and now includes effects of CNS-targeting drugs such as antidepressants, antipsychotics, psychostimulants, or sedatives drugs as potential modulators of connexin function and thus astrocyte networking activity. In physiological situations, neuroglial networking is consequently resulting from a two-way interaction between astrocyte gap junction-mediated networks and those made by neurons. As both cell types are modulated by CNS drugs we postulate that neuroglial networking may emerge as new therapeutic targets in neurological and psychiatric disorders.

Keywords: astrocyte network, connexin, gap junction, neuroglial interaction, glia

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cognitive processes such as sleep (Franco-Perez et al., 2012), through the modulation of their network organization (Petit and Magistretti, 2016).

Confounding Effects of Antipsychotics and Anti-Epileptics

Psychiatric disorders such as schizophrenia, bipolar disorders, or autism are characterized by marked signs of glial dysfunction (Yamamuro et al., 2015). More precisely, in autistic patients astroglial Cx43 expression is increased in superior frontal cortex (Fatemi et al., 2008a). Antipsychotic drugs have been developed since the 50s to reduce the symptoms of those disorders, and among them haloperidol, lithium, clozapine, or chlorpromazine. Those drugs generally reduce the expression of astrocyte Cxs (Orellana et al., 2006; Fatemi et al., 2008b). Concerning chlorpromazine, kinetic studies indicate that this reduction is achieved through an indirect mechanism involving a complex signaling cascade from drug application to effects on GJ channels, including modulation by alteredCx43 phosphorylation. Alternatively, a cellular redistribution of GJs by chlorpromazine has also been suggested to be at the origin of the reduction of Cx43 levels (Orellana et al., 2006). Astrocyte Cxs are also involved in the pathophysiology of epilepsy (Steinhauser et al., 2016), as they are often up-regulated in this pathology. In line with this, their blockade is considered to act in an anticonvulsant manner; however, opposite (pro-convulsing) effects have also been reported (Carlen, 2012). Anti-epileptic drugs (valproic acid, carbamazepine, phenytoin, or gabapentin) generally do not modulate Cx expression (Dambach et al., 2014). However, levetiracetam enhances the expression of Cx43 and function of GJ in astrocyte-microglia mixed cultures, allegedly through an anti-inflammatory process (Haghikia et al., 2008). Finally, the effect of anti-epileptic drugs are also known to have an effect on neuronal Cxs (Mylvaganam et al., 2014) but this is beyond the scope of the present review.

CONCLUSION

Astrocytes are highly connected by Cx30 and Cx43, and this cell-cell communication strongly depends on neuronal activity. Conversely, neuronal processes are tightly tuned by astrocyte networks. The implications of neuroglial networking have furthermore been dissected in cognition, sleep-wake cycle, and sensory functions. Over the last decade, drugs that typically target neurons, such as antidepressant, antipsychotics, antiepileptic, psychostimulants, or anesthetics, have been shown to also modulate astroglial Cx expression and function. Additionally, reciprocal interactions, as well as mutual controls operate between neurons and Cx-formed astrocyte networks. Consequently, we envision that neuroglial networking may emerge as a new therapeutic target in neurological and psychiatric disorders. Two demonstrations of the interest in such targeting have been recently published in neuropathic pain (Jeanson et al., 2016a) and narcolepsy (Duchêne et al., 2016; Lu and Chen, 2016; see also Naus and Giaume, 2016). We hypothesized that astrocyte network size and structure should be adapted and optimized to neuronal demand, as a neither too large nor too reduced syncytium might adequately fuel metabolically active synapses, and this remains valid in the presence of CNS drugs (see **Figure 4**).

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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Schwann Cell Phenotype Changes in Aging Human Dental Pulp

E. Couve[1], M. Lovera[1], K. Suzuki[1], and O. Schmachtenberg[2]

Abstract

Schwann cells are glial cells that support axonal development, maintenance, defense, and regeneration in the peripheral nervous system. There is limited knowledge regarding the organization, plasticity, and aging of Schwann cells within the dental pulp in adult permanent teeth. The present study sought to relate changes in the pattern of Schwann cell phenotypes between young and old adult teeth with neuronal, immune, and vascular components of the dental pulp. Schwann cells are shown to form a prominent glial network at the dentin-pulp interface, consisting of nonmyelinating and myelinating phenotypes, forming a multicellular neuroimmune interface in association with nerve fibers and dendritic cells. Schwann cell phenotypes are recognized by the expression of S100, glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), Sox10, GAP43, and p75NTR markers. In young adult teeth, a dense population of nonmyelinating Schwann cells projects processes in close association with sensory nerve terminals through the odontoblast layer, reaching the adjacent predentin/dentin domain. While GAP43 and p75NTR are highly expressed in nonmyelinating Schwann cells from young adult teeth, the presence of these markers declines significantly in old adult teeth. Myelinated axons, identified by MBP expression, are mainly present at the Raschkow plexus and within nerve bundles in the dental pulp, but their density is significantly reduced in old adult versus young adult teeth. These data reveal age-related changes within the glial network of the dental pulp, in association with a reduction of coronal dental pulp innervation in old adult versus young adult teeth. The prominence of Schwann cells as a cellular component at the dentin-pulp interface supports the notion that their association with sensory nerve terminals and immune system components forms part of an integrated multicellular barrier for defense against pathogens and dentin repair.

Keywords: glial network, nociceptors, nerve terminals, p75NTR, myelin, odontoblast

Introduction

Human teeth are dentin units protected by enamel, with a complex root attachment system and a dental pulp that is highly innervated by sensory afferents for temperature, touch, and pain sensation. The appearance of dentin tissue with its sensory function was a key innovation for active tooth protection and regeneration during the early evolution of vertebrates (Hildebrand et al. 1995; Fried and Gibbs 2014; Krivanek et al. 2017). Dental pulp innervation in human teeth localizes essentially to the dentin-pulp interface and comprises a complex nociceptive network formed by unmyelinated and myelinated axons (C- and Aδ-fibers), hierarchically arranged within the Raschkow plexus and projecting nerve endings through the subodontoblastic and odontoblast layers to the adjacent predentin/dentin domains (Fried and Gibbs 2014). Dentin sensitivity is based on stimulation of the plexus of nerve terminals arranged at the dentin-pulp interface, where odontoblasts and nerve fibers interact in a still incompletely understood sensory transduction pathway for dentinal pain (Shibukawa et al. 2015).

The dentin-pulp interface is formed by multiple cell types, with odontoblasts supporting nerve terminals and dendritic cells, which in turn sense potentially damaging perturbations and recognize pathogens, mediating tissue responses to dentin injury by caries or trauma (Couve et al. 2014). Nociceptors have been shown to play a critical role in tissue defense by sensing pathogen-associated molecules, regulating inflammatory responses, and modulating tissue regeneration (Woolf and Ma 2007; Chiu et al. 2013). Moreover, interactions between nociceptors terminals and immune cells are a fundamental aspect protecting tissues from injury and to regulate inflammatory responses (Talbot et al. 2016; Pinho-Ribeiro et al. 2017).

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A supplemental appendix to this article is available online.

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cells expressing immature stem cell markers, such as Sox10 and p75NTR, could generate new dental pulp stem cells and odontoblasts (Kaukua et al. 2014).

In this context, it is interesting to consider the organization of the capillary network at the dentin-pulp interface, as it has been suggested that dental pulp stem cells are associated with perivascular niches (Shi and Gronthos 2003). At the dentin-pulp interface, an organized terminal capillary network is observed in close association with nmSCs. Human dental pulp is a highly vascular tissue, but there are currently insufficient data to relate pulpal vascularity in human teeth with specific markers for dental pulp stem cells (Shi and Gronthos 2003; Krivanek et al. 2017). Blood vessels are, however, a crucial migratory surface that guides SCs as a road during axonal regeneration (Cattin and Lloyd 2016).

However, SCs, sensory neurons, and immune cells are interrelated and react with a complex signaling cascade of cytokines and chemokines to achieve axonal and tissue regeneration in response to nerve injury (Scholz and Woolf 2007). The organization and function of dendritic cells within the dental pulp has been well characterized and related to dental pulp immune responses against injury and infection (Couve et al. 2014; Farges et al. 2015). We observe that SCs are densely intercalated with dendritic cells in dental pulp. Indeed, SCs have been shown to be associated with immune cells, cooperating in the surveillance against pathogens and traumatic injuries and in the regeneration of damaged axons (Scholz and Woolf 2007). SCs might have a key immunomodulatory function, linking innate and adaptive immune responses, and they are crucial as immune-competent cells detecting pathogens through toll-like receptors, expressing MHC-II as do antigen-presenting cells (Meyer zu Horste et al. 2008; Ydens et al. 2013; Tzekova et al. 2014). In dental pulp, the neuroinflammatory activation of SCs was recently reported in response to axonal degeneration during root resorption in deciduous teeth (Suzuki et al. 2015). The data reported here support a close spatial and functional relationship between nmSCs and dendritic cells, suggesting that both cell types have complementary roles in the surveillance and defense of the dentin-pulp interface.

Interactions between nociceptors and immune cells have been shown to modulate coordinated defensive and inflammatory responses to tissue injury and infection (Talbot et al. 2016; Pinho-Ribeiro et al. 2017). We propose that the different cellular components of the dentin-pulp interface function in synergy as a multicellular barrier fundamental to preserve tooth integrity, in many cases for >8 decades. Our observations of the association of SCs with nerve endings and immune components creates a novel perspective for the understanding of the role of glial cells in the modulation of physiologic and inflammatory processes in the dental pulp and the slow but constant debilitation of this defensive concertation by age, which could translate into improved clinical management of tooth pain. Finally, the age-related changes observed at the dentin-pulp interface could explain the reduced physiologic response of the dental pulp to environmental injuries and pathogens in mature and aged teeth (Fig. 5).

Author Contributions

E. Couve, contributed to conception, design, and data acquisition, drafted and critically revised the manuscript; M. Lovera, K. Suzuki, contributed to design and data acquisition, critically revised the manuscript; O. Schmachtenberg, contributed to conception, design, data analysis, and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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ORIGINAL PAPER

IRE1 signaling exacerbates Alzheimer's disease pathogenesis

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Abstract Altered proteostasis is a salient feature of Alzheimer's disease (AD), highlighting the occurrence of endoplasmic reticulum (ER) stress and abnormal protein aggregation. ER stress triggers the activation of the unfolded protein response (UPR), a signaling pathway that enforces adaptive programs to sustain proteostasis or eliminate terminally damaged cells. IRE1 is an ER-located kinase and endoribonuclease that operates as a major stress transducer, mediating both adaptive and proapoptotic programs under ER stress. IRE1 signaling controls the expression of the transcription factor XBP1, in addition to degrade several RNAs. Importantly, a polymorphism in the XBP1 promoter was suggested as a risk factor to develop AD. Here, we demonstrate a positive correlation between the progression of AD histopathology and the activation of IRE1 in human brain tissue. To define the significance of the UPR to AD, we targeted IRE1 expression in a transgenic mouse model of AD. Despite initial expectations that IRE1 signaling may protect against AD, genetic ablation of the RNase domain of IRE1 in the nervous system significantly reduced amyloid deposition, the content of amyloid β oligomers, and astrocyte activation. IRE1 deficiency fully restored the learning and memory capacity of AD mice, associated with improved synaptic function and improved long-term potentiation (LTP). At the molecular level, IRE1 deletion reduced the expression of amyloid precursor protein (APP) in

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Compliance with ethical standards

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The Role of Kappa Opioid Receptors in Glutamate Input Selection in the Ventral Striatum

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Review of Brooks and O'Donnell

The ventral striatum is an important controller of motivated behavior. This nucleus receives dopamine afferents from the ventral tegmental area and glutamate afferents from the basolateral amygdala, thalamus, medial prefrontal cortex (mPFC), and ventral hippocampus, which encode emotional, sensory, executive-control, and contextual information, respectively. The ventral striatum integrates information from these inputs, and its principal neurons, GABAergic medium sized spiny neurons (MSNs), send output to the rest of the basal ganglia. The weight given to the different glutamate sources determines the extent to which contextual, emotional, or executive information influences behavior.

MSNs are segregated into direct and indirect pathways based on their projection target. Direct-pathway MSNs express D1 dopamine receptors, and their activation is thought to promote execution of motivated behaviors. In contrast, indirect-pathway MSNs express D2 dopamine receptors, and their activation is thought to inhibit the performance of motivated behaviors (Kravitz et al., 2012). The integration and processing of glutamate and dopamine inputs to D1 or D2 MSNs constitute the basis for a diverse range of actions.

As mentioned above, MSN activity is controlled by afferents that carry different type of information. Most of the time, the activity of the ventral striatum is synchronized with that of the hippocampus. Hippocampal activity sets MSNs into an active, depolarized state that gates other inputs to ventral striatum. This might be the mechanism by which contextual information is constantly updated in the ventral striatum for use during the execution of motor behavior. Notably, inputs from mPFC evoke firing in MSNs only when the neurons are already in a depolarized state (O'Donnell and Grace, 1995). Nonetheless, the mPFC can take control over MSN activity in certain situations. Specifically, burst activity in mPFC afferents induces heterosynaptic suppression of the MSNs' response to subsequent hippocampal or thalamic activation (Calhoon and O'Donnell, 2013). This effect might allow executive information to transiently take control of the behavior by means of an inhibition of less relevant information, enabling efficient decision-making.

Previous work (Calhoon and O'Donnell, 2013) showed that mPFC inhibition of hippocampal inputs is partially mediated by $GABA_A$ receptors. Intra-MSN blockage of $GABA_A$ receptors *in vivo* attenuated, but did not eliminate, the mPFC-induced inhibition of hippocampal inputs (Calhoon and O'Donnell, 2013), suggesting that other inhibitory mechanisms are recruited during this phenomenon. What mediates the rest of the inhibition was left unanswered.

One mechanism by mPFC activity might produce heterosynaptic suppression of hippocampal input is presynaptic inhibition mediated by the kappa opioid receptors (KORs). KORs are G_i-coupled receptors highly expressed in the ventral striatum, and are mainly found presynaptically in both symmetric and asymmetric synapses (Svingos et al., 1999, 2001). KORs in the ventral striatum are part of a retrograde signaling system, in which the endogenous KOR agonist dynorphin is somatodendritically released from D1-MSNs to act on presynaptic KORs (Gerfen and Young, 1988). Most studies have focused on the impact of KORs on dopamine neurotransmission. Indeed, within the ventral striatum, KORs are preferentially localized in close proximity to the dopamine transporter (Svingos et al., 2001), a localization that is consistent with its inhibitory role over dopamine release (Di Chiara and Imperato, 1988; Chefer et al., 2005). However, increasing evidence points to an inhibitory effect of KOR activation on glutamate neurotransmission. KORs are located presynaptically on asymmetric synapses, indicative of glutamate afferents (Svingos et al., 1999). Early studies of striatal synaptosomes showed that KOR agonists decrease glutamate release (Hill and Brotchie, 1995, 1999). Moreover, electrophysiological studies in brain slices showed

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Original Article

ORIGINAL ARTICLE

Basal Forebrain Gating by Somatostatin Neurons Drives Prefrontal Cortical Activity

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Abstract

The basal forebrain provides modulatory input to the cortex regulating brain states and cognitive processing. Somatostatin-expressing neurons constitute a heterogeneous GABAergic population known to functionally inhibit basal forebrain cortically projecting cells thus favoring sleep and cortical synchronization. However, it remains unclear if somatostatin cells can regulate population activity patterns in the basal forebrain and modulate cortical dynamics. Here, we demonstrate that somatostatin neurons regulate the corticopetal synaptic output of the basal forebrain impinging on cortical activity and behavior. Optogenetic inactivation of somatostatin neurons in vivo rapidly modified neural activity in the basal forebrain, with the consequent enhancement and desynchronization of activity in the prefrontal cortex, reflected in both neuronal spiking and network oscillations. Cortical activation was partially dependent on cholinergic transmission, suppressing slow waves and potentiating gamma oscillations. In addition, recruitment dynamics was cell type-specific, with interneurons showing similar temporal profiles, but stronger responses than pyramidal cells. Finally, optogenetic stimulation of quiescent animals during resting periods prompted locomotor activity, suggesting generalized cortical activation and increased arousal. Altogether, we provide physiological and behavioral evidence indicating that somatostatin neurons are pivotal in gating the synaptic output of the basal forebrain, thus indirectly controlling cortical operations via both cholinergic and non-cholinergic mechanisms.

Key words: basal forebrain, network oscillations, optogenetics, prefrontal cortex

Introduction

The mammalian basal forebrain is a collection of subcortical structures comprising the ventral pallidum, diagonal band of Broca, substantia innominata, medial septum and peripallidal region, which provides extensive axonal projections to the entire cerebral cortex (Jones 2008; Zaborszky et al. 2012). Damage to the basal forebrain has been implicated in several neurological disorders, including Alzheimer's disease, Parkinson's disease, schizophrenia, and drug abuse (Whitehouse et al. 1982; Conner et al. 2003; Smith et al. 2004; Blanco-Centurion et al. 2007). Under normal physiological conditions, the basal forebrain plays central roles in arousal, attention, motivation, memory, plasticity, sensory processing and sleep-wake cycles (Moruzzi and Magoun 1949; Kilgard and Merzenich 1998; Froemke et al. 2007; Pinto et al. 2013; Lin et al. 2015; Xu et al. 2015). These actions are achieved by the complementary roles of a heterogeneous mixture of cell types that differ in neurotransmitter content, somato-dendritic morphology, axonal projections and spike timing (Brashear et al. 1986;

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Notes

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RESEARCH ARTICLE

Cannabinoids Prevent the Amyloid β-Induced Activation of Astroglial Hemichannels: A Neuroprotective Mechanism

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The mechanisms involved in Alzheimer's disease are not completely understood and how astrocytes and their gliotransmission contribute to this neurodegenerative disease remains to be fully elucidated. Previous studies have shown that amyloid-β peptide (Aβ) induces neuronal death by a mechanism that involves the excitotoxic release of ATP and glutamate associated to astroglial hemichannel opening. We have demonstrated that synthetic and endogenous cannabinoids (CBs) reduce the opening of astrocyte Cx43 hemichannels evoked by activated microglia or inflammatory mediators. Nevertheless, whether CBs could prevent the astroglial hemichannel-dependent death of neurons evoked by Aβ is unknown. Astrocytes as well as acute hippocampal slices were treated with the active fragment of Aβ alone or in combination with the following CBs: WIN, 2-AG, or methanandamide (Meth). Hemichannel activity was monitored by single channel recordings and by time-lapse ethidium uptake while neuronal death was assessed by Fluoro-Jade C staining. We report that CBs fully prevented the hemichannel activity and inflammatory profile evoked by Aβ in astrocytes. Moreover, CBs fully abolished the Aβ-induced release of excitotoxic glutamate and ATP associated to astrocyte Cx43 hemichannel activity, as well as neuronal damage in hippocampal slices exposed to Aβ. Consequently, this work opens novel avenues for alternative treatments that target astrocytes to maintain neuronal function and survival during AD.

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Key words: neuroinflammation, connexin43, neuron, glia, alzheimer

Introduction

Dementia encompass a wide range of symptoms of cognitive decline that impairs quality of life and well-being. The major features include complete or partial loss of memory and reasoning, as well as emotional, language and personality changes leading, eventually, to death (Fotuhi et al., 2009). Different metabolic, vascular, and neurological disorders might produce dementia, but Alzheimer's disease (AD) is the most prevalent cause responsible for the 50–70% of cases worldwide. Although the origin of AD remains unknown, the abnormal accumulation of amyloid-β peptide (Aβ) at amyloid plaques constitutes one of the major histopathological hallmarks observed in postmortem AD brains (Selkoe and Hardy, 2016). Although the diverse forms of Aβ have been linked to neurotoxicity by affecting synaptic transmission, trophic support, mitochondrial function, Ca^{2+} homeostasis, antioxidant defense, and inflammatory response (Querfurth and LaFerla, 2010), the full underlying mechanisms associated to AD remain to be elucidated.

In a previous work, using cell cultures and acute hippocampal slices we demonstrated that Aβ induces neuronal death by a mechanism that involves the excitotoxic release of ATP and glutamate linked to glial cell hemichannel opening (Orellana et al., 2011b). These plasma membrane channels

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alterations in electrophoretic mobility, because Cx43 total levels and pattern of immunoreactive bands were similar in Aβ-stimulated and control astrocytes. In addition, immunofluorescence labeling showed no differences in the distribution of structures compatible with gap junction plaques in astrocytes treated with Aβ or WIN, suggesting that internalization or degradation of gap junctions do not explain the modulation of astroglial coupling.

Up to now, various studies have demonstrated the presence of functional hemichannels and pannexons in astrocytes and neurons investigated in brain slices (Abudara et al., 2015; Karpuk et al., 2011; Orellana et al., 2014, 2015; Santiago et al., 2011). Here, we show that CBs entirely abolished the previously described activity of astrocyte Cx43 hemichannels and neuronal Panx1 channels evoked by Aβ in acute hippocampal slices (Orellana et al., 2011b). The combination of this integrated preparation, where all cell types are present, allowed us to confirm the preventive effect of CBs on hemichannel activity found in cell cultures. Unlike to cultures, in acute hippocampal slices the effect of SR2 on the preventing action of synthetic and endogenous CB agonists was present in addition to the effect of SR1 (except in the case of 2-AG). This is likely due to a cumulative action of these compounds since in slices antagonists act simultaneously in several cell types.

Glutamate and ATP are considered crucial transmitters on astrocyte-neuron communication and thereby their release through membrane proteins and vesicles is tightly regulated (Fields and Burnstock, 2006; Perea and Araque, 2010). Indeed, high concentrations of glutamate and ATP at the synaptic cleft could be neurotoxic under pathological conditions (Arbeloa et al., 2012; Ashpole et al., 2013; Lau and Tymianski, 2010). In this context, glutamate and ATP released by a mechanism involving the opening of astroglial Cx43 hemichannels could reduce neuronal survival by triggering the activation of NMDA/$P2X_7$ receptors and further opening of Panx1 hemichannels in neurons (Avendano et al., 2015; Orellana et al., 2011b). Here, we found that CBs strongly prevent the Aβ-induced death in pyramidal neurons, indicating that they are neuroprotective and likely act by inhibiting the inflammatory profile of astrocytes and the subsequent activation of Cx43 hemichannels and further release of excitotoxic gliotransmitters. Supporting this notion, the Aβ-induced overexpression of GFAP and release of glutamate and ATP via Cx43 hemichannels was totally reduced by WIN, 2-AG and Meth. Since activation of NMDA and $P2X_7$ receptors lead to opening of Panx1 channels in neurons (Iglesias et al., 2008; Thompson et al., 2008), it is plausible that Aβ-induced neuronal death could be associated with an ionic, osmotic and intracellular Ca^{2+} imbalance, as well as caspase activation evoked by NMDA/$P2X_7$ receptor and Panx1 channel activation, as previously described in neurons subjected to different pathological conditions (Gulbransen et al., 2012; Nishida et al., 2012; Weilinger et al., 2016).

Different mechanisms have been proposed to mediate neuroprotective features of CBs as for instance antioxidant, antiglutamatergic, and anti-inflammatory effects and are known to be of common interest for understanding many neurodegenerative processes (Micale et al., 2007). Here, we report that CBs prevent Aβ-induced neuronal death by preventing the inflammatory condition that rise Cx43 hemichannel activity in astrocytes. Such pathway might be relevant for AD since the expression of astroglial Cx43 was reported to be increased at amyloid plaques from patients with AD (Koulakoff et al., 2012; Nagy et al., 1996). In addition, in transgenic mouse models that develop amyloid plaques, connexin-mediated astroglial networks are increased in cortical astrocytes located near the plaques (Peters et al., 2009) and increased Cx43 hemichannel activity has been observed in astrocytes (Yi et al., 2016). Altogether, these observations argue for a possible contribution of astrocytes during the Aβ-triggered cascade with hemichannel activation taking place in the early phases of the increase in Aβ production, and being a consequence of this process the resulting neuronal death. Moreover, the regulation of hemichannel activity by CB agonists might represent a strategy against neuronal damage triggered by short-term (hours to few days) increase in Aβ production. This finding should help to generate the design of new CB agonists that could conserve their neuroprotective role without having psychoactive effects (Pertwee, 2009). In conclusion, the presented evidence unveil a new protective mechanism for CBs in the occurrence of a sequential activation of astroglial hemichannels and pannexin channels in neurons, providing concrete basis for definition of alternative pharmacological strategies aiming at preserving neuronal function and survival during AD.

