ABSTRACT'S BOOK

RED DE EXCELENCIA SICI CONSOLIDER International Workshop

El Rancho de la Aldegüela (Torrecaballeros, Segovia) October, 20th-22nd, 2016

Supported by:

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

International workshop

RED DE EXCELENCIA SICI CONSOLIDER

El Rancho de la Aldegüela (Torrecaballeros; Segovia)
October, 20th-22nd, 2016

PROGRAMME

Thursday, October 20th

18:00-18:15 **Welcome by the Organizing Committee**

18:15-19:15 **Keynote Lecture: Pierre Paoletti** (Institut de Biologie de l'ENS- IBENS- Paris-France)
"NMDA receptors: from molecular engineering to synaptic regulation"

20:30 **Welcome dinner**

Friday, October 21st

9:30-11:30 **Session I. Ion Channels in Physiology**
Chairman: Antonio Ferrer Montiel (UMH- Elche)
Speakers:
- John Wesseling (CIMA- Pamplona) *"NMDARs in electrogenesis: A new hypothesis for the special role of NMDARs in neurodegeneration."*
- **Rafael Luján** (Univ. Castilla La Mancha) *"Subcellular localization of ion channels and associated proteins along the neuronal surface"*
- **Antonio Felipe** (Universidad de Barcelona)*"Molecular interactions shaping the functional Kv1.3 channelosome"*
- **José A. Lamas** (Universidad de Vigo)*"Modulation of potassium TREK-channels by bradykinin".*

11:30-12:00 **Poster session and coffee break**

12:00-14:00 **Session II. Ion channels in disease**
Chairman: Rosario González Muñiz (IQM-Madrid)
Speakers:
- **Xavier Altafaj** (Idibell- Barcelona) *"Rett-like severe encephalopathy caused by a de novo GRIN2B mutation is attenuated by D-serine dietary supplement"*
- **Alvaro Villarroel** (Instituto Biofisika CSIC-Bilbao) *"The assembly domain couples calmodulin binding and PIP2 regulation of Kv7.2 channels"*
- **Diego Alvarez de la Rosa** (Universidad de la Laguna-Tenerife) *"Ion channels and vascular dysfunction in chronic kidney disease"*
- **Luis Carlos Barrio** (Hospital Ramón y Cajal- Madrid) *"Neuronal connexin-36 protects against sudden infant death syndrome"*

14:00-15:30 **Lunch**

15:30-17:30 **Session III. Structure-function Ion channels**

Chairman: *Pilar de la Peña (Universidad de Oviedo)*

Speakers:

- **Antonio Alcaraz** (Universidad Jaume I-Castellón) *"Fluctuation-driven transport in bacterial channels under acidic stress"*
- **Oscar Millet** (CIC Biogune- Bilbao) *"Structural basis for the calmodulin mediated Ca2+ inhibition of the Kv7.2 channel"*
- **Teresa Giráldez** (Universidad La Laguna- Tenerife) **"***Using unnatural aminoacids to probe the molecular basis of BK channel modulation by Ca2+"*
- **Alfonso Martínez de la Cruz** (CIC Biogune- Bilbao) *"Structural basis of the oncogenic interaction of PRL-1 with the Magnesium transporter CNNM2"*

17:30-18:00 **Poster session and coffee break**

18:00-19:30 **Oral communications (15min.each)**

Chairman: *Asia Fernández Carvajal (UMH- Elche)*

- **Alex Perálvarez** (Universidad Autónoma de Barcelona) **"***Interactomic profiles reveal neuropathological implications for TRPV2 channel."*
- **Mar Orzaez** (CIPF-Valencia)*"The Bax transmembrane domain interacts with pro-survival Bcl-2 proteins in biological membranes"*
- **Felipe Ortega** (Universidad Complutense de Madrid) **"***Unravelling the mechanisms controlling neurogenic and oligodendrogliogenic lineages in the adult subependymal zone."*
- **Sonia Marco** (CIMA- Pamplona)*"Silencing GluN3A-containing NMDA receptors to treat Huntington´s disease"*
- **Katarzyna Styrczewska** (Universidad de Barcelona) *"Poliubiquitination mediates the voltage-gated potassium channel Kv1.3 endocytosis"*
- **Rosa Gómez-Villafuertes** (Universidad Complutense de Madrid) *"Upregulation of ionotropic P2X7 receptor facilitates the survival of neuroblastoma cells under limiting growth conditions: implication of PI3K/Akt signaling pathway."*