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Review article

Connexins and pannexins in Alzheimer's disease

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ABSTRACT

By 2040 neurodegenerative diseases will become the world's second leading cause of death after cardiovascular disease (WHO). Major efforts are required to elucidate the underlying molecular and cellular mechanisms of neurodegenerative diseases. Connexin and pannexin membrane channel proteins are conduits through which neuronal, glial, and vascular tissues interact. In the normal brain, this interaction underlies homeostasis, metabolic supply and neuroprotection. In models of neuroinflammation these channels present aberrant functioning. Validation of the molecular mechanisms by which these membrane channels influence neurodegeneration particularly in Alzheimer's disease could lead to new and alternative therapeutic strategies targeting these channels.

1. Introduction and overview

1.1. Overview of AD

Alzheimer's Disease (AD) is the most common neurodegenerative disorder leading to dementia, affecting 11% of the population in North America over the age of 65 and about one-third of the people 85 and older [3]. Considering that mean life expectancy is increasing, the world is facing a dementia epidemic; unfortunately the causes and mechanisms leading to AD remain elusive. So far, no definitive marker of early AD has been identified and no effective prevention and disease-modifying treatment for AD has been described. Less than 1% of AD cases are familial with early-onset and genetic studies have shown mutations in three genes cause autosomal dominantly-inherited familial AD (FAD): *amyloid β precursor protein (APP)* on chromosome (Chr) 21, *presenilin1* (*PSEN1*) on Chr14, and *presenilin* 2 (*PSEN2*) on Chr1 [26,107]. A majority of AD cases are sporadic, late-onset and the causes are unknown. Variants in the *ApoE* gene on chromosome 19 are associated with AD, and people with *ApoE* e4 allele have a higher risk to develop late-onset AD. Neuritic plaques, neurofibrillary tangles, and neuronal loss in the brain are the hallmarks of AD neuropathology. So far, amyloid plaque formation is the pathological feature prominent in AD. Although preclinical studies have shown anti-amyloid and anti-tau strategies are effective to improve cognition in AD animal models, there have been no successful human trials on new anti-amyloid- or anti-tau-based Alzheimer's drug development within the last 15 years [50,76,84].

The severity of cognitive impairment in AD patients is correlated with neuronal dysfunction, damage and finally loss. Exposure of neuronal cultures to Aβ induces apoptosis and activation of caspase-2, 8 and 12 have been reported to mediate Aβ-induced cell death [64], caspase-6 activation has also been linked to AD pathogenesis [34]. Aβ has also been reported to increase regulator of calcineurin-1 (RCAN1) expression and triggers apoptosis [91]. However, the mechanism underlying neuronal loss in AD remains not well understood. While a contribution of connexins and pannexins to neurodegeneration have been documented over the past few years, their role in AD is only beginning to be explored [43,70,78]. Understanding how connexins and pannexins may participate in AD is complicated since different cell types express different (and usually several) isoforms [79].

1.2. Why gap junctions, hemichannels and pannexins are important in Alzheimer's disease

Electron microscopy [63] and immunocytochemistry [58] approaches have demonstrated that gap junction plaques and connexin expression were increased in glial cells in brain samples from AD patients, particularly in astrocytes [43]. These observations were also confirmed in a murine model of AD [58,105], the APP/PS1 mouse, that presents several features of the pathology, including: Aβ deposits,

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This treatment results in the rescue of neuronal damage in a similar way to that in the APP/PS1-Cx43$^{fl/fl}$-GFAP/cre mouse [104]. This observation opens the way for the development of treatment targeting hemichannel activity in glia to prevent neuronal sufferance in the context of the amyloid pathology.

5.2. Validation of connexins and pannexins as potential therapeutic targets

When considering the literature regarding connexins/pannexins and brain diseases, the general perspective is that in many cases gap junctional communication has a beneficial role by maintaining homeostasis and allowing dissipation of toxic compounds within large astroglial networks. By contrast, connexin (and pannexin) hemichannel activity is currently best characterized in terms of its toxic outcome. Indeed, both connexin hemichannels and pannexin channels are associated with the release (and possibly the uptake) of substances which may lead to ionic fluxes and release of gliotransmitters like ATP, glutamate and D-serine. Ionic fluxes include Na^+ and Ca^{2+} entry, and K^+ escape, which will result in volume changes/cell swelling [18] and cellular Ca^{2+} entry. This last property suggest that the release of gliotransmitters can also be indirect based on Ca^{2+}-dependent pathways triggering *e.g.* vesicular release [55]. Also, hemichannel opening has a firm link to cellular Ca^{2+} signaling, as cytoplasmic Ca^{2+} may act as a trigger of channel opening, which in its turn will influence the cytoplasmic Ca^{2+} concentration [10,20–22]. Interestingly, altered Ca^{2+} homeostasis is an integral part of the pathophysiology of AD [9,14,25,44,92], with glial Ca^{2+} wave activity in the vicinity of plaques supporting this concept [45].

So far, there is no final demonstration concerning the mechanism by which acute and chronic (in the APP/PS1 mouse model) exposure to amyloid peptides leads to the activation of Cx43 hemichannels and Panx1 channels. What we know is that in the APP/PS1 mouse the intracellular Ca^{2+} is increased [41] and that the activation of Cx43 hemichannels is related to this while the activation of Panx1 channels results from the release of pro-inflammatory cytokines by microglia [95]. In addition, there are reports indicating that the amyloid can activate so called "Aβ calcium channels" that allow the influx of Ca^{2+} [27,48,49] and Ca^{2+} release from the endoplasmic reticulum [24]. Accordingly, based on these studies we cannot exclude that the activation of Cx43 hemichannels could be a secondary step to these reported increases in intracellular Ca^{2+} [83].

Beside these considerations it is noteworthy that there is a general consensus that hemichannel opening may disturb cell function and lead to cell injury/cell death in brain disease. Indeed up to now, abnormal and sustained activation of glial hemichannels has been reported in a number of brain pathologies with detrimental effects on neuronal function and survival [11,19,70]. To this end, several strategies aimed at blocking hemichannel activity using genetic or pharmacological tools have been developed [37,66,82,93]. Most of these suppressed Cx43 expression and/or function, which is considered as the main hemichannel constituent based on the use of KO of connexin genes, antisense tools, antibodies or mimetic peptides. However, all these attempts likely impacted as well astroglial gap junctional communication making mechanistic interpretation of the observations problematic. Thus, an approach blocking only hemichannel functions without compromising gap junctional coupling may represent a unique strategy that should tune down their deleterious effects in brain pathologies. Also for neurodegenerative diseases, the design of therapeutics has to take into account the necessity of a chronic treatment and the condition that the concerned agent is able to pass the blood brain barrier. Again, these requirements have been met with the use of boldine hydrochloride that is water soluble, crosses the brain blood barrier and can be delivered chronically for 3 months in the water drinking of the APP/PS1 mouse [104].

As indicated above connexin and/or pannexin channels are suggested to play a role in degenerative processes involved in AD. They do not seem to be critical in the triggering of the pathology since the expression of connexin and pannexin in glia increases when the amyloid plaques are already present. However, once such enhancement takes place hemichannel activity is detected in the AD mouse model [105]. In addition, *in vitro* and *ex vivo* experiments also indicate that acute treatment with the amyloid peptide leads to neurodegeneration [72]. Taking into consideration that pharmacological and genetic approaches inhibiting Cx43 hemichannel activity in astrocytes results in either a reduction in neuronal death [72] or neuronal damage [105] depending on the AD models, it is expected that therapeutic strategies targeting hemichannel activity in glia and/or neurons should open the way to promising interventions. In terms of potential hemichannel-targeting agents, some interesting substances have recently appeared. Gap19 and L2 are two peptides that inhibit Cx43 hemichannels without inhibiting gap junctions [74,100]. These peptides interfere with loop-tail interaction of the Cx43 protein, an interaction that distinctly modulates the function/gating of gap junction channels and hemichannels (reviewed in [39]). L2/Gap19 have been used in brain slice work [1] and for *in vivo* memory-related investigations [90,98]. Peptide5 is another hemichannel blocker that does not affect gap junctions when applied at low (5 μM) concentration [42]. This peptide has significant neuroprotective effects and inhibits inflammatory processes in several animal models including brain ischemia [19], retinal ischemia [17] and spinal cord injury [67]. Peptides are however difficult to use for chronic treatment and there is active ongoing research to develop small molecule hemichannel inhibitors. Two compounds have been reported to display hemichannel specificity and little effects on gap junctions: boldine [36] and D4 [13]. Boldine is an alkaloid extracted from the boldo tree; the chemical nature of D4 was not revealed. Without any doubt, novel agents that are easy to administer and are endowed with sufficient blood-brain barrier permeation will come up in the next few years.

6. Conclusions

The NIH Clinical Trials website (http://www.clinicaltrials.gov/) indicate there are more than 1900 clinical trials for AD that have been or are being conducted. With no promising treatments in sight, there is an urgent need for novel therapeutic strategies. Given the role of connexins and pannexins in mouse models of AD, as well as proof of principle regarding the neuroprotective effects of specific hemichannel blocking reagents, validation of these channels as potential therapeutic targets for AD is warranted. However, the identification of efficient molecules and protocols still depends on several requirements such as their specificity (connexin and pannexin molecular nature; hemichannels versus gap junction channels; neuronal, glial and MC targeting), their availability to be used for long-term treatment and appropriate animal models to investigate AD symptoms.

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Chapter 14
I_{KD} Current in Cold Transduction and Damage-Triggered Cold Hypersensitivity

Alejandro González, Gaspar Herrera, Gonzalo Ugarte, Carlos Restrepo, Ricardo Piña, María Pertusa, Patricio Orio, and Rodolfo Madrid

Abstract In primary sensory neurons of the spinal and trigeminal somatosensory system, cold-sensitivity is strongly dependent on the functional balance between TRPM8 channels, the main molecular entity responsible for the cold-activated excitatory current, and *Shaker*-like Kv1.1–1.2 potassium channels, the molecular counterpart underlying the excitability brake current I_{KD}. This slow-inactivating outward K^+ current reduces the excitability of cold thermoreceptor neurons increasing their thermal threshold, and prevents unspecific activation by cold of neurons of other somatosensory modalities. Here we examine the main biophysical properties of this current in primary sensory neurons, its central role in cold thermotransduction, and its contribution to alterations in cold sensitivity triggered by peripheral nerve damage.

Keywords Primary sensory neurons • Cold thermotransduction • Kv1 channels • TRPM8 • 4-AP • α-DTx • Cold hypersensitivity

Abbreviations

4-AP	4-AminoPyridine
CCI	Chronic Constriction Injury
CIN	Cold-Insensitive Neuron

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Neurobiology of Disease

Role of the Excitability Brake Potassium Current I_{KD} in Cold Allodynia Induced by Chronic Peripheral Nerve Injury

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Cold allodynia is a common symptom of neuropathic and inflammatory pain following peripheral nerve injury. The mechanisms underlying this disabling sensory alteration are not entirely understood. In primary somatosensory neurons, cold sensitivity is mainly determined by a functional counterbalance between cold-activated TRPM8 channels and Shaker-like Kv1.1–1.2 channels underlying the excitability brake current I_{KD}. Here we studied the role of I_{KD} in damage-triggered painful hypersensitivity to innocuous cold. We found that cold allodynia induced by chronic constriction injury (CCI) of the sciatic nerve in mice, was related to both an increase in the proportion of cold-sensitive neurons (CSNs) in DRGs contributing to the sciatic nerve, and a decrease in their cold temperature threshold. I_{KD} density was reduced in high-threshold CSNs from CCI mice compared with sham animals, with no differences in cold-induced TRPM8-dependent current density. The electrophysiological properties and neurochemical profile of CSNs revealed an increase of nociceptive-like phenotype among neurons from CCI animals compared with sham mice. These results were validated using a mathematical model of CSNs, including I_{KD} and TRPM8, showing that a reduction in I_{KD} current density shifts the thermal threshold to higher temperatures and that the reduction of this current induces cold sensitivity in former cold-insensitive neurons expressing low levels of TRPM8-like current. Together, our results suggest that cold allodynia is largely due to a functional downregulation of I_{KD} in both high-threshold CSNs and in a subpopulation of polymodal nociceptors expressing TRPM8, providing a general molecular and neural mechanism for this sensory alteration.

Key words: α-DTx; 4-AP; Kv1 channels; PBMC; thermotransduction; TRPM8

Significance Statement

This paper unveils the critical role of the brake potassium current I_{KD} in damage-triggered cold allodynia. Using a well-known form of nerve injury and combining behavioral analysis, calcium imaging, patch clamping, and pharmacological tools, validated by mathematical modeling, we determined that the functional expression of I_{KD} is reduced in sensory neurons in response to peripheral nerve damage. This downregulation not only enhances cold sensitivity of high-threshold cold thermoreceptors signaling cold discomfort, but it also transforms a subpopulation of polymodal nociceptors signaling pain into neurons activated by mild temperature drops. Our results suggest that cold allodynia is linked to a reduction of I_{KD} in both high-threshold cold thermoreceptors and nociceptors expressing TRPM8, providing a general model for this form of cold-induced pain.

Introduction

Painful hypersensitivity to innocuous cold, or cold allodynia, is a prevalent symptom of neuropathic and inflammatory pain induced by peripheral nerve damage. Despite major advances in our understanding of the mechanisms underlying the diverse forms of cold-induced pain in response to axonal damage, including cold allodynia, the molecular and neural bases of this

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RESEARCH ARTICLE Open Access

Neurochemical and behavioral characterization of neuronal glutamate transporter EAAT3 heterozygous mice

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Abstract

Background: Obsessive–compulsive disorder (OCD) is a severe neuropsychiatric condition affecting 1–3% of the worldwide population. OCD has a strong genetic component, and the *SLC1A1* gene that encodes neuronal glutamate transporter EAAT3 is a strong candidate for this disorder. To evaluate the impact of reduced EAAT3 expression in vivo, we studied male EAAT3 heterozygous and wild-type littermate mice using a battery of behavioral paradigms relevant to anxiety (open field test, elevated plus maze) and compulsivity (marble burying), as well as locomotor activity induced by amphetamine. Using high-performance liquid chromatography, we also determined tissue neurotransmitter levels in cortex, striatum and thalamus—brain areas that are relevant to OCD.

Results: Compared to wild-type littermates, EAAT3 heterozygous male mice have unaltered baseline anxiety-like, compulsive-like behavior and locomotor activity. Administration of acute amphetamine (5 mg/kg intraperitoneally) increased locomotion with no differences across genotypes. Tissue levels of glutamate, GABA, dopamine and serotonin did not vary between EAAT3 heterozygous and wild-type mice.

Conclusions: Our results indicate that reduced EAAT3 expression does not impact neurotransmitter content in the corticostriatal circuit nor alter anxiety or compulsive-like behaviors.

Keywords: EAAT3, *SLC1A1*, Neuronal glutamate transporter, Obsessive–compulsive disorder

Background

Obsessive–compulsive disorder (OCD) is a persistent, disabling neuropsychiatric condition affecting 1–3% of the worldwide population. OCD is characterized by persistent intrusive thoughts (obsessions), repetitive ritualistic behaviors (compulsions) and excessive anxiety [1]. Family, twin and case–control studies have shown that genetic factors play a major role in OCD (for a review, see [2]).

Altered glutamatergic neurotransmission has been postulated in the etiology of OCD. The glutamatergic hypothesis has accumulated evidence from neuroimaging studies [3, 4], animal models with altered glutamatergic neurotransmission exhibiting compulsive-like behaviors [5–7] and reports of beneficial effects of anti-glutamatergic agents on treatment-resistant OCD [8]. Genetic linkage and association studies have implicated glutamate system genes in OCD; among them, the most consistent candidate gene in OCD is *SLC1A1* (solute carrier, family 1, member 1) gene [1, 9–15]. *SLC1A1* encodes for the neuronal excitatory amino acid transporter EAAT3, with reported roles in controlling glutamate spillover which affects extrasynaptic NMDA and metabotropic glutamate receptors activity [16, 17].

Mice lacking EAAT3 (KO) were first reported 20 years ago; the original report showed that EAAT3 KO mice have reduced locomotor activity, but no neurological or cognitive impairments [18]. Given its role in cysteine

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Authors' contributions
PRM, DLM and RSZ designed the study. LFG, CDA, FHB and MCO collected the data. LFG, GA, PRM and RSZ carried out the statistical analyses. LFG, GA, PRM and RSZ prepared the figures. All authors wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The datasets from current study are available from the corresponding author on reasonable request.

Consent for publication
Not applicable.

Ethics approval
All experimental procedures were approved by the Animal Ethics Committee (Protocol BEA-024-2013, Universidad de Valparaíso) and adhered to the guidelines of the American Association for Accreditation of Laboratory Animal Care and National Institutes of Health. Efforts were made to minimize the number of animals used and their suffering.

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Dynamin-2 mutations linked to Centronuclear Myopathy impair actin-dependent trafficking in muscle cells

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Dynamin-2 is a ubiquitously expressed GTP-ase that mediates membrane remodeling. Recent findings indicate that dynamin-2 also regulates actin dynamics. Mutations in dynamin-2 cause dominant centronuclear myopathy (CNM), a congenital myopathy characterized by progressive weakness and atrophy of skeletal muscles. However, the muscle-specific roles of dynamin-2 affected by these mutations remain elusive. Here we show that, in muscle cells, the GTP-ase activity of dynamin-2 is involved in *de novo* actin polymerization as well as in actin-mediated trafficking of the glucose transporter GLUT4. Expression of dynamin-2 constructs carrying CNM-linked mutations disrupted the formation of new actin filaments as well as the stimulus-induced translocation of GLUT4 to the plasma membrane. Similarly, mature muscle fibers isolated from heterozygous knock-in mice that harbor the dynamin-2 mutation p.R465W, an animal model of CNM, exhibited altered actin organization, reduced actin polymerization and impaired insulin-induced translocation of GLUT4 to the sarcolemma. Moreover, GLUT4 displayed aberrant perinuclear accumulation in biopsies from CNM patients carrying dynamin-2 mutations, further suggesting trafficking defects. These results suggest that dynamin-2 is a key regulator of actin dynamics and GLUT4 trafficking in muscle cells. Our findings also support a model in which impairment of actin-dependent trafficking contributes to the pathological mechanism in dynamin-2-associated CNM.

Dynamins are mechano-chemical large GTP-ases, whose catalytic activity is required in several membrane-based processes including endocytosis, vesicle trafficking, and exocytosis[1–4]. These proteins also exhibit a critical role in actin cytoskeleton dynamics by promoting elongation[5], remodeling[6] and stabilizing actin filaments[7].

Dynamins are composed of five conserved domains: an N-terminal GTP-ase domain, a middle structural domain, a pleckstrin homology (PH) domain that binds phosphoinositides, a GTP-ase effector domain (GED), and a C-terminal proline/arginine-rich domain (PRD) that binds SH3-domain-containing partners[1–4]. Three dynamin isoforms have been described in mammals, which share approximately 80% of sequence homology

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The F-Actin Binding Protein Cortactin Regulates the Dynamics of the Exocytotic Fusion Pore through its SH3 Domain

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Upon cell stimulation, the network of cortical actin filaments is rearranged to facilitate the neurosecretory process. This actin rearrangement includes both disruption of the preexisting actin network and *de novo* actin polymerization. However, the mechanism by which a Ca^{2+} signal elicits the formation of new actin filaments remains uncertain. Cortactin, an actin-binding protein that promotes actin polymerization in synergy with the nucleation promoting factor N-WASP, could play a key role in this mechanism. We addressed this hypothesis by analyzing *de novo* actin polymerization and exocytosis in bovine adrenal chromaffin cells expressing different cortactin or N-WASP domains, or cortactin mutants that fail to interact with proline-rich domain (PRD)-containing proteins, including N-WASP, or to be phosphorylated by Ca^{2+}-dependent kinases, such as ERK1/2 and Src. Our results show that the activation of nicotinic receptors in chromaffin cells promotes cortactin translocation to the cell cortex, where it colocalizes with actin filaments. We further found that, in association with PRD-containing proteins, cortactin contributes to the Ca^{2+}-dependent formation of F-actin, and regulates fusion pore dynamics and the number of exocytotic events induced by activation of nicotinic receptors. However, whereas the actions of cortactin on the fusion pore dynamics seems to depend on the availability of monomeric actin and its phosphorylation by ERK1/2 and Src kinases, cortactin regulates the extent of exocytosis by a mechanism independent of actin polymerization. Together our findings point out a role for cortactin as a critical modulator of actin filament formation and exocytosis in neuroendocrine cells.

Keywords: exocytosis, fusion pore, actin polymerization, cortactin, N-WASP, neuroendocrine cells, chromaffin cells, catecholamines

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Abbreviations: ACCs, adrenal chromaffin cells; 2A, ERK1/2 non-phosphorylatable cortactin mutant S405,418A; CF, cortical fluorescence; DAPI, 40,6-diamidino-2-phenylindole; DMPP, 1,1-dimethyl-4-phenyl-pierazinium; ERK1/2, extracellular signal-regulated protein kinases 1 and 2; 3F, Src non-phosphorylatable cortactin mutant Y421,466,482F; FL-W525K, full-length cortactin mutant W525K; LatA, latrunculin A; MEK, MAP and ERK kinase; NCF, cell fluorescence with no cell cortex; NPF, nucleation promoting factor; NTA, N-terminal acidic domain; N-WASP, neural Wiskott-Aldrich syndrome; PBS, phosphate-buffered saline; active or phosphorylated ERK1/2 (pERK1/2), PFA, *p*-formaldehyde; PRD, proline-rich domain; Q, spike charge; $t_{1/2}$, half-width; TF, total fluorescence intensity; TRITC, tetramethyl-rhodamine-B-isothiocyanate; VCA, verprolin-cofilin homology-acidic; WAVE; WASP-family verprolin-homologous protein; WGP, Wiskott-Aldrich GTPase proline-rich domain; WT, cortactin wild-type.

cortactin/N-WASP association. Effectively, the 2A mutant prolonged the duration of the initial fusion pore and increased the amplitude of the foot signal, similarly to that observed with the injection of the N-WASP PRD or with the expression of the FL-W525K. Interestingly, the same effects also were observed in cells expressing the 3F mutant, indicating that this type of cortactin phosphorylation can also regulate fusion pore dynamics. Given that cortactin can be simultaneously phosphorylated by both pERK1/2 and tyrosine kinases (Kelley et al., 2011), it is probable that both types of phosphorylation contribute together to regulate fusion pore expansion in ACCs. On the other hand, and contrary to that observed with the SH3 domain of cortactin, both non-phosphorylatable mutants 2A and 3F significantly increased $t_{1/2}$, suggesting that cortactin is involved in the regulation of the duration of the release events.

The effects of the cortactin non-phosphorylatable mutant 3F on $t_{1/2}$ and foot duration correlate well with the effects of the pharmacological inhibition of Src kinases on those parameters (Olivares et al., 2014). On the other hand, both the expression of the non-phosphorylatable mutant 2A and the ERK1/2 signaling inhibitor U0126 prolonged foot duration. However, they have opposite effects on foot amplitude; whereas the 2A mutant prolongs foot amplitude, U0126 decreases it (**Figure 5C** and **Table 1**). pERK1/2 also phosphorylates the MLCK (Klemke et al., 1997), and inhibition of ERK1/2 signaling decreases the function of MLCK, as well as the phosphorylation of its substrate myosin light chain (Klemke et al., 1997). Expression of a non-phosphorylatable mutant of myosin II regulatory light chain in ACCs hinders the fusion pore expansion, limiting the release of catecholamines through the initial fusion pore (Neco et al., 2008). Therefore, the effects of U0126 on fusion pore conductance could be a consequence of an inhibition of MLCK.

Both the expression of the phosphorylatable mutant 2A and the cell treatment with the ERK1/2 signaling inhibitor U0126 reduced the number of exocytotic events, suggesting that cortactin phosphorylation by pERK1/2 influences the amount of exocytosis. As discussed above, MLCK is a cortactin partner involved in vesicle transport and priming (Kumakura et al., 1994; Neco et al., 2002). Furthermore, and as aforementioned, MLCK is also a substrate of ERK1/2 (Klemke et al., 1997). Then these findings point out to ERK1/2, MLCK and cortactin as regulators of the amount of exocytosis.

CONCLUSION

Together, the present findings point out a role of cortactin as a modulator of Ca^{2+} regulated exocytosis. Cortactin actions on exocytosis depend on the interaction of its SH3 domain with PRD-containing partners, such as N-WASP and MLCK. Whereas the association of cortactin with N-WASP appears to be critical for the Ca^{2+}-induced actin filament formation and fusion pore expansion, a different mechanism seems to determine the role of cortactin in the extent of exocytosis. These cortactin actions are regulated by its phosphorylation at serine and tyrosine residues by ERK1/2 and Src kinases, respectively. These new mechanisms are clearly relevant for the tight regulation of transmitter release in neuroendocrine cells.

AUTHOR CONTRIBUTIONS

AG-J: designed and performed experiments, performed statistical analysis, interpreted results, helped draft parts of the manuscript and critically revised the manuscript; MG, MO, VH-A, XB-M, and JV-N: performed experiments and analyzed data; FM: analyzed and interpreted data and critically revised the manuscript; NM-Q: designed constructs, interpreted results and critically revised the manuscript. AC: conceived the study, designed experiments, interpreted results, performed statistical analysis and drafted the manuscript. All authors red and approved the final manuscript.

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Research paper

Accessing the structural and thermodynamic properties of ultra-thin layers of C32 adsorbed on a SiO_2 surface

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ABSTRACT

Medium-chain alkanes are important molecules with applications in biology and industry. Notably, their structural properties are scarcely understood. To assess structural and thermodynamic properties of dotriacontane (C32) molecules adsorbed on a SiO_2 surface, we conducted all-atom molecular dynamics (MD) simulations. By analyzing potentials of mean force, order parameters and self-diffusion, we compared the stability and preferential orientation between ordered and disordered systems. Our data confirm the presence of one parallel layer of C32 followed by a mixture of disordered C32 segments exhibiting no thermodynamic preference. This semi-ordered structural model shed light to the interactions between C32 and a SiO_2 surface.

1. Introduction

Medium-chain alkanes are the main constituents of several molecules with biological and industrial relevance. They are important for biosensing and bio-remediation of fossil fuels [1], as microlubricants, anticorrosive agents, and surfactants [2]. Consequently, their structural and dynamical properties have been the focus of both experimental and theoretical studies [3–10]. Given their simplicity and due to their role as prototypes for more complex polymers including biologically relevant molecules such as membranes, alkanes have been used to study the behavior of nano-scale materials such as polymeric thin films [11,12]. Using a variety of tools including atomic force microscopy, X-ray diffraction, high-resolution ellipsometry and more recently, molecular dynamics, it is currently accepted that the behavior of alkane thin films is mainly dominated by surface effects [5,10,13–16].

According to experimental evidence published by Volkmann et al. [17,18], the growth of dotriacontane ($C_{32}H_{66}$, C32) thin films, a linear medium-chain alkane, supported on amorphous silica surfaces covered with their native oxide layer (SiO_2) begins with the formation of a bilayer of C32 molecules with their long axis orientated parallel to the surface. On top of this parallel bilayer, a perpendicular layer is formed, i.e., a layer of C32 molecules with their long axis lays perpendicular to the surface. This layer will continue to grow adding as much perpendicular layers, one on top of each other, as more C32 molecules are added to the system, until the formation of mesoparticles is achieved. This mechanism is known as Stranski-Krastanov growth and is commonly accepted as the growth mechanism for medium-sized alkanes supported on inorganic surfaces such as SiO_2 [19–21]. Despite the advancements represented by these studies, fundamental questions remain to be answered regarding the nature of the physicochemical properties governing the interaction between inorganic surfaces and organic molecules. Moreover, shading lights on these questions is a key step to support the development of nanotechnological systems with application in biotechnology [9,12].

A relevant tool to gain insights with atomic resolution on the behavior of molecules is by using a whole spectrum of computer simulation techniques called Molecular Dynamics (MD). MD techniques offer a set of methods suitable to investigate complex interactions between molecules in heterogeneous systems. During the last years, it has emerged as a powerful tool to study diverse phenomena at the atomic scale, ranging from protein folding, structure-function relationships in proteins, to inorganic/biological interactions [9,12]. In particular, several molecular dynamics simulations of alkanes have been previously reported [3,4,6,8,11,13–16,22–26]. Most simulations performed to date have used united

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Table 4
Activation energy of self-diffusion for each system.

	257-C32			514-C32			1028-C32		
	XYZ	XY	Z	XYZ	XY	Z	XYZ	XY	Z
Whole film	3.646 ± 0.081	3.591 ± 0.082	4.180 ± 0.147	3.816 ± 0.082	3.760 ± 0.087	4.037 ± 0.066	3.904 ± 0.131	3.851 ± 0.130	4.051 ± 0.149
Upper layer	–	–	–	3.451 ± 0.097	3.411 ± 0.089	3.669 ± 0.201	3.493 ± 0.120	3.440 ± 0.124	3.669 ± 0.123
Middle layer	–	–	–	–	–	–	3.799 ± 0.308	4.230 ± 0.261	0.965 ± 0.260
Bottom layer	–	–	–	3.617 ± 0.192	3.841 ± 0.259	2.463 ± 0.086	3.640 ± 0.293	3.726 ± 0.298	3.127 ± 0.282

Units in kcal/mol.

explain why PMF profiles for 514-C32 and 1028-C32 differ from the bottom-layer and onwards. When comparing these results with the ordered systems, it is clear that 257-C32 behaves as the PO system, although the former has slightly higher diffusion rates than that of the latter. As for the PP system, the bottom-layer of this ordered system has the lowest self-diffusion constants overall, with its axial self-diffusion constant being very similar to the bottom-layers of systems 514- and 1028-C32. As mentioned before, this behavior could be explained by partial confinement produced by the upper and middle layers, respectively.

Temperature dependence of the calculated diffusion constants is properly described by an Arrhenius plot, from which the activation energy of self-diffusion (E_D) can be obtained (Table 4). E_D can be understood as the sum of the energy required to form a void into which the diffusing molecule can move and the energy needed to transfer it from the force field of its nearest neighbors into the void [40]. The obtained energy data reinforces what was already described with the self-diffusion constants: axial diffusion is less favorable than in-plane diffusion, since the activation energy of self-diffusion for the latter is smaller (Table 4). This is observed for all three systems, being more noticeable for 257-C32. When considering each separate layer, a similar behavior is seen for the upper layers compared to the film as a whole: axial diffusion is less favorable than in-plane diffusion. As for the bottom layers, something unexpected is seen for axial diffusion: although rates are slower than for in-plane diffusion (see Table 3), its activation energy is lower, especially for 514-C32 (Table 4). This seems contradictory since lower activation energies should translate into higher diffusion rates. As mentioned earlier, E_D is the sum of two energies: the energy needed to form a void and the energy needed to move a molecule into that void. So, considering that axial diffusion rates are slow, lower activation energies for axial diffusion could be interpreted as one energy term being much higher than the other, while for in-plane diffusion both energy terms could be very similar. Since PMF profiles (Fig. 4) showed practically no energy barriers between consecutive basins, a diffusive process should guide the axial movement of a molecule through the system. Thus, energy needed to form the void should be greater than the energy needed to move a molecule into that void, precluding axial diffusion. Experimental assessment of self-diffusion constants for C32 at different temperatures obtained from nuclear magnetic resonance (NMR) [41] and quasielastic neutron scattering (QENS) experiments [40], shows that our self-diffusions constants are actually 1 or 2 orders of magnitude below such measurements. It is important to note that our self-diffusion rates consider alkanes in close interaction with a SiO_2 surface, which is expected to alter the experimental behavior in terms of diffusion. On the other hand, our estimation of E_D (between 3.64 and 3.90 kcal/mol, see Table 4) is in good agreement with data obtained by QENS (3.87 kcal/mol) [40] as well as with data obtained from other MD simulations with similar time scales (3.91 kcal/mol) [27]. In Smuda et al. [40], it is discussed that NMR and QENS data differ from each other since NMR data covers long-time long-range diffusion (μs to ms), while QENS data covers short-time diffusion (<60 ps). Although our MD simulations span by several nanoseconds length, self-diffusion constants were derived from a ps time scale where we computed the MSD (see Section 2). Therefore, it is expected that E_D obtained from our simulations agree with the one obtained experimentally by QENS.

4. Conclusions

We have performed all-atom molecular dynamics simulations of ordered and disordered thin films of C32 near an amorphous SiO_2 surface considering different temperatures and system sizes. Our results support previous structural conclusions generated from available experimental data [17,34,42] suggesting that, while both ordered systems (PO and PP) could be possible, the perpendicular orientation is more energetically favorable than that of the parallel one. When trying to reach the ordered state from a disordered one, we observe that C32 forms at least one or two parallel layers at the SiO_2/C32 interface. Successive layers of C32 show neither the preferential ordering nor the orientation expected in an ordered model. Instead, a disordered bundle appears from the second parallel layer and above, producing entangled molecular segments that remain at all tested temperatures. As a whole, our results shed lights on the nature of physicochemical properties that govern the interaction between SiO_2 and C32.

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Appendix A. Supplementary material

Supporting Methods describing simulation protocol and analyzes performed. Supporting Fig. S1, Density profile for system 1028-C32 at 300 K. Supporting Figs. S2–S4, PMF and order profiles for the disordered systems at all temperatures above C32's melting point. Supporting Fig. S5, Detail of a C32 molecule trapped in the SiO_2 surface. Supporting Table S1, Self-diffusion constants for each layer of the disordered systems. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cplett.2017.01.065.

Neuron
Article

LTP at Hilar Mossy Cell-Dentate Granule Cell Synapses Modulates Dentate Gyrus Output by Increasing Excitation/Inhibition Balance

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SUMMARY

Excitatory hilar mossy cells (MCs) in the dentate gyrus receive inputs from dentate granule cells (GCs) and project back to GCs locally, contralaterally, and along the longitudinal axis of the hippocampus, thereby establishing an associative positive-feedback loop and connecting functionally diverse hippocampal areas. MCs also synapse with GABAergic interneurons that mediate feed-forward inhibition onto GCs. Surprisingly, although these circuits have been implicated in both memory formation (e.g., pattern separation) and temporal lobe epilepsy, little is known about activity-dependent plasticity of their synaptic connections. Here, we report that MC-GC synapses undergo a presynaptic, NMDA-receptor-independent form of long-term potentiation (LTP) that requires postsynaptic brain-derived neurotrophic factor (BDNF)/TrkB and presynaptic cyclic AMP (cAMP)/PKA signaling. This LTP is input specific and selectively expressed at MC-GC synapses, but not at the disynaptic inhibitory loop. By increasing the excitation/inhibition balance, MC-GC LTP enhances GC output at the associative MC-GC recurrent circuit and may contribute to dentate-dependent forms of learning and epilepsy.

INTRODUCTION

The dentate gyrus, the principal input region of the hippocampus, plays a key role in memory formation by transforming patterns of cortical inputs into new patterns of output to the CA3 area (Kesner and Rolls, 2015; Knierim and Neunuebel, 2016). Although the cellular and synaptic basis of this transformation remains unclear, the two glutamatergic cell types in the dentate gyrus, granule cells (GCs) and hilar mossy cells (MCs), likely play a major role. GCs receive excitatory inputs from the entorhinal cortex via the perforant path (PP) and send excitatory output to CA3 pyramidal neurons via the mossy fibers (Amaral et al., 2007). MCs mediate an intrinsic (or associative) excitatory loop, receiving powerful input from a relatively small number of GCs and providing highly distributed excitatory output to a large number of GCs (Amaral et al., 2007; Buckmaster and Schwartzkroin, 1994; Buckmaster et al., 1996; Scharfman and Myers, 2013). In addition to the recurrent circuit, MCs also contact GABAergic interneurons, which mediate feed-forward inhibition onto GCs (Larimer and Strowbridge, 2008; Scharfman, 1995). Although MCs were first identified over a century ago (Lorente De Nó, 1934; Ramón y Cajal, 1911), there are still significant gaps in our knowledge about their function (Scharfman, 2016), and little is known about activity-dependent plasticity of their synaptic outputs.

MCs project their associational and commissural axons to the ipsi- and contralateral inner molecular layer (IML) of the dentate gyrus, where they synapse onto proximal dendrites of GCs (Scharfman, 2016; Scharfman and Myers, 2013). Because of their proximity to the GC soma, MC-GC synapses are in an ideal position to influence the activity of GCs. Moreover, MCs not only contact GCs locally (same lamella) but also project widely along the longitudinal axis of the hippocampus, both septally and temporally from the point of origin (Amaral et al., 2007; Buckmaster et al., 1996). It has been estimated that a single MC may innervate as much as 75% of the septotemporal axis (Amaral and Witter, 1989) and establish ~35,000 synapses in the IML onto putative GC dendrites (Buckmaster et al., 1996). The hippocampus is functionally heterogeneous along this axis; the dorsal/septal hippocampus is primarily involved in spatial memory, while the ventral/temporal hippocampus is associated with emotional memory (Fanselow and Dong, 2010; Strange et al., 2014). Thus, the translamellar projection of MCs could modulate GC activity throughout the hippocampus, thereby linking functionally diverse areas (Scharfman and Myers, 2013). Based on the wide distribution of their axons along the septotemporal

SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures and one table and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2017.07.028.

AUTHOR CONTRIBUTIONS

Y.H., K.N., K.R.J., and A.E.C. performed research and analyzed the data. D.C. performed initial BDNF puff experiments. Y.H., K.N., K.R.J., A.E.C., and P.E.C. designed the experiments. Y.H, K.N. K.R.J., and P.E.C. wrote the manuscript. All authors read and edited the manuscript.

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MOLECULAR DETERMINANTS OF BK CHANNEL FUNCTIONAL DIVERSITY AND FUNCTIONING

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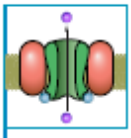

Latorre R, Castillo K, Carrasquel-Ursulaez W, Sepulveda RV, Gonzalez-Nilo F, Gonzalez C, Alvarez O. Molecular Determinants of BK Channel Functional Diversity and Functioning. *Physiol Rev* 97: 39–87, 2017. Published November 2, 2016; doi:10.1152/physrev.00001.2016.—Large-conductance Ca^{2+}- and voltage-activated K^+ (BK) channels play many physiological roles ranging from the maintenance of smooth muscle tone to hearing and neurosecretion. BK channels are tetramers in which the pore-forming α subunit is coded by a single gene (*Slowpoke, KCNMA1*). In this review, we first highlight the physiological importance of this ubiquitous channel, emphasizing the role that BK channels play in different channelopathies. We next discuss the modular nature of BK channel-forming protein, in which the different modules (the voltage sensor and the Ca^{2+} binding sites) communicate with the pore gates allosterically. In this regard, we review in detail the allosteric models proposed to explain channel activation and how the models are related to channel structure. Considering their extremely large conductance and unique selectivity to K^+, we also offer an account of how these two apparently paradoxical characteristics can be understood consistently in unison, and what we have learned about the conduction system and the activation gates using ions, blockers, and toxins. Attention is paid here to the molecular nature of the voltage sensor and the Ca^{2+} binding sites that are located in a gating ring of known crystal structure and constituted by four COOH termini. Despite the fact that BK channels are coded by a single gene, diversity is obtained by means of alternative splicing and modulatory β and γ subunits. We finish this review by describing how the association of the α subunit with β or with γ subunits can change the BK channel phenotype and pharmacology.

I. INTRODUCTION

Evidence of Ca^{2+}-activated conductance in cells was initially reported in *Helix* and *Aplysia* in the 1970s (300, 301). Ca^{2+} injections into neurons and an increase in intracellular Ca^{2+} induced by metabolic poisoning promoted an increase in the K^+ conductance, and Meech (299) in a seminal review proposed that this Ca^{2+}-activated conductance was the link between cell metabolism and cell electrical activity. The improvements on the patch-clamp technique in the early 1980s (166) allowed the characterization of the electrical activity of single channels activated by voltage and internal Ca^{2+}, observations that were reported the same year by Marty in chromaffin cells (288) and Pallota et al. (342) in cultured muscle cells (see also Ref. 19). Almost at the same time, these channels were incorporated into lipid bilayers from a muscle membrane preparation enriched in t tubules, and they were found to have almost identical properties as those described in cells (245). Dubbed BK (for big K^+) (35) or Maxi K^+ (242), these channels proved to have unsuspected characteristics compared with other K^+ channels, such as an extremely large single-channel conductance (~250 pS in symmetrical 100 mM K^+) and an exquisite K^+ selectivity (34, 111, 496). Similar to other K^+ channels, BK channels were blocked by internal and external tetraethylammonium (TEA) and Ba^{2+} (34, 329, 330, 451-454, 496). These ions, as well as scorpion toxins [charibdotoxin and iberiotoxin (58, 143, 144, 277, 278, 307, 310)] were fundamental in unveiling the BK channel's gross architecture, which proved to contain a large internal vestibule and a rather shallow external mouth connected by a narrow selectivity filter. The lack of state-dependent blockage by

By having gating charges distributed in the S2, S3, and S4 transmembrane segments, the voltage sensor of BK channels has been considered as a "descentralized" voltage sensor compared with the VSD of K_v channels, where most of the gating charges are concentrated in S4. Voltage-clamp fluorometry experiments indicate that during activation S2 interacts with S4 since, neutralization in one segment modifies the effective valence on the other. Electrical field focusing or mechanical coupling can account for these observations. The S0 segment appears also to be part of the VSD in BK channels as this structure also undergoes voltage-dependent changes similar to S2 and S4. The evidence indicates that the S6 segment is an important player in the coupling between the VSD and the pore gate. However, the molecular details of this process are still lacking.

The crystal structure of the BK gating ring gave a clear picture of one of the high-affinity Ca^{2+} binding sites, the Ca^{2+} bowl, and identified the important residues involved in divalent cation binding. However, the structure of the second high-affinity Ca^{2+} binding site is still a mystery. The evidence we have so far indicates that the coupling between the Ca^{2+} sensors and the activation gate is mediated by the S6-RCK1 linker. Expansion of the gating ring is thought to pull the linker, initiating the opening of the pore. The Mg^{2+} binding site has attracted special attention because of its particular location at the interface between the VSD and the COOH-terminal domain (CTD). Mg^{2+}, through long-range electrostatic interactions, modifies the workings of the voltage sensor, enhancing voltage sensor-activation gate coupling. It remains to be determined to what extent the VSD/CTD interface is involved in pore opening.

The physiological versatility of the BK channel is greatly increased by alternative splicing and its association with β and γ subunits. Alternative splicing gives rise to many different BK phenotypes, and it also appears that certain BK splice variants have as a target not the plasma membrane but organelles, as it occurs in the case of the mitochondrial BK channel. β Subunits, on the other hand, allow BK channels to be important players in the normal as well as the pathological function of smooth muscle and the nervous system and in determining their pharmacological profile. The more recently discovered γ subunits have revealed new roles for this ubiquitous channel, allowing it to open without much Ca^{2+} at negative voltages. Splicing, modulatory subunit interactions, and metabolic regulation allow BK channels to be involved in a wide array of physiological functions.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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Research Article

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Nanomedicine

Molecular determinants for cyclo-oligosaccharide-based nanoparticle-mediated effective siRNA transfection

Aim: To study the structural requirements that a cyclooligosaccharide-based nanoparticle must fulfill to be an efficient siRNA transfection vector. **Materials & methods:** siRNA protection from degradation by RNAses, transfection efficiency and the thermodynamic parameters of the nanoparticle/siRNA interactions were studied on pairs of amphiphilic molecules using biochemical techniques and molecular dynamics. **Results:** The lower the siRNA solvent accessible surface area in the presence of the nanoparticle, higher the protection from RNAse-mediated degradation in the corresponding nanocomplex; a moderate nanoparticle/siRNA binding energy value further facilitates reversible complexation and binding to the target cellular mRNA. **Conclusion:** The use, in advance, of these parameters will provide a useful indication of the potential of a molecular nanoparticle as siRNA transfecting vector.

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Keywords: endosomal escape • molecular modeling • molecular nanoparticle • protection from RNAses • protein knock down • siRNA transfection

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The use of RNA interference technology provides a very effective gene silencing mechanism that represents an innovative approach to study the role of certain proteins in the physiology or pathology of different organs by knocking down the protein of interest and studying the behavior of the system in the absence of such a protein [1]. It represents a good alternative to knock-out mice since it does not generate compensatory pathways during development, it is faster and it can be used even with proteins whose removal is lethal at embryonic stages [2]. In addition, siRNA-mediated knockdown of proteins involved in cancer cell survival has been proposed to potentiate antitumoral actions of drugs [3], establishing another potential therapeutic approach for cancer treatment. Several exogenous activators of the RNA interference system can silence specific sequences involved in cellular signaling, being siRNAs the most widely used. siRNAs are double-strand RNAs each one formed by about 21 nucleotides that degrade homologous mRNAs [4]. They are highly effective for protein knockdown, but they are quickly degraded in the extracellular medium and they do not enter the cell in naked form, requiring the use of carriers (vectors) to protect them from degradation and to transport them to the cell interior [5].

Different types of nanoparticles (NPs) have been used to efficiently transfect siRNA into different cell types [1,6] and whole animals [7]. To produce an efficient transfection, the NPs, together with the siRNA cargo, must circumvent several barriers. First, it should interact with the siRNA, generally by electrostatic interactions involving positive charges located at the NP periphery [8]. The nanoparticles should also be able to protect the siRNA from degradation by RNAses present in the culture medium or in plasma [6]. Nanoparticle/siRNA complexes must then

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tial as siRNA transfection system of a given molecular vector prototype *a priori*, before it is synthesized; it is therefore well suited for computer-assisted design strategies.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/nnm-2017-0123

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No writing assistance was utilized in the production of this manuscript.

Summary points

- A combination of directed chemical synthesis, biological experiments and in silico modeling has been used to identify some of the key molecular determinants for efficient siRNA transfection by a cyclooligosaccharide-based nonviral vector.
- Two closely related amphiphilic cyclotrehalan derivatives, EMA5 and EMA6, were synthesized and showed striking differences in siRNA protection from degradation by RNAses.
- EMA6 fully protects siRNA against RNAse-mediated degradation while EMA5 does not.
- Molecular modeling of the molecular NP/siRNA interactions indicates that the siRNA solvent accessible surface area is significantly smaller in the presence of EMA6 as compared with EMA5.
- The smaller solvent accessible surface area translates into a more efficient covering of the siRNA and a lower accessibility to RNAses for EMA6 as compared with EMA5 formulations.
- Two very closely related amphiphilic β-cyclodextrin derivatives, AMC6 and AMC36, were also synthesized and showed very different transfection efficiency for siRNA targeting p42-MAPK in several tumoral cell lines.
- AMC6-siRNA complexes reduced p42-MAPK protein levels to about 25% of control values at 72 h while AMC36-siRNA complexes did not cause any significant reduction in p42-MAPK protein levels.
- No differences were found between both NPs regarding their ability to induce endosomal escape, excluding this as a possible cause of the observed differences in transfection efficiency.
- Molecular modeling studies indicated that AMC36/siRNA complexes were much more stable than AMC6/siRNA complexes under conditions simulating full protonation state of the vectors, suggesting that dissociation and release of the siRNA cargo would be significantly hampered in the first case, precluding its biological action.

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• **Proposes that siRNA delivered by nanoparticles might be useful in cancer therapeutics.**

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ORIGINAL RESEARCH

The complex of PAMAM-OH dendrimer with Angiotensin (1–7) prevented the disuse-induced skeletal muscle atrophy in mice

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Abstract: Angiotensin (1–7) (Ang-(1–7)) is a bioactive heptapeptide with a short half-life and has beneficial effects in several tissues – among them, skeletal muscle – by preventing muscle atrophy. Dendrimers are promising vehicles for the protection and transport of numerous bioactive molecules. This work explored the use of a neutral, non-cytotoxic hydroxyl-terminated poly(amidoamine) (PAMAM-OH) dendrimer as an Ang-(1–7) carrier. Bioinformatics analysis showed that the Ang-(1–7)-binding capacity of the dendrimer presented a 2:1 molar ratio. Molecular dynamics simulation analysis revealed the capacity of neutral PAMAM-OH to protect Ang-(1–7) and form stable complexes. The peptide coverage ability of the dendrimer was between ~50% and 65%. Furthermore, an electrophoretic mobility shift assay demonstrated that neutral PAMAM-OH effectively bonded peptides. Experimental results showed that the Ang-(1–7)/PAMAM-OH complex, but not Ang-(1–7) alone, had an anti-atrophic effect when administered intraperitoneally, as evaluated by muscle strength, fiber diameter, myofibrillar protein levels, and atrogin-1 and MuRF-1 expressions. The results of the Ang-(1–7)/PAMAM-OH complex being intraperitoneally injected were similar to the results obtained when Ang-(1–7) was systemically administered through mini-osmotic pumps. Together, the results suggest that Ang-(1–7) can be protected for PAMAM-OH when this complex is intraperitoneally injected. Therefore, the Ang-(1–7)/PAMAM-OH complex is an efficient delivery method for Ang-(1–7), since it improves the anti-atrophic activity of this peptide in skeletal muscle.