21:00 **Dinner at Rancho de la Aldegüela**

Saturday, October 22nd

9:30-11:30 **Session IV. Ion Channel drug discovery**

Chairman: Angel Messeguer (IQAC- Barcelona)

Speakers:

-**Mª Jesús Pérez de Vega** (IQM-Madrid) *"Chalcones and Chalconoids: New Allosteric Modulators of the α7 Nicotinic Acetylcholine Receptor (α7 nAChR)"*

- **Ricardo Borges** (Universidad de la Laguna-Tenerife) *"The vesicular transporter of nucleotides its role in the accumulation of neurotransmitters."*

-**Antonio Rodríguez Artalejo** (Universidad Complutense de Madrid) *"Alpha 2 adrenergic and P2X3 receptors from adrenomedullary chromaffin cells as novel targets for the treatment of neuropathic pain"*

-**Andrés Morales** (Universidad de Alicante) *"Deciphering the complex modulation of nAChRs by local anesthetics: looking for modulating binding sites"*

11:30-12:00 **Poster session and coffee break**

12:00-13:00 **Keynote Lecture: Juan Tamargo** (Universidad Complutense de Madrid)
"Translational Research on Cardiac Arrhytmias secondary to Channelopathies (The ITACA Consortium)"

13:00-13:30 **Concluding remarks and farewell**

13:30-14:00 **SICI Network meeting**

14:00-15:00 **Lunch**

Organized by:

Sponsored by:

Organizing contact: Ms. Raquel Jorquera (rjorquera@umh.es)

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KEYNOTE LECTURES

Keynote Lecture 1:
'NMDA receptors: from molecular engineering to synaptic regulation'

Pierre Paoletti
IBENS, ENS, Paris

The function of the human brain and its capacity for experience-dependent change hinges on the dynamics of chemical synapses. My team has a long-standing interest in studying the molecular principles underpinning the structure and function of chemical synapses. Our research focuses on NMDA receptors (NMDARs), a family of glutamate-gated ion channel receptors that are essential mediators of excitatory neurotransmission and synaptic plasticity. NMDARs have also been implicated in a plethora of neuropathological conditions thus receiving strong interest as potential therapeutic targets. Recent years have witnessed major progress in our understanding of the structure, mechanisms and regulation of NMDARs, with highlights including the decoding of the first full-length receptor crystal structures. Studies from our team have also contributed to the emerging view that NMDARs are particularly complex molecular machines, endowed with unique allosteric capacity and exquisitely sensitive to their native microenvironment. I will present the key role of the large extracellular N-terminal domains (NTDs) in the subunit-specific regulation of receptor function and how the allosteric capacity of these domains is intimately linked to their high structural dynamics. I will also present results obtained with genetically-modified mice showing how endogenous brain modulators, such as zinc ions, interact with specific NMDAR NTDs and populations to regulate excitatory synapses and behavior. I will conclude by showing our latest developments in optogenetic pharmacology with the design of optically-controlled NMDAR subunits for precise manipulation and interrogation of receptor structure and function.

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Keynote Lecture 2: Translational Research on Cardiac arrhythmias secondary to Channelopathies (The ITACA Consortium)

Juan Tamargo
Department of Pharmacology, School of Medicine, University Complutense of Madrid, Spain

The ITACA Consortium was created and coordinate the basic and clinical research of the Services of Cardiac Electrophysiology and Cardiology of seven University Clinical Hospitals of Madrid (La Paz, Ramón y Cajal, Puerta de Hierro, 12 de Octubre, Clínico San Carlos, Gregorio Marañón and Getafe), NIMGenetics (New Integral Medical Genetics) and the Laboratory of Cardiac Electrophysiology (Department of Pharmacology, School of Medicine, University Complutense of Madrid). The main aims of the ITACA Consortium are:

1. To recruit patients (probands) and their immediate relatives at these Hospitals with the diagnosis of a primary arrhythmogenic syndromes (PASs) in whom we suspect that the arrhythmia is caused by a mutation in genes encoding a constitutive protein of a cardiac ion channel or that participate in the channelosome, transcription factors and scaffold proteins. The large sample size (around 5 million people) ensures the identification of probands even when the prevalence of some of the primary arrhythmogenic syndromes is low.
2. To perform functional electrophysiological studies of the mutations. In my talk I shall present the most important findings of the ITACA Consortium, including the identification of new genes and their role in the control of cardiac electrophysiology.
3. To create a collection of blood samples of the probands and their immediate relatives who meet all the requirements stated in the Biomedical Research Act (14/2007).
4. To create a Registry of patients with the diagnosis of PASs. The database developed by the services of Cardiology of the Hospitals Puerta de Hierro and 12 de Octubre will allow future clinical and epidemiological research on the PASs.

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ORAL COMMUNICATIONS

Oral 1

NMDARs in electrogenesis: A new hypothesis for the special role of NMDARs in neurodegeneration

John F. Wesseling, Kashif Mahfooz, Sonia Marco, Rebeca Martínez-Turrillas, Mathan K. Raja, and Isabel Pérez-Otaño

KEY WORDS: NMDA; NMDA receptors; upstate; NMDA spike; Huntington's disease; HD

NMDA receptors (NMDARs) are ligand-gated ion channels than are also gated by voltage in some situations because of the near complete blockade by Mg^{2+} at resting membrane potentials. Some dendrites express them at high enough density to fire regenerative electrogenic events known as NMDA spikes. NMDA spikes induce a heavy intracellular Ca^{2+} load, and Ca^{2+} influx through NMDARs has long been thought to be involved in a variety of neurodegenerative diseases. I will discuss our recent work showing that: the neurons that are selectively vulnerable in Huntington's disease (HD) can fire exceptionally large NMDA spikes; that the threshold is effectively reduced several fold in a mouse model of HD; that the reduction in threshold depends on the aberrant expression of the GluN3A subunit of NMDARs; and that HD signs and symptoms can be prevented by suppressing the aberrant expression. Moreover, memantine - a medicine used to treat Alzheimer's disease (AD) and in clinical trials for HD - could return the threshold to control levels. The results are all in-line with the possibility that NMDA spikes may play an important role in a range of neurodegenerative diseases.

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Oral 2
Two- and three-dimensional subcellular localization of ion channels and associated proteins along the neuronal surface

Rafael Luján

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KEY WORDS: electron microscopy, hippocampus, cerebellum, Alzheimer, GABAB receptors, AMPA, NMDA

One of the main goals in neurosciences has been to determine the precise subcellular localization of neurotransmitter receptors and ion channels in the brain, as a crucial step for the understanding of their function. In the last decade, considerable effort has been dedicated to define immunohistochemical methods that accurately estimate the density or proportion of those signalling molecules in different subcellular compartments, as well as the changes that take place during the course of normal life and under pathological. In the central nervous system, a number of neurotransmitter receptors and ion channels have been localised relying on the analysis of single sections, but very little is known about their two- and three-dimensional organization. This gap in our knowledge seriously hampers our understanding of neuronal signalling mechanisms. Now, two revolutionary immunoelectron microscopy techniques have been developed: FIB/SEM combined with pre-embedding immunogold (FIB/SEM immunogold) and the SDS-FRL technique. FIB/SEM allows obtaining long series of ultrastructural images that allows providing three-dimensional information. The SDS-FRL technique allows localising proteins on the whole surface of neurons in a two-dimensional fashion. Using these techniques we have found that $GABA_B$ colocalise with GIRK2 and Cav2.1 in a compartment-dependent manner both at pre- and post-synaptic sites. At presynaptic sites, $GABA_B$ colocalise with Cav2.1 along the active zone of axon terminals, whereas colocalise with GIRK2 at the edge of the active zone. We also studied possible changes of receptors and ion channels in pathological conditions like Alzheimer´s disease. For instance, in control animals, we detected a large density of immunoparticles for AMPA, NMDA or $GABA_{B1}$ along the surface of dendritic shafts of pyramidal cells, distributed clustered. However, in APP/PS1 mice, we detected a significant reduction in the number and density of immunoparticles for the three proteins along the neuronal surface, suggesting that alteration of receptors/ion channels in Alzheimer´s disease is taking place both at synaptic sites and at extrasynaptic sites. In summary, the two techniques represent a major advance in the study of the microanatomy of the brain, opening up new horizons and opportunities to unravel its complexity in normal and pathological conditions.