Keywords: muscle wasting, peptide delivery, carrier, anti-atrophic peptide

Introduction

The biological activities of several proteins and peptides can be exploited for therapeutic applications. Furthermore, the controlled release of oligopeptides has many potential applications in biomedical and pharmaceutical areas, including in the treatment of diabetes, cancer and tumors, and metabolic, cardiovascular, and infectious diseases. However, peptide use is limited due to low hydrolytic stability, poor systemic distribution, short half-life resulting from rapid metabolization, and low ability to cross physiological barriers.

Consequently, many peptide-delivery strategies have been studied, including direct injection into the malignant tissue and administration using osmotic pumps. Alternatively, hyperbranched polymers and dendrimers exhibit properties that make them attractive candidates for oligopeptide delivery. The chemical properties of dendrimer terminal groups and branch flexibilities play critical roles in interactions with cargo molecules. Indeed, different poly(amidoamine) (PAMAM) dendrimer terminal groups are involved,

effects of an injectable Ang-(1–7)/PAMAM-OH complex on sepsis-induced muscle wasting.

The presently reported findings could be biologically important in the treatment of numerous chronic and highly prevalent diseases, including cardiovascular, renal, and inflammatory conditions, as well as diabetes, cancer, and glaucoma.[36] The effect of Ang-(1–7) has been demonstrated in cardiovascular pathologies such as cardiac remodeling, fibrosis, hypertension, and endothelial dysfunction.[36,38] In the case of plasma glucose handling, target tissues evidence beneficial effects of Ang-(1–7), including in skeletal muscle.[35] Furthermore, several kidney conditions can be improved by Ang-(1–7) treatment, especially those involving fibrosis.[39] Therefore, novel treatments against several chronic diseases that utilize Ang-(1–7) as a principal molecule are also potentially beneficial therapeutic strategies against induced skeletal muscle atrophy.

Conclusion

We determined the feasibility of using neutral dendrimers as therapeutic peptide carriers. This study was based on the biological activity of Ang-(1–7) on skeletal muscle atrophy, and it demonstrated that a novel formulation of Ang-(1–7) with a dendrimer could improve the anti-atrophic properties of Ang-(1–7) when IP injected. Therefore, the Ang-(1–7)/dendrimer complex could serve as a protective delivery system that improves the biological functions of the peptide in vivo. Non-toxic effects were observed in in vivo assays using Ang-(1–7)/PAMAM-OH. At the same time, this study is a contribution to the design of novel dendrimers decorated with neutral (hydroxyl) and/or charged groups (amine, carboxylate, etc.) with the aim of improving or modulating the half-life or kinetic release of peptides and therefore improving the biological effect of peptides, as was demonstrated with Ang-(1–7) in this study.

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Disclosure

The authors report no conflicts of interest in this work.

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LoTo: a graphlet based method for the comparison of local topology between gene regulatory networks

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ABSTRACT

One of the main challenges of the post-genomic era is the understanding of how gene expression is controlled. Changes in gene expression lay behind diverse biological phenomena such as development, disease and the adaptation to different environmental conditions. Despite the availability of well-established methods to identify these changes, tools to discern how gene regulation is orchestrated are still required. The regulation of gene expression is usually depicted as a Gene Regulatory Network (GRN) where changes in the network structure (i.e., network topology) represent adjustments of gene regulation. Like other networks, GRNs are composed of basic building blocks; small induced subgraphs called graphlets. Here we present *LoTo*, a novel method that using Graphlet Based Metrics (GBMs) identifies topological variations between different states of a GRN. Under our approach, different states of a GRN are analyzed to determine the types of graphlet formed by all triplets of nodes in the network. Subsequently, graphlets occurring in a state of the network are compared to those formed by the same three nodes in another version of the network. Once the comparisons are performed, *LoTo* applies metrics from binary classification problems calculated on the existence and absence of graphlets to assess the topological similarity between both network states. Experiments performed on randomized networks demonstrate that GBMs are more sensitive to topological variation than the same metrics calculated on single edges. Additional comparisons with other common metrics demonstrate that our GBMs are capable to identify nodes whose local topology changes between different states of the network. Notably, due to the explicit use of graphlets, *LoTo* captures topological variations that are disregarded by other approaches. *LoTo* is freely available as an online web server at http://dlab.cl/loto.

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Keywords Differential analysis, Gene Regulatory Network, Metric, Graphlet

INTRODUCTION

In biological sciences, networks are becoming one of the main tools to study complex systems (*Newman, 2010*). Networks are employed to represent metabolic pathways (*Palumbo et al., 2005*), signaling cascades (*Pescini et al., 2012*; *Ben Hassen, Masmoudi & Rebai, 2008*), and protein-protein interactions (*Wuchty, Oltvai & Barabási,*

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Author Contributions

- Alberto J. Martin conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Sebastián Contreras-Riquelme performed the experiments, wrote the paper, reviewed drafts of the paper.
- Calixto Dominguez analyzed the data, wrote the paper, reviewed drafts of the paper.
- Tomas Perez-Acle wrote the paper, reviewed drafts of the paper.

Data Availability

The following information was supplied regarding data availability:
The raw data has been supplied as a Supplementary File.

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High Glucocorticoid Levels During Gestation Activate the Inflammasome in Hippocampal Oligodendrocytes of the Offspring

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ABSTRACT: **Exposure to high levels of glucocorticoids (GCs) during early life induces long-lasting neuroinflammation. GCs induce rapid degranulation of mast cells, which release proinflammatory molecules promoting activation of microglia and astrocytes. The possible involvement of oligodendrocytes, however, remains poorly understood. It was studied whether high GC levels during gestation activates the inflammasome in hippocampal oligodendrocytes of mouse offspring. Oligodendrocytes of control pups showed expression of inflammasome components (NLRP3, ACS, and caspase-1) and their levels were increased by prenatal administration of dexamethasone (DEX), a synthetic GC. These cells also showed high levels of IL-1β and TNF-α, revealing activation of the inflammasome. Moreover, they showed increased levels of the $P2X_7$ receptor and pannexin1, which are associated to inflammasome activation. However, levels of connexins either were not affected (Cx29) or reduced (Cx32 and Cx47). Nonetheless, the functional states of pannexin1 and connexin hemichannels were elevated and directly associated to functional $P2X_7$ receptors. As observed in DEX-treated brain slices, hemichannel activity first increased in hippocampal mast cells and later in microglia and macroglia. DEX-induced oligodendrocyte hemichannel activity was mimicked by urocortin-II, which is a corticotropin-releasing hormone receptor (CRHR) agonist. Response to DEX and urocortin-II was inhibited by antalarmin (a CRHR blocker) or by mast cells or microglia inhibitors. The increase in hemichannel activity persisted for several weeks after birth and cross-fostering with a control mother did not reverse this condition. It is proposed that activation of the oligodendrocyte inflammasome might be relevant in demyelinating diseases associated with early life exposure to high GC levels.** © 2016 Wiley Periodicals, Inc.

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Keywords: **dexamethasone; NLRP3; hemichannels; connexins; pannexin1**

INTRODUCTION

In the last decade, prenatal stress and maternal exposure to an excess of glucocorticoids (GCs) have been associated with a variety of alterations in newborns. These changes include increased susceptibility to developing physical, mental, and social disorders, which can be unveiled and/or enhanced by new stress episodes during adulthood (Barker, 2004; Pincus-Knackstedt et al., 2006; Drake et al., 2007; Ehrlich

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Microbial Cell Factories

RESEARCH

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Structure and application of antifreeze proteins from Antarctic bacteria

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Abstract

Background: Antifreeze proteins (AFPs) production is a survival strategy of psychrophiles in ice. These proteins have potential in frozen food industry avoiding the damage in the structure of animal or vegetal foods. Moreover, there is not much information regarding the interaction of Antarctic bacterial AFPs with ice, and new determinations are needed to understand the behaviour of these proteins at the water/ice interface.

Results: Different Antarctic places were screened for antifreeze activity and microorganisms were selected for the presence of thermal hysteresis in their crude extracts. Isolates GU1.7.1, GU3.1.1, and AFP5.1 showed higher thermal hysteresis and were characterized using a polyphasic approach. Studies using cucumber and zucchini samples showed cellular protection when samples were treated with partially purified AFPs or a commercial AFP as was determined using toluidine blue O and neutral red staining. Additionally, genome analysis of these isolates revealed the presence of genes that encode for putative AFPs. Deduced amino acids sequences from GU3.1.1 (gu3A and gu3B) and AFP5.1 (afp5A) showed high similarity to reported AFPs which crystal structures are solved, allowing then generating homology models. Modelled proteins showed a triangular prism form similar to β-helix AFPs with a linear distribution of threonine residues at one side of the prism that could correspond to the putative ice binding side. The statistically best models were used to build a protein-water system. Molecular dynamics simulations were then performed to compare the antifreezing behaviour of these AFPs at the ice/water interface. Docking and molecular dynamics simulations revealed that gu3B could have the most efficient antifreezing behavior, but gu3A could have a higher affinity for ice.

Conclusions: AFPs from Antarctic microorganisms GU1.7.1, GU3.1.1 and AFP5.1 protect cellular structures of frozen food showing a potential for frozen food industry. Modeled proteins possess a β-helix structure, and molecular docking analysis revealed the AFP gu3B could be the most efficient AFPs in order to avoid the formation of ice crystals, even when gu3A has a higher affinity for ice. By determining the interaction of AFPs at the ice/water interface, it will be possible to understand the process of adaptation of psychrophilic bacteria to Antarctic ice.

Keywords: Antifreeze proteins, Antarctica, Psychrophiles, Frozen food, Ice binding proteins

Background

Antarctica is an extreme continent with the coldest temperatures, low precipitations, dryness, and almost completely covered by ice. In this continent, microorganisms dominate the genetic pool and biomass playing an important role maintaining the operation of the ecosystem. Although different microorganisms have been isolated from several places, biological records are available for only a miniscule fraction of them from land and surrounding waters [1].

Psychrophilic microorganisms dominate the majority of Antarctic habitats [2]. For their survival, they have developed several growing strategies on ice including the production of compatible solutes, exopolysaccharides, and antifreeze proteins [3].

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Abbreviations
AFP: antifreeze proteins; TH: thermal hysteresis; TBO: toluidine blue O; NR: neutral red; CV: cell viability.

Authors' contributions
PM and JB participate in sample collection and AFPs purification and characterization. Both authors are corresponding authors. PM isolated and characterized microorganisms from environmental samples. SM modeled AFPs. SM, FG and VM performed docking and molecular dynamics simulations. All authors contributed equally in writing to manuscript. All authors read and approved the final manuscript.

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Enaction

Missing Colors: The Enactivist Approach to Perception

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> Context • Part of Varela's work focused on the study of visual perception, particularly on the grounds of an enactivist theory of vision. **> Problem** • Varela held that the problem of misrepresentation and the comparability of visual experience were crucial. We live with other creatures in sensory worlds that are not tractable, so could we share color-similar experiences? We are still missing an integrative enactive framework to tackle the problems of misrepresentation and comparability related to animal color experience. **> Method** • We carried out a literature survey to draw attention to the status of the enactivist theory of vision and to explore how the problems of misrepresentation and comparability may be tackled. **> Results** • As shown, philosophy and computational science have recently incorporated concepts from neurobiology that close gaps between disciplines and support aspects of the enactivist approach of vision. **> Implications** • Epistemological problems related to perception are here tackled, considering some controversial assumptions related to vision. We argue that an enactivist theory of visual perception may not only clarify the problematic consequences of those assumptions, but also fruitfully guide future philosophical and empirical research on this topic. **> Constructivist content** • The presence of singular "visual channels", as well as physical, sensorimotor and evolutionary factors, constrains our own perceptual experience as proposed by enactivism.
> Key words • **Enaction, perception, misrepresentation, comparability, high color space dimensionality, objectivism, subjectivism, computational science**.

Introduction

« 1 » Francisco Varela's work was highly original. He introduced, together with Humberto Maturana, the notion of *autopoiesis* to elaborate on the biological roots of living organisms (Maturana & Varela 1973). Moreover, he introduced the concept of *enaction* to explain how perception as a "perceptually guided action" leads to embodied cognition (Varela, Thompson & Rosch 1991). According to enactivism, single perceptual states are determined on the basis of factors including sensorimotor structure and ecological interactions. In this sense, action shapes perception, meaning that the ability to perceive is constructed via several previous instances, actions, and abilities.

« 2 » Enaction engages the structure of the visual system, i.e., a set of different visual channels, to guide behavior in interplay with the environment. The enactive approach to perception opposes representationalism, a perspective that confronts two main questions: Is misrepresentation possible? Are visual experiences comparable? In what follows, we develop key aspects of the concept of enaction and visual perception, focusing on color perception as a case study.

« 3 » The notion of color perception is related to the dimensionality of color space in the visual system (Figure 1). Cone photoreceptors are associated with chromatic perception. There are three types of cones in the human retina, Long (L), Middle (M) and Short (S) type, which are identified by wavelength selectivity (visual pigment) and represent a normative case for trichromacy. However, in most lineages of avians, reptiles and fishes, the presence of a fourth cone type constitutes tetrachromacy and a high-dimensional color space (Thompson, Palacios & Varela 1992; henceforth TPV).

« 4 » Although it has been reported that a minority of cone pathways process chromatic signals at low resolution, the majority process achromatic signals at high resolution (Sabesan et al. 2016). In contrast, a digital camera splits the captured input light (see Figure 2) into three channels acting as pass-band filters tuned to different wavelengths and determinate proportions of light receptors in each channel, producing an RGB (red/green/blue) image. Consequently, even if at the level of photoreceptor (cone-types) natural and artificial devices are comparable, the representation of the outer world, due to e.g., the uncoupling sensory-motor system characteristic of most artificial systems, still remains unapproachable from an enactivist point of view.

« 5 » The main argumentative structure of our article will be the following. In the next sections, we introduce two well-known philosophical problems associated with perception: misrepresentation and comparability. We consider how some perspectives within the field of computer science and machine vision, with a focus on color perception, may tackle them. Then, the enactive account of visual perception will be characterized, as opposed to computational objectivism. A key aim is to emphasize that the incompatibility between enactivism and objectivism does not imply that enactivism must be incompatible with every account of perception that involves computational notions, such as the notion of representation.

http://constructivist.info/13/1/117.palacios

color) does not eliminate an illusory perceptual attribute (e.g., two squares have different colors). They tend to neglect the fact that

a the conflict is not between a belief and a percept, but between two beliefs (and, correspondingly, between two percepts) and

b observers are deprived of interactions with the image that would resolve the conflict between the two sets of beliefs/percepts.

Thus, the illusory attributes typically provide strong ground for the supposedly incorrect belief, which is in conflict with the supposedly correct belief. Not only do these cases not support the notion of modularity, in some examples – when the incorrect belief is one that is supported by memory, and contradicts the physical properties of the image – they even contradict modularity (e.g., Hansen et al. 2006). In contrast to illusory attributes, when a percept does not provide strong ground for a particular belief, i.e., when the perceptual state is ambiguous, then the perceiver's thought can influence perception (e.g., the case of intentionally switching between two states of a bi-stable image). That is to say, in the case of perceptual ambiguity, an observer can engage in the activity of switching between multiple beliefs that could be associated with the image.

« 11 » In sum, the enactivist perspective offers a broader and more inclusive paradigm in which the common-sense view of color perception – with its associated puzzles – remains intelligible. The common-sense view of veridical perception is one based on the match between subjective experiences and objective states of affairs. Once we recognize that "objective" is a shorthand for an infinite possibility of other activities (different ways of interacting with an object of perception), and recognize the "subjective-objective match" as a shorthand for a coherence among those activities (including interaction with other perceivers), we arrive at different conceptions of misperception and individual differences. As I tried to argue, the enactivist view of an illusion might be best characterized in terms of attributes that do not survive transitions across different modes of activity. A sustained illusion, therefore, might be best characterized in terms of a sustained deprivation from activities that would otherwise test/remove the illusion. These new conceptions no longer pose the same paradoxes and unbridgeable gaps as they did within a naïve model of color perception.

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Authors' Response

Is a Weak Notion of Representation not Compatible with a Contextualist and Enactivist Account of Perception?

Adrián G. Palacios, María-José Escobar & Esteban Céspedes

> Upshot • We argue that the notion of basic perception could help to develop a general enactivist account of perception, without compromising the compatibility between our approach to this theory and the notion of weak representation. To support this, we elaborate on the contextual and normative aspects of our enactivist proposal, on perception, and on how these aspects may be crucial for understanding misrepresentation and comparability.

« 1 » We respond to the inspiring and challenging comments our article about the enactive account of perception sparked, mainly clarifying the compatibility between that account and a weak notion of representation. Such a notion may help to explore different ways of associating enactivism with related approaches and research proposals. We also focus on the importance of the contextual and normative aspects of our enactive approach to perception, responding to challenging cases related to misrepresentation and comparability.

A radical notion of perception could form the basis for a broader and more flexible enactivism

« 2 » Laura Nascimento and Erik Myin argue that the notion of contentless interaction should be considered to understand color perception. We cannot disagree with this suggestion. Actually, this might be one of the best ways to arrive at a non-circular definition of the notion of contentful representation. However, we find it hard to accept that this idea could ground an argument against our claim that enactivism and a non-objectivist version of representationalism are compatible, as they seem to do. As we try to show, we can account for different features of color perception without dismissing entirely the concept of representation. We do not have to provide a naturalistic definition of that concept within an account of color vision. But if that were necessary, the radical enactivist account defended by Nascimento & Myin would help.

« 3 » The authors further point out that our proposal can be confronted with the hard problem of content, i.e., the challenge of providing a scientifically adequate explanation of how representations occur (§4). We agree that such a problem is crucial if we want to arrive at a general theory of cognition and color perception. Nascimento & Myin characterize basic color perception on the basis of the idea that "organisms tend to show similar perceptual reactions to similar stimuli" (§9). With this characterization, we can account for color perception mainly in terms of how organisms interact with their environments. The notion of contentful representation should be characterized at the non-basic level of human communication, by considering linguistic and cultural interactions between individuals and their environment.

« 4 » Nascimento and Myin dismiss the idea that all cases of perception involve contentful representation (§11). The fact that they mention this thesis in the com-

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RESEARCH ARTICLE

Transgenerational Diapause as an Avoidance Strategy against Bacterial Pathogens in *Caenorhabditis elegans*

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ABSTRACT The dynamic response of organisms exposed to environmental pathogens determines their survival or demise, and the outcome of this interaction depends on the host's susceptibility and pathogen-dependent virulence factors. The transmission of acquired information about the nature of a pathogen to progeny may ensure effective defensive strategies for the progeny's survival in adverse environments. Environmental RNA interference (RNAi) is a systemic and heritable mechanism and has recently been linked to antibacterial and antifungal defenses in both plants and animals. Here, we report that the second generation of *Caenorhabditis elegans* living on pathogenic bacteria can avoid bacterial infection by entering diapause in an RNAi pathway-dependent mechanism. Furthermore, we demonstrate that the information encoding this survival strategy is transgenerationally transmitted to the progeny via the maternal germ line.

IMPORTANCE Bacteria vastly influence physiology and behavior, and yet, the specific mechanisms by which they cause behavioral changes in hosts are not known. We use *C. elegans* as a host and the bacteria they eat to understand how microbes trigger a behavioral change that helps animals to survive. We found that animals faced with an infection for two generations could enter a hibernationlike state, arresting development by forming dauer larvae. Dauers have closed mouths and effectively avoid infection. Animals accumulate information that is transgenerationally transmitted to the next generations to form dauers. This work gives insight on how bacteria communicate in noncanonical ways with their hosts, resulting in long-lasting effects providing survival strategies to the community.

KEYWORDS *Caenorhabditis elegans*, RNA interference, defense, diapause, pathogenesis, survival strategies

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Caenorhabditis elegans is exposed to a wide variety of bacterial species. Soil bacteria represent a diverse pool of pathogenic and nonpathogenic food, and the nematode's responses to different bacteria can contribute to diverse effects on its survival (1). *C. elegans* has previously been used to identify virulence mechanisms of bacteria and to characterize host responses to infection (2). Host and microbe contribute to the outcome, and therefore, the magnitude of the host damage results from the host-microbe interaction, explaining why infection with a particular microbe can drive different effects on the same host (3).

Animals challenged with infectious microbes have three main options: to fight the microbial attack by activating physiological cellular defenses, a costly approach in terms

statistical analysis. George O'Toole kindly provided plasmid pSMC2 for GFP expression in *P. aeruginosa* PAO1. Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (grant number P40 OD010440).

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Intracellular trafficking and cellular uptake mechanism of PHBV nanoparticles for targeted delivery in epithelial cell lines

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Abstract

Background: Nanotechnology is a science that involves imaging, measurement, modeling and a manipulation of matter at the nanometric scale. One application of this technology is drug delivery systems based on nanoparticles obtained from natural or synthetic sources. An example of these systems is synthetized from poly(3-hydroxybutyrate-co-3-hydroxyvalerate), which is a biodegradable, biocompatible and a low production cost polymer. The aim of this work was to investigate the uptake mechanism of PHBV nanoparticles in two different epithelial cell lines (HeLa and SKOV-3).

Results: As a first step, we characterized size, shape and surface charge of nanoparticles using dynamic light scattering and transmission electron microscopy. Intracellular incorporation was evaluated through flow cytometry and fluorescence microscopy using intracellular markers. We concluded that cellular uptake mechanism is carried out in a time, concentration and energy dependent way. Our results showed that nanoparticle uptake displays a cell-specific pattern, since we have observed different colocalization in two different cell lines. In HeLa (Cervical cancer cells) this process may occur via classical endocytosis pathway and some internalization via caveolin-dependent was also observed, whereas in SKOV-3 (Ovarian cancer cells) these patterns were not observed. Rearrangement of actin filaments showed differential nanoparticle internalization patterns for HeLa and SKOV-3. Additionally, final fate of nanoparticles was also determined, showing that in both cell lines, nanoparticles ended up in lysosomes but at different times, where they are finally degraded, thereby releasing their contents.

Conclusions: Our results, provide novel insight about PHBV nanoparticles internalization suggesting that for develop a proper drug delivery system is critical understand the uptake mechanism.

Background

Nanotechnology is the science of engineering materials and systems on a molecular scale. Its application to medicine, "nanomedicine", has enabled the development of nano-sized drug-delivery vehicles. These nanocarriers are generally <200 nm in size and have the ability to carry and deliver therapeutics to discrete sites into the cells [1].

Due to their small size, increased stability and sustained drug release properties, biodegradable polymeric nanocarriers display several advantages, being more effective for cancer treatment than other nanoparticles (e.g. metallic ones) [2]. Nanoparticles are being developed for in vivo tumor imaging, targeted drug delivery and biomolecular profiling of cancer biomarkers. Biodegradable polymeric nanoparticles (NPs) have been shown promissory as controlled drug delivery systems, showing high therapeutic potential [3]. Currently, delivery technologies using cell-targeting [4, 5] or specific targeting of organelles inside a cell [6] are becoming increasingly important as an area of scientific investigation. In cancer,

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When cells were incubated with PHBV NPs, after trafficking through the endo-lysosome pathway, which finally ends at lysosomes, these organelles enter into a special stage where they fused and get polarized, resembling what normally happens during autophagy and immunological synapsis [52, 53]. To investigate whether NPs/cell incubation may trigger autophagy, we analyze LC3 expression by western blot in the two different cell lines, which showed no LC3 expression (data not shown) suggesting that lysosomal fusion not be triggering by an autophagy event [54]. These findings lead to propose the recruitment of a possible MTOC (Microtubule Organizing Center) towards a particular area, allowing the local regulation of potential exocytosis and endocytosis processes by concentrating at a specific place the required molecular machinery. A scheme of PHBV NPs entry pathway for HeLa and SKOV-3 cell lines suggested in this article is depicted in Fig. 13.

Conclusion

We investigated the cellular uptake mechanism of PHBV for intracellular delivery into HeLa and SKOV3 cells. Our experimental results showed that this process is time, concentration and energy-dependent, and the internalization mechanism depends on the cell line. Results described above, give us a general understanding of the cellular processes required for nanoparticle internalization, which contributes to understanding further targeting properties of PHBV enhancing their targeting efficiency.