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Oral 3

Molecular interactions shaping the functional Kv1.3 channelosome

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KEY WORDS: Channelosome, traffic, heterooligomers, association, regulation.

The idea of ion channels as a simple association of conducting subunits located at the cell membrane is over. Functional voltage dependent potassium channels are a complex of heterooligomeric associations which control channel activity. For the last 25 years, the knowledge of the physiological role of voltage-dependent potassium channels (Kv) in the immune system has grown exponentially. Leukocytes express a limited repertoire of Kv channels, which contribute to the membrane potential. These proteins are involved in the immune response and are therefore considered good pharmacological targets. Leukocyte channels participate in divergent physiological functions such as activation proliferation and apoptosis, further adding complexity to the knowledge of their physiology. Although there is a clear consensus about the physiological relevance of Kv1.3, the expression and the role of Kv1.5 are controversial. Recent reports from our lab indicate that certain heteromeric Kv1.3/Kv1.5 associations may provide insight on Kv1.5. In addition, the presence of other unknown interactions with ancillary subunits (Kv
kinases (ERK1/2) and scaffolding proteins (caveolin 1) which influence the channel activity as well as the location of the functional complex, named channelosome, may shape the ion channel function. Here, we summarize what is known about this issue and highlight the role of Kv1.5 and other partnership interactions that could be responsible for this debate. The Kv1.3/Kv1.5 heterotetrameric composition of the channel and their possible differential associations with accessory modulatory proteins warrant further investigation.

Supported by the Ministerio de Economía y Competitividad (MINECO), Spain (BFU2014-54928-R and BFU2015-70067-REDC) and Fondo Europeo de Desarrollo Regional (FEDER).

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Oral 4
Modulation of potassium TREK-channels by bradykinin

Lamas JA, *Rivas-Ramírez P, Conesa-Buendía F, Rueda L, Herrera-Pérez S, Guillán-Fresco,M, Reboreda A*
Centro de Investigación Biomédica (CINBIO). Universidad de Vigo.

KEY WORDS: Bradykinin, TREK channels, sympathetic neurons, PIP2, SCG

Bradykinin (BK) is a small peptide with a significant role in vasodilatation, pain and inflammation after tissue injury. This peptide has been shown to produce an increase in excitability, by depolarizing the membrane potential and reducing adaptation, in different neuronal types. In sympathetic neurons of the superior cervical ganglion (SCG), this increase in excitability has been ascribed to the inhibition of the potassium M-current through B2 receptors, Gq proteins, PLC, calcium increase and binding to calmodulin. Recently we have shown that two pore domain potassium channels (K2P) of the TREK subfamily can be modulated by muscarinic agonists in mouse SCG neurons, much like it was demonstrated for the M-current before, and it was tempting to speculate that these background K2P channels can also be modulated by BK.

The main objectives of this work were to investigate whether bradykinin can modulate the activity of TREK channels and if so, to characterize the second messenger pathway involved.

We cultured SCG neurons from young adult mice and used the whole-cell perforated patch and cell-attached single channel recording to study the effect of BK on the riluzole activated current (mainly TREK-2 current) and on the current through TREK-2 single channels. Drugs were applied to a continuous perifusion system.

We reported before that, with the appropiate protocol (clamping the neuron at -30 mV and adding several ion channel blockers), the application of riluzole to SCG neurons induces an outward current (I_{RIL}) carried out mainly through TREK-2 channels. Bath application of BK (250-750 nM) induced a strong reduction of TREK-2 single channel NPo and a 45% reduction of I_{RIL}. The inhibition of I_{RIL} was completely abolished by pretreatment with HOE140 indicating an effect through B2 receptors. The application of bisindolylmaleimide did not affect the inhibition of I_{RIL} by BK, indicating that PKC is not involved. Similar results were found when blocking IP3 receptors with 2-APB. However when PIP2 levels were kept high, applying PIP2 coupled to a histone carrier, BK was unable to inhibit I_{RIL}. Our results demonstrate that bradikinin inhibits TREK-2 potassium channels through a reduction of PIP2 in sympathetic neurons.