Additional files

Additional file 1: Figure S1. Physicochemical characterization of nanoparticles. A) Size, B) Z Potential and C) Polydispersion Index.

Additional file 2: Figure S2. PHBV nanoparticles stability. Synthesized nanoparticles were stored at 4 °C for four week period and then analyzed by DLS (Size and zeta potential). Results are expressed as the mean ± standard deviation of triplicate determinations from three independent experiments. One-way ANOVA with Bonferroni test as statistical analysis was performed. ns = not significant, * P <0.05, ** P <0.01, *** P <0.001.

Additional file 3: Figure S3. Cytotoxicity of PHBV nanoparticles at 1, 10, 100 and 1.000 µg/mL against HeLa cells using the MTT assay. Cells were incubated with the respective concentrations and left untreated to measure cell viability by MTT assay.

Authors' contributions

JPP, CV and CO designed the study; JPP, VMM, MCB, RRR, FML performed experiments; FMB. collected and analysed data; JPP, VMM and CO wrote the manuscript; LAV, JAF, FDGN and MRD gave technical support and conceptual advice. All authors read and approved the final manuscript.

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Availability of data and material

Generated data and material are available in the manuscript and supplemental data.

Competing interests

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Stochastic simulation of multiscale complex systems with PISKaS: A rule-based approach

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ABSTRACT

Computational simulation is a widely employed methodology to study the dynamic behavior of complex systems. Although common approaches are based either on ordinary differential equations or stochastic differential equations, these techniques make several assumptions which, when it comes to biological processes, could often lead to unrealistic models. Among others, model approaches based on differential equations entangle kinetics and causality, failing when complexity increases, separating knowledge from models, and assuming that the average behavior of the population encompasses any individual deviation. To overcome these limitations, simulations based on the Stochastic Simulation Algorithm (SSA) appear as a suitable approach to model complex biological systems. In this work, we review three different models executed in PISKaS: a rule-based framework to produce multiscale stochastic simulations of complex systems. These models span multiple time and spatial scales ranging from gene regulation up to Game Theory. In the first example, we describe a model of the core regulatory network of gene expression in *Escherichia coli* highlighting the continuous model improvement capacities of PISKaS. The second example describes a hypothetical outbreak of the Ebola virus occurring in a compartmentalized environment resembling cities and highways. Finally, in the last example, we illustrate a stochastic model for the prisoner's dilemma; a common approach from social sciences describing complex interactions involving trust within human populations. As whole, these models demonstrate the capabilities of PISKaS providing fertile scenarios where to explore the dynamics of complex systems.

1. Introduction

Complex Systems (CSs) encompass a variety of phenomena where the interaction between constituent elements produces emergent properties. Among other characteristics, CSs exhibit degeneracy being highly robust to random failures [1]. Such systems are ubiquitous in nature and society and, when it comes to their analysis, they are usually represented as networks. In these networks, edges represent the interactions occurring between the entities composing the system, which are typically depicted as nodes. Nevertheless, networks are static pictures of CSs: they disregard its dynamic behavior precluding the study of some of its fundamental properties such as evolvability [2]. Among other methods, two main approaches for the dynamic modeling of CSs prevail: deterministic methods based on ordinary differential equations (ODEs) or agent-based modeling, and stochastic approaches based either on stochastic differential equations (SDEs), or on the Stochastic Simulation Algorithm (SSA).

Approaches based on ODEs and SDEs require the definition of an

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PISKaS, our rule-based stochastic simulation engine. As a whole, PISKaS versatility provides a suitable framework to the study of complex multiscale systems with explicit spatial definitions. As discussed above, these features are particularly important to model biological systems where the spatial heterogeneity and the stochasticity generated by individual components are both key elements to understand the dynamics of the system.

Conflicts of interest

All authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrc.2017.11.138.

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Calcium binding and voltage gating in Cx46 hemichannels

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The opening of connexin (Cx) hemichannels in the membrane is tightly regulated by calcium (Ca^{2+}) and membrane voltage. Electrophysiological and atomic force microscopy experiments indicate that Ca^{2+} stabilizes the hemichannel closed state. However, structural data show that Ca^{2+} binding induces an electrostatic seal preventing ion transport without significant structural rearrangements. In agreement with the closed-state stabilization hypothesis, we found that the apparent Ca^{2+} sensitivity is increased as the voltage is made more negative. Moreover, the voltage and Ca^{2+} dependence of the channel kinetics indicate that the voltage sensor movement and Ca^{2+} binding are allosterically coupled. An allosteric kinetic model in which the Ca^{2+} decreases the energy necessary to deactivate the voltage sensor reproduces the effects of Ca^{2+} and voltage in Cx46 hemichannels. In agreement with the model and suggesting a conformational change that narrows the pore, Ca^{2+} inhibits the water flux through Cx hemichannels. We conclude that Ca^{2+} and voltage act allosterically to stabilize the closed conformation of Cx46 hemichannels.

Connexins (Cxs) are transmembrane proteins that can form two types of non-selective channels, gap junction channels and hemichannels. Gap junction channels connect the cytoplasm of two adjacent cells and are involved in electrical coupling. Hemichannels, on the other hand, connect the cell interior with the extracellular milieu allowing the influx and release of signaling molecules for autocrine and paracrine cell communication. These proteins control many cellular processes and play an important role in human pathophysiology, as they are expressed in almost every tissue of the human body and are related to several hereditary diseases[1–3] (Reviewed in[4]).

Cx based channels display two gating mechanisms termed "loop" or "slow" gating and "Vj" or "fast" gating[5]. Both gating mechanisms are regulated by voltage. Each gate is also differentially regulated by several factors. Ca^{2+} and several other polyvalent cations[6–8] and extracellular pH affect the slow gate[9], while intracellular pH[10,11], post-translational modification of the C-terminal domain[8,12] and metabolic inhibition[13] modulate the activation of the fast gate. Regulation of Cx hemichannels by extracellular cations has been extensively studied[14–18]. Among these cations, Ca^{2+} is key for the regulation of Cxs. The extracellular media contains Ca^{2+} in the millimolar range, concentrations which have been reported to maintain Cx hemichannels mainly closed at resting potentials[6,16]. Thus, regulation of hemichannels by Ca^{2+} can be important to prevent their opening under physiological conditions, and subsequent leakage of the intracellular content[19]. The affinity of connexins for divalent cations has been measured by analyzing the steady-state currents at different Ca^{2+} concentration and fitting the normalized ionic currents to a Hill equation of inhibition[7,9,16]. Using this methodology, the half maximal inhibitory concentration (IC 50) of Ca^{2+} for Cx32 and Cx26 are 1.3 mM and 0.33 mM, respectively.

Additionally, Ca^{2+} generates a shift in the activation of Cx hemichannels to higher voltages[6,14,18,20]. In the case of Cx46, the effects of voltage on the channel sensitivity to Ca^{2+} and Mg^{2+} was first interpreted as a voltage-dependent block followed by a voltage-dependent stabilization of the blocked state[6]. However, Verselis and Srinivas found that Cx46 hemichannels close even in absence of divalent cations, ruling out the possibility of a divalent block as the gating mechanism, and proposed that external divalent cations stabilize the slow gate closures induced by hyperpolarization[18]. Moreover, AFM imaging of Cx hemichannels shows that in the presence of Ca^{2+} the extracellular mouth of the channel narrows[17,18,21]. Taken together, these results support the hypothesis that voltage-dependent gating is an intrinsic property of the slow gate and divalent cations stabilize the closed state(s). However, in human Cx26 the results indicate that extracellular Ca^{2+} destabilizes the open state of hemichannels facilitating its closure, a mechanism that would require that Ca^{2+} binds to the open state of the channel[16]. A new crystallographic structure of the Cx26 gap junction channel in a Ca^{2+} bound configuration

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Author Contributions

B.P., R.L., A.M., and C.G., conceived and coordinated the study, and designed the experiments. B.P., A.P., I.G. performed and analyzed the experimental data. B.P., C.G., R.L., and I.G. interpreted the experimental data and formulated the calcium model. All authors co-wrote and approved the final version of the manuscript.

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OSTEOCYTES (T BELLIDO AND J KLEIN-NULEND, SECTION EDITORS)

Connexins and Pannexins in Bone and Skeletal Muscle

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Abstract
Purpose of review To discuss current knowledge on the role of connexins and pannexins in the musculoskeletal system.
Recent findings Connexins and pannexins are crucial for the development and maintenance of both bone and skeletal muscle. In bone, the presence of connexin and more recently of pannexin channels in osteoblasts, osteoclasts, and osteocytes has been described and shown to be essential for normal skeletal development and bone adaptation. In skeletal muscles, connexins and pannexins play important roles during development and regeneration through coordinated regulation of metabolic functions via cell-to-cell communication. Further, under pathological conditions, altered expression of these proteins can promote muscle atrophy and degeneration by stimulating inflammasome activity.

Summary In this review, we highlight the important roles of connexins and pannexins in the development, maintenance, and regeneration of musculoskeletal tissues, with emphasis on the mechanisms by which these molecules mediate chemical (e.g., ATP and prostaglandin E2) and physical (e.g., mechanical stimulation) stimuli that target the musculoskeletal system and their involvement in the pathophysiological changes in both genetic and acquired diseases.

Keywords Gap junctions · Hemichannels · Connexon · Inflammation

This article is part of the Topical Collection on *Osteocytes*

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Introduction

Connexins and pannexins are channel-forming proteins that share similar topology, although they do not exhibit sequence homology [1]. Both connexins and pannexins comprise four transmembrane domains, two extracellular, and one intracellular loop, along with amino- and carboxyl-terminal regions facing the cytoplasm. Connexins form hexamers or connexons in the cell membrane that mediate the exchange of small molecules between the cells and the extracellular compartment, named hemichannels [2]. Hemichannels present in adjacent cells can align to form gap junction channels that allow the exchange of molecules between neighboring cells [3]. Pannexins also form hexamers in the cell membrane, but most investigators agree that they are not able to form functional gap junction channels [4, 5]. The existence of connexin channels in osteoblasts, osteoclasts, and osteocytes has long been recognized, but only very recently has the presence of pannexin channels been described in these bone cells [3]. In skeletal muscle, connexins are only expressed in undifferentiated precursors and upon injury in regenerating muscles, whereas pannexins have been reported to be present in both precursors

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continuous advancements in this field will allow for the development of new strategies that might target the musculoskeletal system to improve bone and skeletal muscle health.

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Compliance with Ethical Standards

Conflict of Interest Juan Sáez, Hannah Davis, Lilian Plotkin, and Bruno Cisterna declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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MINI REVIEW
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Regulation of Connexin-Based Channels by Fatty Acids

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In this mini-review, we briefly summarize the current knowledge about the effects of fatty acids (FAs) on connexin-based channels, as well as discuss the limited information about the impact FAs may have on pannexins (Panxs). FAs regulate diverse cellular functions, some of which are explained by changes in the activity of channels constituted by connexins (Cxs) or Panxs, which are known to play critical roles in maintaining the functional integrity of diverse organs and tissues. Cxs are transmembrane proteins that oligomerize into hexamers to form hemichannels (HCs), which in turn can assemble into dodecamers to form gap junction channels (GJCs). While GJCs communicate the cytoplasm of contacting cells, HCs serve as pathways for the exchange of ions and small molecules between the intra and extracellular milieu. Panxs, as well as Cx HCs, form channels at the plasma membrane that enable the interchange of molecules between the intra and extracellular spaces. Both Cx- and Panx-based channels are controlled by several post-translational modifications. However, the mechanism of action of FAs on these channels has not been described in detail. It has been shown however that FAs frequently decrease GJC-mediated cell-cell communication. The opposite effect also has been described for HC or Panx-dependent intercellular communication, where, the acute FA effect can be reversed upon washout. Additionally, changes in GJCs mediated by FAs have been associated with post-translational modifications (e.g., phosphorylation), and seem to be directly related to chemical properties of FAs (e.g., length of carbon chain and/or degree of saturation), but this possible link remains poorly understood.

Keywords: gap junction channel, hemichannel, connexon, pannexon, G-protein coupled receptor

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INTRODUCTION

Fatty Acids: General Characteristics

Fatty acids (FAs) are carboxylic acids classified into three groups based on the length of their aliphatic carbon tails (Layden et al., 2013). These include: (i) short (<6 carbons), (ii); medium (6–12 carbons); and (iii) long (>12 carbons) aliphatic chains (Talukdar et al., 2011; Layden et al., 2013). In addition, FAs are also classified by the number of double bonds present in their aliphatic chain: saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) or polyunsaturated fatty acids (PUFAs) (Poudyal and Brown, 2015). In turn, PUFAs can be classified into omega-3 (ω-3) and omega-6 (ω-6), based on the location of the last double bond (Schmitz and Ecker, 2008). Despite their structural similarities, ω-3 FAs generally cause biological responses opposing to ω-6 FAs (Senkal et al., 2007). Although traditionally the interest in FAs and their effect on human

possible to develop modified FAs with higher specificities for Cx docking.

AUTHOR CONTRIBUTIONS

All authors listed have made substantial, direct and intellectual contributions to the work, and approved it for publication.

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Research Article

RESEARCH ARTICLE

Increasing the intracellular isoprenoid pool in *Saccharomyces cerevisiae* by structural fine-tuning of a bifunctional farnesyl diphosphate synthase

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One sentence summary: Fine-tuning farnesyl diphosphate synthase in *Saccharomyces cerevisiae* allows the modification of substrate affinity and product selectivity regarding the production and accumulation of dimethylallyl diphosphate, geranyl diphosphate or farnesyl diphosphate.

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ABSTRACT

Farnesyl diphosphate synthase (FPPS) is a key enzyme responsible for the supply of isoprenoid precursors for several essential metabolites, including sterols, dolichols and ubiquinone. In *Saccharomyces cerevisiae*, FPPS catalyzes the sequential condensation of two molecules of isopentenyl diphosphate (IPP) with dimethylallyl diphosphate (DMAPP), producing geranyl diphosphate (GPP) and farnesyl diphosphate (FPP). Critical amino acid residues that determine product chain length were determined by a comparative study of strict GPP synthases versus strict FPPS. *In silico* ΔΔG, i.e. differential binding energy between a protein and two different ligands—of yeast FPPS mutants was evaluated, and F96, A99 and E165 residues were identified as key determinants for product selectivity. A99X variants were evaluated *in vivo*, *S. cerevisiae* strains carrying A99R and A99H variants showed significant differences on GPP concentrations and specific growth rates. The FPPS A99T variant produced unquantifiable amounts of FPP and no effect on GPP production was observed. Strains carrying A99Q, A99Y and A99K FPPS accumulated high amounts of DMAPP-IPP, with a decrease in GPP and FPP. Our results demonstrated the relevance of the first residue before FARM (First Aspartate Rich Motif) over substrate consumption and product specificity of *S. cerevisiae* FPPS *in vivo*. The presence of A99H significantly modified product selectivity and appeared to be relevant for GPP synthesis.

Keywords: single-point mutation; FPPS; protein engineering; isoprenoid; LC-MS

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Saccharomyces cerevisiae expresses the phosphatases LPP1 and DPP1, which have the ability to dephosphorylate GPP and FPP mainly generating prenyl alcohols such as geraniol, linalol and farnesol (Chambon *et al.* 1990). Thus, the limited accumulation of GPP in the A99T strain could result from the action of these phosphatases and the consequent consumption of GPP. In order to confirm this assumption, it is necessary to quantify the production of geraniol.

The expression of the A99G mutation caused an increase in the accumulation of FPP, which was the only detectable product. The substitution of alanine for glycine increased the volume of the active site, particularly in the area where the terminal end of the aliphatic chain of FPP is located, which probably led to an increase in the stability of FPP in this region.

The residue A99 of FPPS in *S. cerevisiae* corresponds to the first residue before FARM. The analyzed mutations show that this residue is crucial for the chain extension of the product. Moreover, some modifications of this residue decrease the catalytic activity of this enzyme, accumulating DMAPP/IPP and reducing the intracellular concentration of GPP and FPP to undetectable levels.

In view of the mixed effect over isoprene phosphates of the different variants analyzed, we propose to evaluate the A99T variant as a potential candidate for monoterpene overproduction. Indeed, the latter showed exclusive accumulation of GPP, without detectable FPP, suggesting an important redirection of the metabolic flux to the monoterpene precursor GPP.

Results for the A99G variant suggest a substantial improvement of the FPPS activity, which translated in a significant increment of FPP concentration. Further work on the impact of this mutation in sesquiterpenes—or larger terpenes—overproducing strains is underway.

Unexpectedly, the strains expressing A99P, A99Q and A99Y mutations did not show detectable levels of GPP or FPP. However, these strains accumulated considerable amounts of DMAPP and/or IPP, i.e they are potential candidates for isoprene-producing strains.

CONCLUSION

The present study allowed us to characterize the effect of the expression of various FPPS mutants *in vivo*, completely eliminating the effect of *wt* FPPS activity, preventing its action as a homo- or heterodimer, as the native erg20 gene was deleted.

Using this current approach, it was possible to identify various FPPS mutants that increase the concentration or the relative abundance of DMAPP-IPP, GPP or FPP in *S. cerevisiae in vivo*. This provided several variants of FPPS, which favor the availability of precursors for the production of hemiterpenes, monoterpenes and sesquiterpenes in strains expressing these terpene synthases.

It is important to consider repressing or deleting the native erg20 gene for the production of mono- and hemiterpenes to decrease the FPPS activity in the strain to be used, which is similar to the previously described need to inhibit other enzymes of the ergosterol pathway, such as squalene synthase for producing sesquiterpenes, diterpenes and carotenoids.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSYR online.

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Conflict of interest. None declared.

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PERSPECTIVES

Unravelling a novel mechanism for the up-regulation of connexin43 gap junctions between cells derived from the blood–brain barrier

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Endothelial cells from different vascular territories in mammals express at least three connexins (Cx37, Cx40 and Cx43), which are protein subunits of gap junction channels. In an article in this issue of *The Journal of Physiology*, however, Bader *et al.* (2017) detected only Cx43 at gap junction plaques from hCMEC/D3 cells, which is a cell line derived from the blood–brain barrier. The existence of one Cx type forming gap junctions should simplify the understanding of mechanisms that regulate this intercellular communication pathway. But many years have passed since the up-regulation of gap junctions by increased intracellular cAMP concentrations was first described (i.e. two reports from Dr Werner R. Loewenstein's laboratory in 1981). The research group of Dr Anaclet Ngezahayo have now unravelled an unsuspected intracellular signalling step (Bader *et al.* 2017), on which I comment here, focusing exclusively on studies about gap junctions channels formed by Cx43 and their regulation by cAMP.

Numerous studies have described a direct correlation between elevated intracellular cAMP concentrations and gap junctional communication in different cell types. Several of them have illuminated the phenomenon thanks to the formation of new reagents (e.g. specific mRNA primers and antibodies) as well as the implementation of complementary techniques (e.g. transfection of a specific Cx type in cells deficient in Cx expression, and recording of functional gap junctions using the dual-voltage clamp technique). These advances have deepened our knowledge in this regard and cannot all be quoted in this article due to space limitation.

Several putative mechanisms that might explain the upregulation of gap junctions by increased intracellular cAMP concentrations have been proposed. One of them focuses on the possible gating of pre-existing gap junction channels found at cell–cell interfaces (e.g. opening of gap junction channels induced by protein phosphorylation). Studies designed to demonstrate this possibility have revealed that direct phosphorylation of Cx43 by cAMP-dependent kinase (protein kinase A; PKA) does not occur, since post-translational modification and functional analysis at the single-molecule level (unitary current) have failed to detected significant changes. For example, recombinant Cx43 or synthetic peptides containing the putative Cx43 phosphorylation site were shown to be poor substrates for PKA, and the molar ratio of Cx43 phosphorylation was not significantly affected in cells treated with 8Br-cAMP. In addition, activation or inhibition of PKA was not seen to affect single channel conductance of Cx43 gap junction channels expressed in SKHep cells (Kwak et al. 1995). Therefore, in the latter study it seemed unlikely that activation of Cx43 gap junction channels could result from direct interactions with cAMP or via phosphorylation of Cx43 channels by PKA. However, it should be kept in mind that the possible involvement of changes in cytoplasmic Ca^{2+} concentrations may have been overlooked, since the solution of the recording pipettes contained 10 mM EGTA. These findings, however, do not completely discard the participation of cAMP-dependent protein phosphorylation at other cellular levels by changing the activity of other proteins that regulate Cx43 levels and/or trafficking of preformed Cx43 (see below).

Whether increased synthesis or reduced degradation rates of Cx43 may play a role has also been assessed, considering that changes in gap junctional communication associated with elevated intracellular cAMP concentration occurs within the range of a few dozen minutes to a couple of hours, and that the half-life of Cx43 is relatively short (about 3 h). With regard to the first possibility, cycloheximide (a protein synthesis inhibitor in eukaryotic cells) did not prevent the enlargement of Cx43 gap junctional plaques in cells treated with adenosine, which increases cAMP concentration, as observed through immunofluorescence in hCMEC/D3 (Bader *et al.* 2017). Curiously, though, cAMP does increase the transcriptional rate of Cx43 mRNA in a communication-deficient Morris hepatoma cell line through a mechanism also resistant to cycloheximide (Mehta *et al.* 1992). While in some studies transcription inhibition reduced the expression of Cx43, this effect has not been associated to changes in gap junctional communication. In neonatal rat ventricular myocytes, a rise in cAMP concentration increases the size of Cx43 gap junctions without affecting the Cx43 synthesis rate (Darrow *et al.* 1996). Some studies have reported that cAMP induces *de novo* gap junctional communication, where Cx43 mRNA transcription and translation most likely play a critical role. In general, the cAMP effect on Cx43 gap junctions seems to depend on the cell type and cell stage, and as mentioned above, it is independent of protein synthesis in several cells. Consequently, an alternative mechanism of action that has been proposed is that cAMP promotes the insertion of preformed Cx43 in the cell membrane. Cx43 could be found as dispersed hemichannels on the cell surface or in vesicles located between the Golgi apparatus and the cell membrane. Several phosphorylation sites located in the Cx43 C-terminal domain have been proposed to affect gap junction size and communication. One of them is the phosphorylation of the amino acid residue Ser373 by Akt, which increases gap junction size and communication by eliminating interactions between Cx43 and the scaffolding protein ZO-1 (Dunn & Lampe, 2014). In addition, the same group showed that phosphorylation of Cx43 by casein kinase 1 (CK1) at Ser325, Ser328 and Ser330 promotes gap junction assembly. As a matter of controversy, phosphorylation of Cx43 might not be needed for gap junction formation, since truncated Cx43 lacking most of the cytoplasmic C-terminal domain would form functional gap junction channels anyway. But that is a black and white demonstration, and the role of cAMP on gap junction size and communication might only be regulatory. Thus, it remains to be studied whether the coordinated

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cross-talk between a cAMP-dependent pathway might activate Akt and/or CK1. A possible alternative was recently discovered in a systematic study by Bader et al. (2017) performed on hCMEC/D3 cells. These cells were treated with agents that increase cAMP intracellular concentration (e.g. agonists of A2 receptors, agonist of adenylyl cyclase or membrane-permeant derivatives of cAMP), after which increases in gap junction size and gap junctional communication were consistently observed. However, such a response was independent of PKA because inhibition of this kinase did not even reduce the gap junction response. Instead, the response required the activation of cyclic nucleotide-gated (CNG) channels, which are directly activated by cAMP binding to a channel domain located in the intracellular side of the cell membrane. Since CNG channels are permeable to Ca^{2+}, their activation led to Ca^{2+} influx, hence raising intracellular free Ca^{2+} concentration. This response was completely eliminated by inhibiting the CNG channel, knocking down the CNG channel or chelating intracellular free Ca^{2+}. This systematic analysis thus revealed the role of cytosolic Ca^{2+} concentration increments in positively regulating gap junctions, which had most frequently been associated to reductions in gap junctional communication.

Increases in cytosolic Ca^{2+} could lead to cytoskeleton disruption, promoting the release of Cx43-containing vesicles anchored to the cytoskeleton that serve as conduits to the cell membrane. Consequently, greater vesicle fusion with the cell membrane and consequent insertion of new hemichannels could occur, which would favour the formation of gap junction channels that would then aggregate and increase gap junction size. This mechanism might operate in conjunction with Cx43 phosphorylation by Akt or CK1, as described above, but further studies need to be performed to prove this possibility.

Many of the above findings are consistent with the role of cAMP as a second messenger, which amplifies signal transduction and has a pleiotropic effect on numerous molecular targets found at different levels of the cellular regulatory machinery. Nevertheless, the studies mentioned could present different temporal outcomes. Systematic studies are hence required in order to demonstrate the relative contribution of each of the findings in gap junction size growth and communication. Finally, it remains to be demonstrated whether this relay in second messengers (cAMP to Ca^{2+}) also operates in different cell types and in the blood–brain barrier *in vivo*. It could be of interest to investigate whether the relay from cAMP to Ca^{2+} might be accomplish via Ca^{2+} influx through a different type of membrane channel or through Ca^{2+} release from intracellular stores. Thus, as generally occurs in scientific research, the discovery of an important issue opens several new questions.

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SCIENCE SIGNALING | RESEARCH ARTICLE

CELL MIGRATION

ATP promotes the fast migration of dendritic cells through the activity of pannexin 1 channels and $P2X_7$ receptors

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Upon its release from injured cells, such as infected, transformed, inflamed, or necrotic cells, extracellular adenosine-5′-triphosphate (ATP) acts as a danger signal that recruits phagocytes, such as neutrophils, macrophages, and dendritic cells (DCs), to the site of injury. The sensing of extracellular ATP occurs through purinergic (P2) receptors. We investigated the cellular mechanisms linking purinergic signaling to DC motility. We found that ATP stimulated fast DC motility through an autocrine signaling loop, which was initiated by the activation of $P2X_7$ receptors and further amplified by pannexin 1 (Panx1) channels. Upon stimulation of the $P2X_7$ receptor by ATP, Panx1 contributed to fast DC motility by increasing the permeability of the plasma membrane, which resulted in supplementary ATP release. In the absence of Panx1, DCs failed to increase their speed of migration in response to ATP, despite exhibiting a normal $P2X_7$ receptor–mediated Ca^{2+} response. In addition to DC migration, Panx1 channel– and $P2X_7$ receptor–dependent signaling was further required to stimulate the reorganization of the actin cytoskeleton. In vivo, functional Panx1 channels were required for the homing of DCs to lymph nodes, although they were dispensable for DC maturation. These data suggest that $P2X_7$ receptors and Panx1 channels are crucial players in the regulation of DC migration to endogenous danger signals.

INTRODUCTION

Dendritic cells (DCs) are bone marrow–derived cells that are sparsely but widely distributed in peripheral tissues. Upon encounter with danger-associated signals, DCs migrate to draining lymph nodes to activate T lymphocytes and initiate adaptive immunity (*1*). DC migration is therefore instrumental to the onset of the immune response. Danger signals include pathogen-associated molecular patterns (PAMPs), such as bacterial lipopolysaccharide (LPS), and damage-associated molecular patterns (DAMPs), such as adenosine-5′-triphosphate (ATP) (*2*, *3*). The case of extracellular ATP is particularly intriguing because it regulates DC migration at different stages (*4*). Upon tissue damage, ATP released into the extracellular milieu induces the rapid recruitment of immune cells (*4*, *5*). Simultaneously, but with reduced kinetics, ATP activates DCs and stimulates the cell surface expression of CCR7, a chemokine receptor that facilitates DC migration to lymph nodes (*6*). ATP therefore emerges as a danger signal that links local sensing to adaptive immunity.

In tissues, the steady-state extracellular concentration of ATP is maintained in the nanomolar range but markedly increases (up to the millimolar range) upon tissue damage, inflammation, infection, and other pathological conditions (*7*–*9*). The presence of extracellular ATP is sensed through purinergic receptors (P2), which are located at the cell surface and classified into two families, P2X or P2Y, which correspond to ion channels and metabotropic receptors, respectively (*10*). P2 receptors are differentially activated and are involved in a plethora of cellular responses depending on the exposure time and the concentration of ATP (*11*, *12*). In particular, ATP released from damaged cells to the extracellular milieu promotes cell migration through paracrine signaling (*4*, *5*). In addition, ATP released from migrating cells acts in an autocrine manner, enhancing the motility of the ATP-releasing cells (*4*, *12*, *13*); however, whether such signaling mechanisms function in DCs is unclear.

Different mechanisms of ATP release might contribute to autocrine signaling that is stimulated during cell migration, including membrane channels constituted of pannexin 1 (Panx1) (*4*, *12*–*15*). Panx1 channels enable the passage of small metabolites, signaling molecules, and fluorescent dyes, and their opening is associated with the activation of $P2X_7$ (*16*) and some P2Y receptors (*17*). Panx1 is ubiquitously expressed in the immune system and is involved in various cellular processes, such as cell migration, T cell activation, and cell death (*14*). In DCs, the presence of functional Panx1 channels is suggested from experiments in which dye uptake was observed after the ATP-dependent activation of $P2X_7$ receptors (*18*–*24*). However, functional evidence of the activity of those channels and their potential influence on immune surveillance by DCs is still missing.

Here, we combined the use of microfabricated devices and live-cell microscopy to show that the ATP-stimulated activation of DCs promoted their fast migration, which likely occurred through a sustained autocrine purinergic signaling loop. This loop depended on the opening of Panx1 channels, $P2X_7$ receptor activation, and subsequent Ca^{2+} signaling and resulted in the rearrangement of the actin cytoskeleton to maintain fast migration. Our results identify Panx1 channels and the $P2X_7$ receptor as players in the regulation of cytoskeletal organization and DC locomotion. Thus, modulation of Panx1 channels and $P2X_7$ receptor activity in DCs might help modulate the immune sentinel functions of these cells in a pathological context, such as asthma or cancer, in which ATP concentrations are dysregulated.

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RESULTS

ATP-stimulated membrane permeability in DCs involves Panx1 channels

Upon activation, Panx1 channels and other large pore–forming proteins enable the diffusion of ions, small molecules, and also fluorescent

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Phosphoethanolamine addition to the Heptose I of the Lipopolysaccharide modifies the inner core structure and has an impact on the binding of Polymyxin B to the *Escherichia coli* outer membrane

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ABSTRACT

Phosphoethanolamine (pEtN) decoration of *E. coli* Lipopolysaccharide (LPS) provides resistance to the antimicrobial Polymyxin B (PolB). While EptA and EptB enzymes catalyze the addition of pEtN to the Lipid A and Kdo (pEtN-Kdo-Lipid A), EptC catalyzes the pEtN addition to the Heptose I (pEtN-Hept$_I$). In this study, we investigated the contribution of pEtN-Hept$_I$ to PolB resistance using *eptA*/*eptB* and *eptC* deficient *E. coli* K12 and its wild-type parent strains. These mutations were shown to decrease the antimicrobial activity of PolB on cells grown under pEtN-addition inducing conditions. Furthermore, the 1-*N*-phenylnapthylamine uptake assay revealed that in vivo PolB has a reduced OM-permeabilizing activity on the Δ*eptA*/*eptB* strain compared with the Δ*eptC* strain. In vitro, the changes in size and zeta potential of LPS-vesicles indicate that pEtN-Hept$_I$ reduce the PolB binding, but in a minor extent than pEtN-Kdo-Lipid A. Molecular dynamics analysis revealed the structural basis of the PolB resistance promoted by pEtN-Hept$_I$, which generate a new hydrogen-bonding networks and a denser inner core region. Altogether, the experimental and theoretical assays shown herein indicate that pEtN-Hept$_I$ addition promote an LPS conformational rearrangement, that could act as a shield by hindering the accession of PolB to inner LPS-targets moieties.

1. Introduction

The Gram-negative Outer Membrane (OM) Lipopolysaccharide (LPS) is the first molecular barrier for a variety of molecules such as metabolites, drugs and antimicrobial peptides (AP). Each LPS molecule is composed of Lipid A, a core oligosaccharide and a repetitive oligosaccharide termed O-Antigen. The core oligosaccharide could be further divided into inner and outer core [1]. The Lipid A and the inner core are usually described as conserved regions; nevertheless, both could be modified by nonstoichiometric substitutions, which impact on the bacterial susceptibility to several antimicrobials [2].

Almost all AP have a net cationic (positive) charge, a large proportion of hydrophobic residues, an amphipathic structure and exhibit a variety of structures as well as biological activity. AP activity is directly related with the electrostatic properties of the LPS, as example, Polymyxin B (PolB) mode of action is mediated by the binding of its diaminobutyric acid residues (DAB) to the Lipid A, attracted by the negative charge of the Kdo [3,4], the concomitant displacement of Ca^{2+} and Mg^{2+} ions and the insertion of its hydrophobic tail into the OM acyl chain core. Altogether, these molecular events result in the rupture of the cell membrane and promote the binding of new PolB molecules to the OM, thus, it is a highly efficient OM-permeabilizing compound [5].

As defense strategy, the bacteria decorate the LPS with different chemical moieties such as acyl chains, aminoarabinose and phosphoethanolamine (pEtN), which shields the negative charges of the LPS carboxyl and phosphoryl groups and impede the antimicrobial

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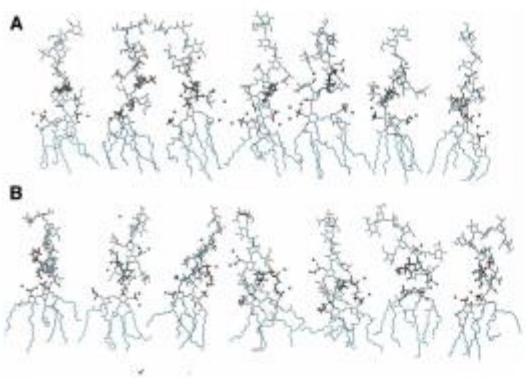

Fig. 3. Representative structures of (A) LPS_{abc} and (B) LPS_C molecules that were randomly chosen from 250 ns molecular dynamics simulation. Phosphoethanolamine and phosphate moieties are highlighted.

the negative charge shielding. Moreover, the influence of pEtN-$Hept_I$ on the LPS core compactness may convert this region into a hydrophilic barrier to hydrophobic molecules, far from the acyl core of the OM. This protective role of pEtN-$Hept_I$ against PolB is in agreement with the zeta potential and reduced LPS disaggregation activity, which suggest that Polymyxin B might not fully access the negative charges of the LPS, furthermore, is in agreement with the NPN assays that shows that pEtN-Hep_I contribute to decrease the destabilization of the hydrophobic acyl core of the OM by PolB.

4. Conclusions

The Lipopolysaccharide layer of the OM is the first molecular barrier of Gram-Negative bacteria. The LPS is usually decorated with pEtN moieties at the Lipid A and Kdo, which reduce the penetration of antimicrobial such as PolB. Our study shows that the recently described addition of a pEtN moiety to the $Hept_I$ also contribute to reduce the activity of Polymyxin B in vivo and *in vitro*. We combined experimental assays and theoretical methods to describe how this addition modifies the LPS core structure, and its implications on the hydrophobicity and the electrostatic interactions of the LPS core with different molecules. Finally, the analysis of molecular simulations of LPS bilayers indicates that when pEtN-$Hept_I$ is added the LPS molecules tend to be in a compacted conformation, which contribute to electrostatic shielding the charge of the inner core phosphate groups and appears to act as a steric hindrance for PolB binding.

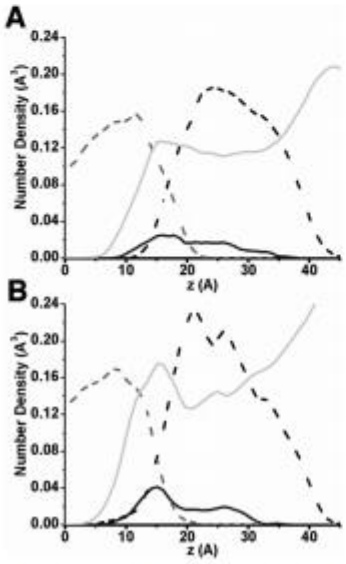

Fig. 4. Density profiles of lipid A (dashed line, gray), inner core (dashed line, dark gray), water (solid line, gray) and Ca^{+2} (solid line, dark gray) atoms along the Z axis in the (A) LPS_{abc} and LPS_C bilayers. The Z-axis is truncated to show the water distribution around the inner core region in detail.

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Appendix A. Supplementary data

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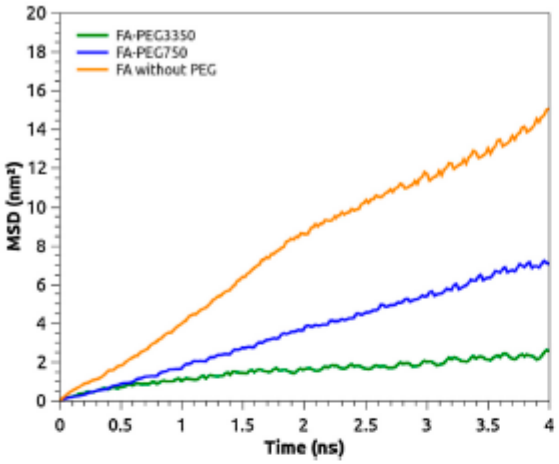

Fig. 8. Self-diffusion coefficients of FA-PEG750 and FA-PEG3350 dendrimers.

retain the folic acid inside the binding pocket (Fig. S1 in Supporting information). The main amino acids involved in this binding are Asp81, Trp102, Arg103, Arg106, Trp140, His135 and Gly137. The side chains of the first five amino acids directly interact with folic acid, and the last two with the backbone part of the amino acid. On the other hand, π–π stacking interactions over the pterin ring were observed, formed by His135 and Trp171 amino acids. As it was expected, when we evaluated the interactions of the complete dendrimers against the folate receptor, we observed several variations in comparison with the FA-FRα crystal reference. Which means that the dendrimers have an effect over the interactions with the aminoacids of the receptor evaluated at 200 ns of MD.

In addition, we can assume that the folate fragment from the dendrimer with PEG750 remains inside of the receptor, as we can observe a low number of hydrogen bonds (Fig. 7) very close or equal to zero through the whole trajectory. Also, when the whole dendrimer with PEG750 was in contact with the FR-α, it showed the presence of H-bonds and as a result, some interaction with the receptor. Meanwhile, the dendrimer with PEG3350 presented slightly more H-bonds than the dendrimer with PEG750.

As a result, we can imply that FA-PEG3350 dendrimer has a slightly better interaction and stability with the receptor than FA-PEG750 dendrimer. However, the dendrimer with PEG750 is also an interesting vehicle for further experimentation.

In addition, histidine (HIS135) was the amino acid with higher percent of hydrogen bonds occupancy (Table S2: Supporting Information) between the receptor and the folic acid fragment from both dendrimers with PEG750 and PEG3350. According to Müller et al., histidine residues can help improve stability. [29]

Finally, FA-PEG3350 dendrimer showed the least diffusion coefficient (MSD) (Fig. 8) along the molecular dynamics trajectory with a mean value of 1.34×10^3 nm^2/ns in comparison with FA-PEG750 dendrimer and folic acid itself, which presented MSD mean values of 4.35×10^3 nm^2/ns and 2.068×10^4 nm^2/ns respectively.

As a result, PEG 3350 has a better chance to interact with folate receptor FR-α, being a better alternative in conjugation with folic acid for drug delivery systems.

As reported before by Schmidtke et al. [53], when a ligand and a receptor interact via H-bonds shielded from water by surrounding hydrophobic regions, as in this case, the resulting complex tends to be more kinetically stable than if the hydrogen bonds were less shielded. However, the difficulty with which water diffuses into and away from the hydrophobic sites appears to create a kinetic barrier to ligand binding and unbinding. In other words, low diffusion could affect the kinetic properties of the dendrimers.

4. Conclusions

This molecular dynamics study validated that PEG chain lengths (750 and 3350 Da) did not interfere over ligand-receptor binding functionality. Although kinetic binding may be affected, especially for the FA-PEG3350 dendrimer, the folate fragment from both dendrimers remained exposed to the solvent before approaching to folate receptor, so it could selectively direct the dendrimer towards FR-α, where interaction and internalization would be able to occur. As a result, theoretical evidence supports that the proposed PEG-folic acid dendrimers represent a new viable alternative as drug delivery systems for cancer therapies. Nevertheless, experimental corroboration is required to verify if PEG fragment length of this dendrimer allows an adequate diffusion in water and does not affect its application in cancer therapy.

Therefore, *in vitro* and *in vivo* experimental corroboration is necessary by isothermal titration calorimetry, fluorescence microscopy to probe the effectivity of the dendrimer against cell death and to corroborate dendrimer-receptor binding. Flow cytometry and MTT assays can also help to determine cell viability and cytotoxicity of FA-PEG systems.

Conflict of interest

The authors declare no competing financial interest.

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ARTICLE

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Central and peripheral clocks are coupled by a neuropeptide pathway in *Drosophila*

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Animal circadian clocks consist of central and peripheral pacemakers, which are coordinated to produce daily rhythms in physiology and behaviour. Despite its importance for optimal performance and health, the mechanism of clock coordination is poorly understood. Here we dissect the pathway through which the circadian clock of *Drosophila* imposes daily rhythmicity to the pattern of adult emergence. Rhythmicity depends on the coupling between the brain clock and a peripheral clock in the prothoracic gland (PG), which produces the steroid hormone, ecdysone. Time information from the central clock is transmitted via the neuropeptide, sNPF, to non-clock neurons that produce the neuropeptide, PTTH. These secretory neurons then forward time information to the PG clock. We also show that the central clock exerts a dominant role on the peripheral clock. This use of two coupled clocks could serve as a paradigm to understand how daily steroid hormone rhythms are generated in animals.

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Author contributions

M.S., C.M., A.P.-M., F.R., L.U., J.C., G.B., C.I., V.S., C.W. and J.E. performed the experiments. M.S., C.M., A.P.-M., F.R., C.W. and J.E. analysed the data. J.E. and C.W. wrote the paper, with feedback from all authors. All authors edited and approved the manuscript.

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On Biophysical Properties and Sensitivity to Gap Junction Blockers of Connexin 39 Hemichannels Expressed in HeLa Cells

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Although connexins (Cxs) are broadly expressed by cells of mammalian organisms, Cx39 has a very restricted pattern of expression and the biophysical properties of Cx39-based channels [hemichannels (HCs) and gap junction channels (GJCs)] remain largely unknown. Here, we used HeLa cells transfected with Cx39 (HeLa-Cx39 cells) in which intercellular electrical coupling was not detected, indicating the absence of GJCs. However, functional HCs were found on the surface of cells exposed to conditions known to increase the open probability of other Cx HCs (e.g., extracellular divalent cationic-free solution (DCFS), extracellular alkaline pH, mechanical stimulus and depolarization to positive membrane potentials). Cx39 HCs were blocked by some traditional Cx HC blockers, but not by others or a pannexin1 channel blocker. HeLa-Cx39 cells showed similar resting membrane potentials (RMPs) to those of parental cells, and exposure to DCFS reduced RMPs in Cx39 transfectants, but not in parental cells. Under these conditions, unitary events of ~75 pS were frequent in HeLa-Cx39 cells and absent in parental cells. Real-time cellular uptake experiments of dyes with different physicochemical features, as well as the application of a machine-learning approach revealed that Cx39 HCs are preferentially permeable to molecules characterized by six categories of descriptors, namely: (1) electronegativity, (2) ionization potential, (3) polarizability, (4) size and geometry, (5) topological flexibility and (6) valence. However, Cx39 HCs opened by mechanical stimulation or alkaline pH were impermeable to Ca^{2+}. Molecular modeling of Cx39-based channels suggest that a constriction present at the intracellular portion of the para helix region co-localizes with an electronegative patch, imposing an energetic and steric barrier, which in the case of GJCs may hinder channel function. Results reported here demonstrate that Cx39 form HCs and add to our understanding of the functional roles of Cx39 HCs under physiological and pathological conditions in cells that express them.

Keywords: Cx39, gap junction, electrical coupling, membrane potential, unitary conductance, dye-uptake, permeability

intracellular portion shows an electropositive confluence, whereas the extracellular side tends to be electronegative, showing a diffuse pattern in which negative charges are mixed primarily with neutral regions and, to a lesser extent, with positive regions (**Figure 9**). Interestingly, mCx39 escapes this trend by exhibiting a more neutral intracellular side, showing a diffuse pattern of positive charges, and a markedly electronegative confluence at the extracellular side flanked by two neutral regions located at both sides of this negative charge accumulation (**Figure 9**). Moreover, this electronegative patch co-localizes with the unique constriction exhibited by mCx39 at the intracellular region of the PH (**Figure 9**). Considering the importance of the localization of charges for the ionic selectivity of Cx-based channels (Ek-Vitorin and Burt, 2013; Escalona et al., 2016) it is expected that any alteration to this pattern could hinder ion currents or voltage sensing, hence affecting channel function. A possible functional consequence of the homotypic docking could be cell–cell addition, which is an important Cx function frequently overlooked

CONCLUSIONS

Results reported here demonstrate that Cx39 forms functional HCs with distinct unitary conductance (75 ± 5 pS) and inhibited by extracellular Ca^{2+}, however it does not form functional GJCs. Cx39 HCs are preferentially permeable to molecules characterized by six categories of descriptors, namely (1) electronegativity, (2) ionization potential, (3) polarizability, (4) size and geometry, (5) topological flexibility and (6) valence. Unlike other Cx HCs, Cx39 HCs are not permeable to Ca^{2+} and show different in sensitivity to classical HC and GJC blockers.

AUTHOR CONTRIBUTIONS

BAC performed permeability experiments, FS performed electrophysiological experiments, CU and LAC performed permeability experiments, AHV designed and performed electrophysiological experiments. SEGM, AJMM analyzed bioinformatics data of dyes and wrote the paper, CPB, YE performed *in silico* model and wrote the paper, TPA designed bioinformatics experiments and wrote the paper, OS and CFL wrote the paper, and AAV designed and performed electrophysiological and permeability experiments and wrote the paper and JCS designed research and wrote the paper.

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SUPPLEMENTARY MATERIAL

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Selective binocular vision loss in two subterranean caviomorph rodents: *Spalacopus cyanus* and *Ctenomys talarum*

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To what extent can the mammalian visual system be shaped by visual behavior? Here we analyze the shape of the visual fields, the densities and distribution of cells in the retinal ganglion-cell layer and the organization of the visual projections in two species of facultative non-strictly subterranean rodents, *Spalacopus cyanus* and *Ctenomys talarum*, aiming to compare these traits with those of phylogenetically closely related species possessing contrasting diurnal/nocturnal visual habits. *S. cyanus* shows a definite zone of frontal binocular overlap and a corresponding area centralis, but a highly reduced amount of ipsilateral retinal projections. The situation in *C. talarum* is more extreme as it lacks of a fronto-ventral area of binocular superposition, has no recognizable area centralis and shows no ipsilateral retinal projections except to the suprachiasmatic nucleus. In both species, the extension of the monocular visual field and of the dorsal region of binocular overlap as well as the whole set of contralateral visual projections, appear well-developed. We conclude that these subterranean rodents exhibit, paradoxically, diurnal instead of nocturnal visual specializations, but at the same time suffer a specific regression of the anatomical substrate for stereopsis. We discuss these findings in light of the visual ecology of subterranean lifestyles.

It is generally accepted that subterranean environments favor magnetic, sound, tactile, vibratory and olfactory cues to the detriment of visual ones[1,2]. Nevertheless, the cumulative body of anatomical and behavioral evidence suggests that even in strictly subterranean mammals, light perception and low acuity vision play indispensable roles in photic entrainment of activity rhythms, photic control of seasonal reproduction, predator avoidance and tunnel maintenance[3–6]. Subterranean mammals show a great diversity of ocular arrangements, including variations in eye and corneal size, retinal thickness, and number/distribution of ganglion cells[2,7–10]. In addition, though less extensively studied, the central visual nuclei follow similar trends, showing varying levels of regression in different subterranean species[11–14]. For example, the blind mole-rat *Spalax ehrenbergi* has very small subcutaneous eyes with highly reduced numbers of ganglion cells and vestigial visual nuclei, except for the suprachiasmatic nucleus (SCN)[13]. On the other hand, the northern mole-vole *Ellobius talpinus* has normal sized eyes with a moderately reduced number of retinal ganglion cells and fairly well-developed central visual nuclei[14].

Moreover, the degree of visual system reduction seems to correlate with the fraction of daily time spent underground[1,2]. Strictly subterranean species, such as the blind mole-rat, which live almost exclusively underground, show highly regressive visual systems, whereas facultative non-strictly subterranean species, such as the mole-vole, the coruro (*Spalacopus cyanus*) and the tuco-tucos (Ctenomyidae, Rodentia) which spend a significant fraction of time on above-ground activities, are known for having less regressive visual systems[9,10].

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Author Contributions

Study concept and design: T.V.Z., J.M., A.G.P. Performed the experiments: T.V.Z., F.S.M., P.N., C.E.S. Analyzed the data: T.V.Z., F.S.M., J.M., P.N., C.E.S., G.M., J.C.L. Wrote the main manuscript text: T.V.Z., J.M. Significant comments to the manuscript text: F.S.M., G.M., J.C.L., P.N., C.E.S., A.G.P. All authors reviewed the manuscript.

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REVIEW Open Access

Accessing gap-junction channel structure-function relationships through molecular modeling and simulations

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Abstract

Background: Gap junction channels (GJCs) are massive protein channels connecting the cytoplasm of adjacent cells. These channels allow intercellular transfer of molecules up to ~1 kDa, including water, ions and other metabolites. Unveiling structure-function relationships coded into the molecular architecture of these channels is necessary to gain insight on their vast biological function including electrical synapse, inflammation, development and tissular homeostasis. From early works, computational methods have been critical to analyze and interpret experimental observations. Upon the availability of crystallographic structures, molecular modeling and simulations have become a valuable tool to assess structure-function relationships in GJCs. Modeling different connexin isoforms, simulating the transport process, and exploring molecular variants, have provided new hypotheses and out-of-the-box approaches to the study of these important channels.

Methods: Here, we review foundational structural studies and recent developments on GJCs using molecular modeling and simulation techniques, highlighting the methods and the cross-talk with experimental evidence.

Results and discussion: By comparing results obtained by molecular modeling and simulations techniques with structural and functional information obtained from both recent literature and structural databases, we provide a critical assesment of structure-function relationships that can be obtained from the junction between theoretical and experimental evidence.

Keyword: Connexins, Hemichannels, Gap-junction channels, Structure and function, Molecular simulation, Homology modeling

Background

Gap junctions (GJs) are regions of cellular membranes in which transmembrane proteins belonging to adjacent cells are in close contact, thereby forming hydrophilic dual-membrane channels. These channels allow the exchange of nutrients, metabolites, ions and small molecules up to ~1 kDa. GJ channels (GJCs) are formed by the end-to-end docking of the extracellular portion of two hemichannels (HCs) or connexons [1] each HC being composed of an hexagonal array of connexins (Cx) protomers [2]. GJCs have crucial roles in many processes including differentiation, neuronal activity, development, immune responses and cell synchronization. Moreover, several human diseases are caused by mutations in connexins, including neurodegenerative diseases, skin diseases, deafness and developmental abnormalities [3].

From rough to fine: the early ages of GJC structure

The discovery of GJs began with the seminal work of Robertson who described them as regular and hexagonal lattices filling the gap between the cellular membranes of adjacent cells [4, 5]. Benedetti and Emmelot [6] described

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α subunits in $GABA_A$ receptors are dispensable for GABA and diazepam action

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The major isoform of the $GABA_A$ receptor is $\alpha_1\beta_2\gamma_2$. The binding sites for the agonist GABA are located at the β_2+/α_1- subunit interfaces and the modulatory site for benzodiazepines at α_1+/γ_2-. In the absence of α_1 subunits, a receptor was formed that was gated by GABA and modulated by diazepam similarly. This indicates that alternative subunits can take over the role of the α_1 subunits. Point mutations were introduced in β_2 or γ_2 subunits at positions homologous to α_1- benzodiazepine binding and GABA binding positions, respectively. From this mutation work we conclude that the site for GABA is located at a β_2+/β_2- subunit interface and that the diazepam site is located at the β_2+/γ_2- subunit interface. Computational docking leads to a structural hypothesis attributing this non-canonical interaction to a binding mode nearly identical with the one at the α_1+/γ_2- interface. Thus, the β_2 subunit can take over the role of the α_1 subunit for the formation of both sites, its minus side for the GABA binding site and its plus side for the diazepam binding site.

γ-Aminobutyric acid type A ($GABA_A$) receptors are the major inhibitory neurotransmitter receptors in the mammalian central nervous system. The $GABA_A$ receptor is a pentameric protein complex, whose subunits are drawn from the following different isoforms: α(1–6), β(1–4), γ(1–3), δ, ε, θ, π and ρ(1–3). The five subunits form a chloride selective ion channel[1–3]. The most common isoform of this receptor consists of two α_1, two β_2 and one γ_2 subunit(s)[4–6] arranged $\alpha_1\gamma_2\beta_2\alpha_1\beta_2$ counterclockwise when viewed from the extracellular space[7–9]. These receptors have two agonist GABA binding sites and one benzodiazepine binding site[10]. By using *in vitro* mutagenesis the binding sites for the agonist GABA were located to the β_2+/α_1- subunit interfaces[11,12], and the modulatory site for benzodiazepines was at the α_1+/γ_2- subunit interface[13]. Thus, the α_1 subunit is commonly accepted to contribute to the formation of both sites.

The $GABA_A$ receptors can be activated by the agonist GABA and modulated by many drugs[14]. Among these drugs are the benzodiazepines, such as diazepam, that have sedative, anxiolytic, anticonvulsant, hypnotic, and muscle relaxant properties[15]. Coexpression of different combinations of recombinant subunits has generated $GABA_A$ receptors with distinct pharmacological and electrophysiological properties.

As early as 1990, we observed that $\beta_2\gamma_2$ $GABA_A$ receptors, lacking the α_1 subunit, were activated by GABA and potentiated by diazepam[16]. Later this observation was confirmed by several groups for GABA and diazepam[17–19] or other modulators[20]. Expression was also documented for $\beta_1\gamma_2$[21,22] and $\beta_3\gamma_2$[22,23] $GABA_A$ receptors. In the present study, we tried to understand this apparent contradiction and decided to investigate whether alternative GABA and benzodiazepine-binding subunit interfaces exist. Site-directed mutagenesis was combined with two-electrode voltage clamp in *Xenopus* oocytes. Our findings suggest that the β_2 subunit may replace the α_1 subunit for the formation of either site. We have previously utilized experimentally guided computational docking that led to a diazepam bound structure model at the α_1+/γ_2- interface[24]. Computational docking at the β_2+/γ_2- interface yielded structural models which strongly suggest that diazepam can interact with this site in a binding mode nearly identical with the one observed at the canonical α_1+/γ_2- site, thus explaining the similar apparent potency.

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Hyperpolarization-Activated Current Induces Period-Doubling Cascades and Chaos in a Cold Thermoreceptor Model

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In this article, we describe and analyze the chaotic behavior of a conductance-based neuronal bursting model. This is a model with a reduced number of variables, yet it retains biophysical plausibility. Inspired by the activity of cold thermoreceptors, the model contains a persistent Sodium current, a Calcium-activated Potassium current and a hyperpolarization-activated current (I_h) that drive a slow subthreshold oscillation. Driven by this oscillation, a fast subsystem (fast Sodium and Potassium currents) fires action potentials in a periodic fashion. Depending on the parameters, this model can generate a variety of firing patterns that includes bursting, regular tonic and polymodal firing. Here we show that the transitions between different firing patterns are often accompanied by a range of chaotic firing, as suggested by an irregular, non-periodic firing pattern. To confirm this, we measure the maximum Lyapunov exponent of the voltage trajectories, and the Lyapunov exponent and Lempel-Ziv's complexity of the ISI time series. The four-variable slow system (without spiking) also generates chaotic behavior, and bifurcation analysis shows that this is often originated by period doubling cascades. Either with or without spikes, chaos is no longer generated when the I_h is removed from the system. As the model is biologically plausible with biophysically meaningful parameters, we propose it as a useful tool to understand chaotic dynamics in neurons.

Keywords: chaos, hyperpolarization-activated current, conductance-based model, bursting, period doubling

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1. INTRODUCTION

Chaotic behavior in neural systems has been observed for many years. Experimental observations of non-periodic responses range from molluscan neurons (Aihara et al., 1984) to rat sciatic nerves (Gu, 2013), including lobster CPGs (Abarbanel et al., 1996) and fish's Mauthner cells (Faure et al., 2000) (for a review, see Korn and Faure, 2003). In addition, chaotic behavior has been analyzed in detail in several models of neural excitability. Notable examples include the Plant model for the R15 bursting cell in Aplysia (Plant and Kim, 1976) that shows a chaotic regime between bursting and beating firing modes (Canavier et al., 1990); the Chay model of pancreatic β cells (Chay and Rinzel, 1985); and the Huber & Braun (H&B) model of cold thermoreceptors (Braun et al., 1998; Feudel et al., 2000).

found that chaos appears only in certain ranges of slow time constants τ_{sd}, τ_{sr} and τ_h. However, in contrast to the mentioned previous works, our model exhibits chaotic behavior within the biologically plausible values of these parameters.

Our findings emerge as an important contribution to the existing literature on the role of homoclinic bifurcations in concrete neuronal models (Feudel et al., 2000; Shilnikov and Cymbalyuk, 2005; Shilnikov, 2012). Future work on the chaotic behavior of the HB+Ih model can include a more detailed geometrical analysis of the chaotic attractors and a deeper investigation of bifurcations occurring at the onset of chaos. Indeed, chaotic dynamics can also be triggered by a wide range of global bifurcations such as homoclinic and heteroclinic phenomena (Aguirre et al., 2013, 2014) which have yet to be analyzed in the HB+Ih model. The simplicity of this model and the fact that its equations and parameters maintain biophysical meaning, can make it a useful tool to understand how chaotic brain dynamics can be shaped by changes in ion channel expression or to give crucial insight to characterize properties of healthy and ill brains.

AUTHOR CONTRIBUTIONS

KX, JM, MC, and PO performed numerical simulations and analysis. DQ and PO performed bifurcation continuation analysis. KX, JM, PA, and PO wrote the manuscript. KX, JM, MC, DQ, PA, and PO revised and approved the manuscript.

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RESEARCH ARTICLE

Inhibition of glial hemichannels by boldine treatment reduces neuronal suffering in a murine model of Alzheimer's disease

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Abstract

The contribution of reactive gliosis to the pathological phenotype of Alzheimer's disease (AD) opened the way for therapeutic strategies targeting glial cells instead of neurons. In such context, connexin hemichannels were proposed recently as potential targets since neuronal suffering is alleviated when connexin expression is genetically suppressed in astrocytes of a murine model of AD. Here, we show that boldine, an alkaloid from the boldo tree, inhibited hemichannel activity in astrocytes and microglia without affecting gap junctional communication in culture and acute hippocampal slices. Long-term oral administration of boldine in AD mice prevented the increase in glial hemichannel activity, astrocytic Ca^{2+} signal, ATP and glutamate release and alleviated hippocampal neuronal suffering. These findings highlight the important pathological role of hemichannels in AD mice. The neuroprotective effect of boldine treatment might provide the basis for future pharmacological strategies that target glial hemichannels to reduce neuronal damage in neurodegenerative diseases.

KEYWORDS
Alzheimers'disease, astrocytes, connexin, hemichannel, microglia

1 | INTRODUCTION

Altered cell-to-cell communication is now emerging as an important feature of many brain pathologies (Garden & La Spada, 2012). In particular, the crosstalk between glial cells and neurons which is crucial for neuronal function and health is found to be impacted in number of acute, as well as chronic, diseases (Halassa, Fellin, & Haydon, 2007; Kettenmann, Kirchhoff, & Verkhratsky, 2013; Rossi, 2015). Indeed, both astrocytes and microglial cells become reactive and their ability to release a large array of factors including pro-inflammatory cytokines and gliotransmitters is strongly modified, leading to synaptic dysfunctions and neuronal damages. Among the multiple actors involved in this crosstalk, accumulating evidence indicates that hemichannels (HCs) can be important players in pathological processes by providing a paracrine pathway targeting neuronal activity and survival (Bennett et al., 2012; Orellana, Retamal, Moraga-Amaro, & Stehberg, 2016). Indeed, once activated in glial cells, HCs are involved in the release of gliotransmitters like ATP and glutamate as well as chemokines that have deleterious consequences on neurons as reported for murine models of Alzheimer's disease (AD) (Orellana et al., 2011b; Takeuchi et al., 2011; Yi et al., 2016), experimental allergic encephalomyelitis (Shijie et al., 2009), amyotrophic lateral sclerosis (Almad et al., 2016; Takeuchi et al., 2011) and neuropathic pain (Bravo et al., 2014; Chen et al., 2014). HCs can be formed by two families of functionally related proteins, which share the same transmembrane topology but have divergent primary sequences: pannexins (Panxs) and connexins (Cxs) (Giaume, Leybaert, Naus, & Sáez, 2013). They oligomerize into hexameres that isolate a large nonselective pore in the membrane allowing

endothelial cells and, as a consequence, could ameliorate Aβ clearance and reduce Aβ accumulation in boldine treated animals. However, we observed that the progression of the amyloid deposition is not modified by boldine treatment suggesting that if boldine has an effect on BBB, it is only marginal and likely not essential in its neuroprotective action. Second, boldine was also reported to have anti-inflammatory effects (Lau et al., 2015). In agreement with this, we have shown that the level of TNFα was slightly but significantly reduced in APP/PS1 mice treated with boldine while IL-1ß level was not significantly affected. We previously showed that inflammation is involved in Panx1 HC activitation in astrocytes close to amyloid plaques in APP/PS1 mice (Yi et al., 2016). Indeed acute application of minocycline that reduces the level of TNF-α in APP/PS1 brain slices was able to inhibit Panx1 HCs in these reactive astrocytes. Hence, the antiinflammatory effect of boldine can be one of the mechanisms involved in the inhibition of Panx1 HCS observed in APP/PS1 mice.

The absence of toxicity of boldine in a large range of doses including the concentration used presently (Jimenez & Speisky, 2000; O'Brien et al., 2006), its ability to reach the brain after *in vivo* administration, its water solubility and thus its facility to be delivered by oral administration, as well as its relatively low cost compared to mimetic peptides, make it a promising therapeutic molecule to fight deleterious effects of HC activity in diverse brain pathologies. Indeed, a neuroprotective effect of boldine in a model of stroke induced by a permanent MCAO in mice was recently described (de Lima et al., 2017). In mice treated with 25 mg/kg boldine during 5 days after stroke, the size of the infarct area and the neuroinflammatory response were reduced, while cell viability and neurological scores of the mice were improved. The mechanisms underlying such neuroprotection were not examined, but an inhibition of glial HCs that are activated in ischemic conditions (Contreras et al., 2002; Giaume et al., 2013) could likely contribute to its beneficial effects. Moreover, boldine is present in many nutraceutical preparations indicated in the treatment of diverse human disorders in particular digestive ones without secondary deleterious effect (Speisky & Cassels, 1994). Hence, boldine appears as a new neuroprotective drug that could be administered in therapeutical treatment of both acute and chronic brain diseases to reduce neuronal damages.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ORIGINAL RESEARCH
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Mono-Heteromeric Configurations of Gap Junction Channels Formed by Connexin43 and Connexin45 Reduce Unitary Conductance and Determine both Voltage Gating and Metabolic Flux Asymmetry

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In cardiac tissues, the expression of multiple connexins (Cx40, Cx43, Cx45, and Cx30.2) is a requirement for proper development and function. Gap junctions formed by these connexins have distinct permeability and gating mechanisms. Since a single cell can express more than one connexin isoform, the formation of hetero-multimeric gap junction channels provides a tissue with an enormous repertoire of combinations to modulate intercellular communication. To study further the perm-selectivity and gating properties of channels containing Cx43 and Cx45, we studied two monoheteromeric combinations in which a HeLa cell co-transfected with Cx43 and Cx45 was paired with a cell expressing only one of these connexins. Macroscopic measurements of total conductance between cell pairs indicated a drastic reduction in total conductance for mono-heteromeric channels. In terms of Vj dependent gating, Cx43 homomeric connexons facing heteromeric connexons only responded weakly to voltage negativity. Cx45 homomeric connexons exhibited no change in Vj gating when facing heteromeric connexons. The distributions of unitary conductances (γj) for both mono-heteromeric channels were smaller than predicted, and both showed low permeability to the fluorescent dyes Lucifer yellow and Rhodamine123. For both mono-heteromeric channels, we observed flux asymmetry regardless of dye charge: flux was higher in the direction of the heteromeric connexon for MhetCx45 and in the direction of the homomeric Cx43 connexon for MhetCx43. Thus, our data suggest that co-expression of Cx45 and Cx43 induces the formation of heteromeric connexons with greatly reduced permeability and unitary conductance. Furthermore, it increases the asymmetry for voltage gating for opposing connexons, and it favors asymmetric flux of molecules across the junction that depends primarily on the size (not the charge) of the crossing molecules.

Keywords: intercellular communication, heteromeric connexons, gap junctions, permeability, protein kinase C

have been used through the experiments. ADM, EB and APM generated the initial concept of the manuscript and contributed to the initial draft. All have agreed on being accountable for all aspects of the work involved in this manuscript.

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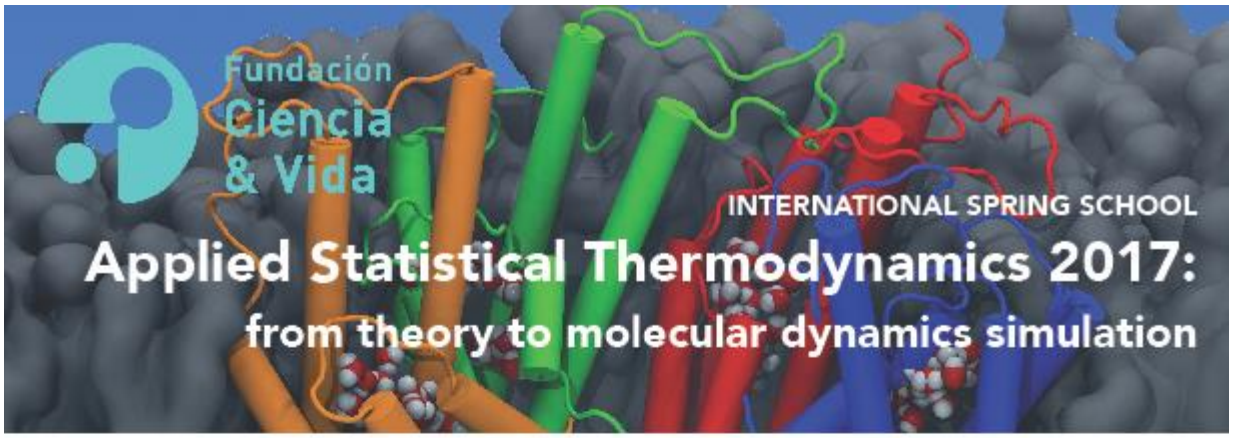
Annex 4
Organization of Scientific Events



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Tomas Perez-Acle
Fundación Ciencia & Vida (FCV), Santiago, Chile

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inscriptions and more info: http://dlab.cl/courses/AST2017


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Wael El-Deredy, Universidad de Valparaíso, Chile
Laurent Perrinet, INT - Institut de Neurosciences de la Timone, Marseille, France
Tatyana Sharpee, Salk Institute, California, USA
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Yasser Iturria, McGill University, Canada
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KEYNOTE SPEAKER

JOSEPH TAKAHASHI
HHMI Investigator, University of Texas Southwestern Medical Center, USA.

PUBLIC LECTURE

CÉLINE VETTER
University of Colorado Boulder, USA.

PLENARY SPEAKERS

NOBEL LAUREATE

- **MICHAEL ROSBASH**
  HHMI Investigator, Brandeis University, USA.

OTHER PLENARY SPEAKERS

- **JAY DUNLAP**
  Dartmouth College, USA.
- **SUSAN GOLDEN**
  HHMI Professor, University of California San Diego, USA.
- **AMITA SEHGAL**
  HHMI Investigator, University of Pennsylvania, USA.

INVITED SPEAKERS

- **TOM DE BOER**
  Leiden University Medical Center, The Netherlands.
- **CARLA GREEN**
  University of Texas Southwestern Medical Center, USA.
- **CHARLOTTE FÖRSTER**
  Würzburg University, Germany.
- **ERIK HERZOG**
  Washington University, USA.
- **JENNIFER LOROS**
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More informations

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Annex 7.1
Outreach activities throughout the period

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CHILE

CENTRO INTERDISCIPLINARIO DE Neurociencia de Valparaíso

DE MEN TE

EL CEREBRO, UN HUESO

DURO DE ROER

DE MEN TE

EL CEREBRO, UN HUESO

DURO DE ROER

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AA studio

Agradecemos en primer lugar a la Universidad de Valparaíso, que alberga al Centro Interdisciplinario de Neurociencia de Valparaíso (CINV), permitiendo hacer ciencia de excelencia desde una universidad pública. A los estudiantes de Magister y Doctorado en Ciencias, mención Neurociencia de esta universidad, quienes escribieron los artículos de este libro, llevando la ciencia de frontera a un lenguaje comprensible por toda la sociedad. Queremos agradecer también al diario electrónico El Mostrador, primer diario online de Chile, por darnos la oportunidad desde 2014 de llegar a cientos de miles de lectores interesados en conocer cómo funciona nuestro sistema nervioso a través de la serie "Los secretos del cerebro", en una experiencia inédita en nuestro país. Por último, queremos agradecer a la Iniciativa Científica Milenio del Ministerio de Economía, cuyo generoso y permanente apoyo ha hecho posible el desarrollo de ciencia competitiva al nivel internacional, además de impulsar su difusión y promoción de modos innovadores, como con esta publicación.

LA ALEGRÍA DE LA CIENCIA

XX Concurso de Proyectos Explora de Valoración y Divulgación de la Ciencia y la Tecnología 2015-2016

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The Falling Walls Lab Chile
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• Start: 2 pm
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QUESTIONS?
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Annex 7.2
Articles and Interviews

1) **"Scientific Center will be raised in historic ruins of Valparaiso"**
   Newspaper: El Mercurio
   Date: April 23, 2017
   Link: http://impresa.elmercurio.com/Pages/NewsDetail.aspx?dt=2017-04-23&dtB=23-04-2017%200:00:00&PaginaId=18&bodyid=3
   Scope: National

EL MERCURIO

Se mantendrá y recuperará la fachada del ex edificio Severín:

Levantarán centro científico en históricas ruinas de Valparaíso

El proyecto para edificar la nueva sede de un instituto de investigación de excelencia a nivel mundial está en licitación.

HERNÁN CISTERNAS ARELLANO

Si todo marcha de acuerdo con lo previsto, en un plazo de 600 días volverán a recobrar vida las ruinas del ex edificio Severín, sitio con valor histórico, que entre febrero y agosto de 1828 acogió al primer Congreso Bicameral de Chile. Está ubicado en el corazón del barrio fundacional de Valparaíso, a pasos de la iglesia La Matriz, uno de los sectores más deteriorados de la ciudad y resurgirá convertido en un refugio científico, que apuesta por revitalizar y recuperar esa zona patrimonial.

La Dirección de Arquitectura del Ministerio de Obras Públicas llamó a licitación para construir en el lugar la sede del Centro Interdisciplinario de Neurociencias de Valparaíso (CINV), que dirige el premio nacional de Ciencias Ramón Latorre.

Se trata de un proyecto que tiene un costo de $7.425 millones, que demoró casi una década en concretarse.

En el siglo XVIII, el ruinoso edificio Severín fue convento jesuita. Posteriormente acogió a la congregación de los Dominicos. El inmueble terminó su vida útil el año 2004, cuando un incendio —el segundo en su historia— destruyó las instalaciones ocupadas en esa fecha por una comisaría de Carabineros. Tras años como sitio eriazo, del inmueble original solo permanece en pie la fachada.

JONATHAN MANCILLA

BARRIO PUERTO.—. El proyecto ayudará a recuperar el deteriorado barrio puerto, el sector fundacional de la ciudad de Valparaíso.

Una vez que se reconstruya, pasará a denominarse "Edificio Juan Ignacio Molina", en honor al abate jesuita considerado como el primer científico chileno, quien residió en ese lugar hasta antes de trasladarse a Bolonia, Italia.

VISITA
Las empresas interesadas en la construcción visitarán el lugar el 4 de mayo.

Para el científico Ramón Latorre, promotor de la idea de instalar el CINV en el barrio fundacional de Valparaíso, no obstante el deterioro del sector, "lo que queremos hacer en el lugar es un faro para la ciencia, un faro para el país y recuperar el barrio".

El desafío es demostrar que a través de un edificio moderno e innovador, convertido en un centro de investigación de excelencia a nivel mundial, es posible recuperar sitios históricos y, paralelamente, reactivar un barrio que forma parte de la zona que ha sido declarada por la Unesco como patrimonio de la humanidad.

El Centro Interdisciplinario de Neurociencias de Valparaíso ostenta desde el año 2011 la categoría de Instituto Milenio.

En el lugar se levantará un edificio de 4.863 m², que contará con instalaciones de alta tecnología, laboratorios para investigación neurocientífica, un auditorio, salas multiuso y de reuniones, espacios de trabajo para 150 personas —entre investigadores y estudiantes de doctorados y post doctorados, chilenos y extranjeros—, un museo interactivo y áreas para la difusión de la ciencia.

Se espera que anualmente el Centro de Neurociencias reciba a más de 2 mil especialistas nacionales e internacionales ligados a la investigación científica.

En julio del 2015, Bienes Nacionales entregó la propiedad en comodato por 40 años a la Universidad de Valparaíso para la construcción del CINV.

Para contar con los $7.425 millones que cuesta el proyecto fue necesario concordar voluntades en pos de un financiamiento compartido entre el Ministerio de Obras Públicas ($1.075 millones), la Universidad de Valparaíso ($1.500 millones) y el Gobierno Regional ($4.850 millones).

La apertura de los antecedentes técnicos que se presenten dentro de la propuesta pública se realizará el 22 de agosto, mientras que las ofertas económicas se conocerán el 13 de septiembre.

2) **"Scientific documentary explains the importance of cuttlefish in the advance of neuroscience in Chile"**
Webpage: Diario electronico el Mostrador
Date: August 27, 2017
Link: http://www.elmostrador.cl/cultura/2017/08/27/documental-cientifico-explica-la-importancia-de-la-jibia-en-el-avance-de-la-neurociencia-en-chile/?php%20bloginfo(%27url%27);%20?%3E/cultura

3) **"Researcher discovers how worms inherit survival mechanisms from their offspring".**
Newspaper: Las Últimas Noticias
Date: December 26, 2017
Link: http://www.lun.com/Pages/NewsDetail.aspx?dt=2017-12-26&PaginaId=32&bodyid=0
Scope: National

Las Últimas Noticias

Según la investigación de la doctora Calixto, el *Caenorhabditi. Elegans* es muy útil para entender cómo funcionan los humanos.

Lo publicaron en la revista "mBio" de la Sociedad Americana de Microbiología

Investigadora descubre cómo gusanos heredan mecanismos de supervivencia a su descendencia

Estudio de doctora Andrea Calixto reveló que el gusano *C. elegans* tiene un mecanismo que lo une a sus antepasados.

CAMILA FIGUEROA

La diferencia entre que un humano haya nacido como tal y no como un gusano es tan mínima, dice Andrea Calixto, doctora en Neurobiología Molecular de la Universidad de Columbia, que solo un porcentaje mínimo de genes hizo la diferencia.

De hecho, asegura, un humano es tan parecido a un gusano, específicamente al de la especie *Caenorhabditis elegans*, que las células y proteínas fabricadas por ambos cuerpos cumplen el mismo rol: decirle al organismo cuándo crecer, cómo reproducirse o cómo defenderse.

Calixto, quien además tuvo como mentor doctoral al mismísimo Martin Chelfie -ganador del Premio Nobel Química del año 2008 por descubrir la proteína verde fluorescente en el mismo gusano (que permite ver procesos celulares que antes eran invisibles. Entre ellos, el desarrollo de las neuronas) incluso afirma que el *C. Elegans* es muy útil para entender cómo funcionan los humanos.

En octubre pasado, luego de cuatro años de estudio, la investigadora del Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso descubrió que el gusano *C. elegans* tiene un mecanismo transgeneracional, es decir que lo une a sus antepasados. Ese mecanismo permite que su descendencia pueda defenderse de las cosas malas de la vida, por ejemplo, de las bacterias patógenas. Una especie de recurso que lo hace evolucionar hacia una especie más resistente, tal cual lo planteó Darwin en "El origen de las especies".

Andrea Calixto es doctora en Neurobiología Molecular.

Mecanismo de defensa

Los *C. elegans* se alimentan de bacterias. Les gusta su olor, pero no pueden distinguir las saludables de las menos saludables. Es más, advierte Calixto, las bacterias patógenas tienen un muy buen olor. "Sin embargo, cuando los gusanos se comen la bacteria y les infecta el intestino, esa infección va al sistema nervioso e informa que deben tomar una decisión que tiene que ver con cerrar la boca, utilizar los ácidos grasos acumulados y ponerse en un estado de letargo", detalla la microbióloga.

Esa decisión vital que deben tomar los gusanos se llama diapausa y es producto del aprendizaje. "Demoran toda una generación en aprenderlo", cuenta.

¿Qué es la diapausa? Calixto dice que cuando los gusanos nacen, pasan por cuatro fases larvarias hasta que finalmente llegan a la adultez. Entre el estadio dos y tres hay una larva que se llama *Dauer*, que tiene la capacidad de ralentizar su metabolismo y endurecerse para así dejar de comer por unos cuatro meses, hasta que pase el estrés de las bacterias malas y vuelva a tener alimento saludable. Eso es la diapausa.

"El hallazgo es que antes no se había descrito que los animales podían hacer diapausa como forma de defensa. Es importante que sea una larva y no un adulto porque los animales jóvenes son los que pueden reproducirse. Si tú sacas del ciclo de vida a un animal viejo, no sirve. La especie necesita cuidar a los jóvenes para que cuando la comunidad salga del estrés, el nicho pueda llenarse nuevamente. Ellos piensan en la comunidad, no son individualistas", advierte.

Agrega que cuando las larvas conocen a la bacteria mala y pasan la información a los hijos, lo hacen de manera transgeneracional. "Eso sirve para preservar la especie porque los parentales le informan a su progenie lo que han aprendido y les pueden salvar la vida. Ahora estamos investigando el mecanismo por el cuál la madre traspasa la información".

Quien se encarga de traspasar el conocimiento es la madre, que en este caso es un hermafrodita que hace de fémina, ya que, según la doctora, solo hay *C. elegans* machos y hermafroditas. "Hicimos cruzas de hermafroditas que tuvieron contacto con los patógenos y con machos que no lo tuvieron. No les importó que el macho no hubiese tenido el contacto con las bacterias. Les basta con la información que les traspasó la madre. Eso se llama línea germinal materna", explica.

La importancia del gusano

Patricio Olguín, académico del Departamento de Neurociencias de la Universidad de Chile e investigador del Instituto de Neurociencia Biomédica (BNI), lo aclara: "Los gusanos tienen muchos genes que están conservados en los humanos y por eso la investigación de Calixto es importante". Agrega que "el uso de *C. elegans* es fundamental porque tiene vías de señalización celular que hacen básicamente lo mismo en humanos, entonces lo que podamos descubrir en ellos, también se puede extrapolar a las personas". Por ejemplo, dice, en los primeros experimentos realizados con este gusano en la década los 60, se encontró el gen de la proteína Netrina, que les dice a las neuronas dónde tienen que hacer contacto en el cerebro. Y tal como pasa en el gusano, sucede en humanos.

» **"Los gusanos tienen muchos genes que están conservados en los humanos y por eso la investigación de Calixto es importante"**
Patricio Olguín, académico del BNI

4) **"Which Chilean City will be the first one to fall down if a pandemic will reach?"**
Newspaper: Las Últimas Noticias
Date: December 12, 2017
Link: http://www.lun.com/Pages/NewsDetail.aspx?dt=2017-12-11&NewsID=388975&BodyID=0&PaginaId=28
Scope: National
Note: This article appeared in diferentt kind of Chilean newspapers

Las Últimas Noticias

Software propone distintos escenarios en caso de enfermedades infecciosas

¿Cuál ciudad chilena sería la primera en caer si llegara una pandemia?

BÁRBARA NÚÑEZ

Investigador concluyó que la conectividad es el factor clave para que una dolencia se disperse en las cuatro urbes más pobladas del país.

¿Qué hubiera pasado en Chile si hubiese llegado una enfermedad como el ébola, el temido mal que asoló África hasta este año? ¿Qué ciudad se hubiera infectado primero? ¿Cómo hubieran reaccionado las autoridades?

Aunque esa es una realidad que no nos tocó vivir, sí ocurrió en el software computacional creado por Tomás Pérez-Acle, director del laboratorio de Biología Computacional de la Fundación Ciencia & Vida e investigador del Centro Interdisciplinario de Neurociencia de Valparaíso. La razón para crear un Chile asolado por una enfermedad infecciosa era simple: prever cómo nos comportaríamos.

¿Cómo simulamos algo que no ha pasado? Con datos. Miles de datos. En este caso, los que se usaron para moldear este futuro distópico vinieron de los ministerios de Salud, Transporte y otras organizaciones internacionales de salud. "Además, nosotros integramos los datos del último brote de ébola en África, es decir, el número de infectados, de muertos, de personas que se recuperaron y el número de cadáveres que no fueron apropiadamente tratados para no propagar la enfermedad", dice Pérez-Acle.

Con esto, se construyó el modelo matemático de la enfermedad. En otras palabras, un software bien parecido a un videojuego apocalíptico.

Para efectos de la simulación, sólo se consideraron las cuatro ciudades más pobladas de Chile: La Serena, Viña del Mar-Valparaíso, Santiago y Concepción.

ABRAHAM MARQUEZ

Tomás Pérez-Acle realizó la simulación.

No existe el paciente cero

"Pusimos un infectado en Santiago en el día cero. Corrimos la simulación y no pasó nada. Luego dos infectados y de nuevo no pasó nada", dice Pérez-Acle. En el software no pasaba nada, pero en la vida real de los cinco investigadores a cargo, bastante.

Cada vez que el software corría una simulación había que esperar tres días para ver los resultados. Finalmente, llegaron a un número mágico: el diez.

"Diez personas tienen que infectarse simultáneamente para que la enfermedad se disperse", dice Pérez-Acle. El número en sí es bastante bajo, pero rompe con la extendida creencia del "paciente cero", típica de película hollywoodense. "Con un solo paciente la probabilidad de que esa enfermedad se disperse es tan baja que habitualmente no ocurre, a menos que ese paciente se dedique a infectar y vaya al estadio y le estornude a todo el mundo", comenta el científico.

Desde Valparaíso a Concepción

Aunque el primer paciente se infecte en la capital, no sería inmediatamente la ciudad en tener más casos. "Descubrimos en nuestra simulación que si bien la infección comienza en Santiago, Valparaíso se infecta muchísimo más rápido, porque la cantidad de habitantes de Valparaíso en términos de la densidad es mucho mayor que la de Santiago".

Tanto en la simulación como en la realidad, Valparaíso es la ciudad con mayores sistemas de conectividad: terminales de buses y el puerto. Cada día de la ciudad porteña salen a Santiago más de 45 buses. Todos a máxima capacidad. "Las ciudades que están más conectadas son las que primero van a caer y las que están en los extremos, las últimas", dice. En este modelo, la última en caer sería Concepción.

> Diez personas tienen que infectarse simultáneamente para que la enfermedad se disperse
> Tomás Pérez-Acle

¿A quién creerle?

Extendida la enfermedad ficticia, el gobierno central ficticio entra en acción. Lo impactante es que, según lo que las autoridades digitales decidan comunicarle a la población, la cantidad de enfermos varía sustancialmente.

Hay tres escenarios posibles: El primero, al entregar un mensaje incorrecto como "no hay epidemia", la cantidad de infectados se dispara.

El segundo, cuando hay mensajes

Sigue en página 30 ›

5) **"Nobel Prize critiscise tendency anti-vaccines"**
Newspaper: El Mercurio de Valparaíso
Date: November 17, 2017
Link: http://www.mercuriovalpo.cl/impresa/2017/11/17/full/cuerpo-principal/3/
Scope: National

PanoraMás. Gepe adelanta su show en el Municipal de Valparaíso

Fútbol. *Págs. 24 y 25* Everton podrá recibir a Colo Colo en Sausalito

Fútbol. *Pág. 26* La historia de la canción que se convirtió en himno caturro

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GUSTAVO ALVARADO

Cruzada política regional para traer foro APEC 2019 a Valparaíso y Viña del Mar

Desarrollo. Alcalde Sharp compromete su apoyo irrestricto, en tanto senadores Lagos Weber (PPD), Walker (DC), Chahuán (RN) y el diputado Urrutia (UDI) presionan a La Moneda desde el Congreso. *Pág. 6*

El porqué del rezago en el presupuesto del FNDR

Estudio. Fundación P!ensa escarbó en nudos de asignación de recursos. *Pág. 4*

Isla de Pascua. *Pág. 2*

Rapa Nui pide intermediación de la ONU para la cesión total del Parque Nacional insular

PREMIO NOBEL CRITICA TENDENCIA ANTIVACUNAS

Genetista Michael Rosbash participa en simposio científico en Valparaíso. *Pág. 3*

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6) **"With this decision, the government is hypothecate the science and to its own knowledge".**
Newspaper: El Mercurio de Valparaíso
Date: October 19, 2017
Link: http://www.mercuriovalpo.cl/impresa/2017/10/19/full/cuerpo-principal/5/
Scope: National

EL MERCURIO
DE VALPARAÍSO

ENTREVISTA. RAMÓN LATORRE, director del CINV, sobre la reducción del 2,2% para la ciencia:

"Con esta decisión el Gobierno está hipotecando el futuro de la ciencia y del conocimiento propio"

Paolo Navia S.
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RAUL GOYCOOLEA

ACADÉMICO CRITICÓ LA REBAJA EN EL PRESUPUESTO 2018 PARA EL DESARROLLO DE LA CIENCIA E INNOVACIÓN.

Como una bofetada en pleno rostro. Así describió Ramón Latorre, Premio Nacional de Ciencias y director del Centro Interdisciplinario de Neurociencia de Valparaíso (CINV), la decisión adoptada por el Gobierno en relación a reducir en un 2,2% los recursos públicos incluidos en el Presupuesto 2018 para el desarrollo de la ciencia, tecnología e innovación (CTI).

La reducción fue tomada como un golpe bajo por parte del mundo científico, más aún si se toma en cuenta que Chile es el país de la Organización para la Cooperación y el Desarrollo Económicos (OCDE) que menos invierte del Producto Interno Bruto (PIB) en ciencia, con sólo el 0,38%.

Ante este escenario, el académico de la Universidad de Valparaíso (UV) fue tajante al señalar que el gobierno de Michelle Bachelet dice una cosa, pero hace otra.

"Acá hay una tremenda diferencia entre lo que se dice y lo que se hace, porque en todos los discursos del Gobierno se habla de la necesidad de crear un país basado en el conocimiento, y cuando se llega al Presupuesto, resulta que no existe fondos para crear tal país", manifestó Latorre.

- Usted dice que existe una contradicción al interior del Ejecutivo en materia científica. ¿Por qué realiza esa afirmación?
- Porque el actual presupuesto destinado para ciencias es de un país subdesarrollado, y a esos recursos, que ya son extremadamente deficientes para echar a andar un país en un mundo moderno, resulta que le recortan el 2%. Entonces, la verdad es que no se entiende cuando se dice que necesitamos conocimiento, que necesitamos un desarrollo de las grandes tecnologías y que necesitamos innovación, pero se recorta un presupuesto que es fundamental para el país.

- ¿Cómo será el impacto que tendrá esta reducción del presupuesto en la escala regional?
- A nosotros, obviamente, nos va a afectar y quizás nos toque una reducción superior al 2%, pero ese no es el tema de fondo, porque al final esto le va a afectar a todo el país, pues si miramos el mundo moderno, no existe ningún país desarrollado que no tenga una ciencia poderosa que permita un desarrollo tecnológico adecuado, ni en Estados Unidos, ni en Europa se da esa situación. Pero nosotros somos un país que se tropieza dos veces con la misma piedra, porque tuvimos el salitre hasta que los alemanes inventaron el sintético, y ahora estamos aún dependiendo del cobre, pero ¿qué pasa si de un día para otro desapareciera por la creación de un sustituto? Por lo tanto, desde la base del conocimiento, ya deberíamos estar ejecutando acciones para enfrentar ese tipo de escenarios.

"Si nosotros como país no producimos conocimiento propio, vamos a seguir dependiendo de las materias brutas, es decir, de vender frutas, vino y cobre, y esto lo hemos dicho una y otra vez, pero las autoridades no nos escuchan"

- ¿Qué tipo de acciones?
- Por ejemplo, los automóviles eléctricos van a utilizar mucho cobre, y por lo tanto, ya deberíamos estar creando fábricas de motores para ese tipo de automóviles, pero para eso se necesita invertir en innovación y conocimientos. Lo mismo con el litio, en donde hace rato tendríamos que estar construyendo baterías.

- Pero ¿existe el potencial científico en Chile para desarrollar este tipo de iniciativas?
- Seguro que sí, pues en Chile tenemos muy buenos ingenieros, y también tenemos gente inteligente y muy capaz, pero esta reducción es como una bofetada en pleno rostro, sobre todo cuando sabemos que dentro del Presupuesto y a raíz de la venta del cobre, las Fuerzas Armadas se van a llevar mil millones de dólares. Entonces, de alguna manera esto da a entender que el país está funcionando de manera inadecuada, y ese es el problema más serio, porque con esta decisión el gobierno está hipotecando el futuro de la ciencia y del conocimiento propio.

- ¿Qué consecuencias puede tener esta decisión en el país?
- Mire, si nosotros como país no producimos conocimiento propio, vamos a seguir dependiendo de las materias brutas, es decir, de vender frutas, vino y cobre, y esto lo hemos dicho una y otra vez, pero las autoridades no nos escuchan, aunque para ser francos, existen personas como el senador Guido Girardi que se la están jugando por apuntar a un sentido y también existe una Comisión del Futuro que todos los días está planteando la necesidad del desarrollo de Chile sobre la base del conocimiento propio, pero al parecer, eso no es suficiente.

- ¿En qué posición quedan las nuevas generaciones de investigadores con esta decisión de reducir el presupuesto para la ciencia?
- Yo creo que se les están cortando las alas, porque acá hay una cadena de reducción presupuestaria. Primero en educación y el recorte a la gratuidad, y ahora en la ciencia, entonces, es como un interés compuesto que da a pensar el tipo de país que estamos creando, con una educación que es muy mala y basada en la posición social, porque la gente con dinero puede mandar a sus hijos a los mejores colegios, y en cambio la gente modesta tiene que hipotecar su futuro. Entonces, la cosa está fallando por todos lados, porque los estudiantes que llegan a primer año de universidad apenas entienden los textos de estudio, y eso algo da a entender.

- ¿Cómo se puede generar una situación que mejore el futuro panorama para el desarrollo de la ciencia en Chile?
- Yo creo que esto va a cambiar cuando el gobierno o el Estado tomen el toro por las astas y acabar con esta falta de consistencia entre lo que se dice y lo que se hace, porque están castigando todos los fondos y programas de desarrollo, inclusive aquellos que representan la base de la ciencia chilena.

7) **"CINV Researcher make an progress in Muscular myopathy studies"**
Newspaper: El Mercurio de Valparaíso
Date: September 16, 2017
Link: http://www.mercuriovalpo.cl/impresa/2017/10/03/full/cuerpo-principal/16/
Scope: National
Note: This article appeared in diferentt kind of Chilean newspapers

EL MERCURIO
DE VALPARAÍSO

Científica del Centro de Neurociencia avanza en estudio de miopatía muscular

VALPARAÍSO. *Doctora Arlek González centra sus estudios en grave patología.*

Desde los laboratorios del Centro Interdisciplinario de Neurociencia, de la Universidad de Valparaíso (CINV), la Dra. Arlek González, investigadora del Instituto Milenio, avanza en la comprensión de las miopatías hereditarias, un conjunto de enfermedades neuromusculares para las cuales no hay cura, y que afectan a alrededor de 6 mil chilenos.

González, bioquímica y oriunda de la ciudad de Los Vilos, descubrió un factor clave en el origen de la miopatía dominante centronuclear, desorden que genera debilidad muscular progresiva en músculos faciales y oculares, así como atrofia irreversible de la musculatura esquelética distal -extremidades-, pudiendo llevar a la invalidez.

Esta enfermedad, que afecta a hombres y mujeres por igual, es causada por diversas mutaciones en un gen encargado de producir una proteína llamada dinamina II, la cual se expresa en distintos tejidos y cumple un rol importante en la formación y funcionamiento de las fibras musculares.

"Los pacientes debutan con la enfermedad en la adultez temprana, alrededor de los 20 años. Los primeros síntomas son debilidad muscular y mucha fatiga. Es una patología progresiva que conlleva a un deterioro paulatino de la función muscular en la que, dependiendo de la severidad con que se manifiesta, los afectados pueden perder la capacidad de caminar, quedando en silla de ruedas", comenta la investigadora.


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DOCTORA ARLEK GONZÁLEZ.

Junto a la Dra. Ana María Cárdenas, investigadora de CINV y quien fuera su tutora de tesis doctoral, la Doctora Arlek González analizó de qué manera estas mutaciones en el gen de dinamina II afectan la función de esta proteína en las fibras musculares.

"En nuestro trabajo observamos que hay una función muy importante que está afectada, relacionada con el tráfico intracelular, es decir, con cómo viajan ciertas proteínas a la membrana de las células. Observamos que en células musculares en las que dinamina II está mutada existe otra proteína, el transportador de glucosa GLUT4, que no está llegando bien a la membrana de la célula y eso no se había descrito anteriormente", comenta.

"Es una patología progresiva que conlleva a un deterioro paulatino de la función muscular"

Arlek González
Científica del CINV

8) " CINV Experter warning: " Sleeping time does not recover it"
Newspaper: El Mercurio de Valparaíso
Date: Agustu 18, 2017
Link: http://www.mercuriovalpo.cl/impresa/2017/08/12/full/cuerpo-principal/8/
Scope: National

EL MERCURIO
DE VALPARAÍSO

Experto del CINV advierte: "La hora de sueño no se recupera"

VALPARAÍSO. *John Ewer advierte sobre el impacto que tendrá el cambio de hora que los chilenos deben hacer esta medianoche y entrega consejos para mitigarlos.*

El impacto que tienen las horas de sueño y vigilia en el cerebro es uno de los temas que el doctor John Ewer maneja a la perfección. Por ello, desde su puesto como investigador del Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso (CINV), se ha elevado como un firme opositor a los cambios de huso horario que enfrentan los chilenos dos veces al año.

Ewer, experto en genética y desarrollo de los sistemas nerviosos, afirma que al adelantar los relojes una hora, lo que se debe realizar esta medianoche (hay que poner la 1 de la madrugada), lejos de ajustar nuestro ciclo laboral con el natural, lo aleja una hora más. A partir de la mañana del domingo, la mayoría de los chilenos tendremos dos horas de diferencia con el huso horario que nos corresponde y estaremos alejados del ciclo que cumple el principal regulador de nuestras respuestas corporales: el sol.

CINV

JOHN EWER ES UN EXPERTO DEL CENTRO DE NEUROCIENCIA PORTEÑO.

"El huso horario en que terminamos es peor que el que tenemos ahora, que ya está equivocado en una hora con respecto a lo que nos corresponde", afirma el especialista que trabaja en los laboratorios del CINV en Valparaíso.

El doctor Ewer explica que con el nuevo horario la salida del sol será más tarde. Como la llegada de la luz del sol regula cuando despertamos en días sin despertador, luego del cambio el despertador nos despertará una hora antes en un día de trabajo, lo cual significa perder una hora de sueño por sobre del déficit de sueño que ya tiene la mayoría de las personas. Esta hora de sueño perdido no se recupera, advierte Ewer, sino que uno no se "acostumbra" a este cambio de horario y su efecto no desapa rece hasta que el sol se levante más temprano, lo cual ocurre naturalmente a medida que nos acercamos al verano.

PARA MITIGAR IMPACTO

Como no hay solución, porque adelantar los relojes es una ley que se debe cumplir, el doctor Ewer plantea algunas medidas capaces de mitigar el daño. "Se recomienda simplemente to mar precauciones, andar con cuidado cuando se desplacen por las calles, idealmente no manejar y no dictar asignatu ras complicadas durante las primeras horas del día, ade más de no realizar tareas o ac ciones que requieran de mu cha atención en la mañana".

En otras palabras, dijo Ewer, sólo se puede minimi zar el daño, pero "hablamos de un daño completamente evitable si cambiaran el hora rio de Chile", es decir, si los re lojes retrocedieran una hora en vez de adelantarse hoy a la medianoche.