AKNOWLEGMENTS: MICINN/MINECO: CSD2008-00005-CONSOLIDER; BFU2011-25371; BFU2014-58999-P; BFU2015-70067-REDC. Xunta de Galicia: INBIOMED-CINBIO CN2012/273; INB1-131H-2; GPC2015/022.
European Commission: FP7-316265-BIOCAPS. Fondos FEDER.

Oral 5

Rett-like severe encephalopathy caused by a *de novo GRIN2B* mutation is attenuated by D-serine dietary supplement

Soto D, Olivella M, Alcón C, Grau C, Gómez de Salazar M, Gratacòs-Batlle E, Ramos-Vicente D, Ciruela F, Bayés À, Sindreu C, López-Sala A, Armstrong J, García-Cazorla À, **Altafaj X**

KEY WORDS: NMDAR, *de novo* mutation, Severe Encephalopathy, D-serine.

Ethyl D-Aspartate subfamily of glutamate ionotropic receptors (NMDARs) are activated during fast excitatory transmission and they have been proved to be key elements in synaptic plasticity, synaptogenesis and neuron survival. Several genetic studies have identified '*de novo*' NMDAR mutations in patients with neurodevelopmental diseases (including severe encephalopathies, autism, intellectual disability) as well as psychiatric disorders. In this work we report a case study of a 4 years-old Rett-like patient with a severe encephalopathy. The genetic studies of this patient (WES and Sanger sequencing) showed the presence of a missense de novo mutation of GRIN2B(p.P553T) coding for the GluN2B subunit of NMDARs. Given the key role of GluN2B subunit in the very early stages of synaptogenesis, we hypothesized that this mutation could be leading to neuronal dysfunction and, subsequently, its normalization would potentially ameliorate the patient's symptomatology. In heterologous expression systems, GluN2B(P553T) mutant construct do neither affected NMDAR oligomerization nor their surface expression in primary neuronal cultures. However, electrophysiological studies showed that although functional, the mutant receptor displayed a significantly reduced channel conductance concomitant with a strong reduction of NMDA-evoked current density.

These data are in agreement with our structural molecular model, and strongly suggest the hypo-functionality of mutant NMDARs that, potentially, could be rescued throughout the enhancement of their activity. In accordance with this hypothesis, in vitro administration of D-serine, a physiological NMDAR co-agonist, displayed a significant increase of NMDA-evoked currents of mutant receptors. Next, a clinical trial with dietary supplement of D-serine was performed. Importantly, after nine-months dietary supplement of D-serine, the patient showed an increase of serine plasma levels, together with a noteworthy clinical improvement. Our results show the possibility to enhance the hypo- functionality of glutamatergic transmission as a therapeutic approach to attenuate cognitive and motor impairment in early childhood.

This work has been supported by Grants PI13/00135 (ISCIII), La Marató (Project N. 20140210), PCIN-2014-105 (MINECO) and Miguel Servet (CP10/00548) to XA; BFU2014-57562-P (MICINN) to DS; PI15/01082 (ISCIII) to AGC; BFU2012-34398 and BFU2015-69717-P (MINECO), Career Integration Grant (ref. 304111), Marie Curie Intra-European Fellowship (ref. 221540), Ramón y Cajal Fellowship (RYC-2011-08391p) to AB; SAF2012-40102 (MINECO/FEDER), FP7 Marie Curie CIG grant (#631035) and Ramón y Cajal (RYC-2011-08026) to CS. CA received a FPI contract (MINECO). We thank the "Medicinal Computational Laboratory" from Universitat Autònoma de Barcelona for providing computing facilities. XA, CS and AB also benefit from the financial support of AGAUR (SGR14-297).

Oral 6
The assembly domain couples calmodulin binding and PIP_2 regulation of Kv7.2 channels

Araitz Alberdi, Carolina Gomis-Perez, Ganeko Bernardo-Seisdedos, Alessandro Alaimo, Covadonga Malo, Pilar Areso and ***Alvaro Villarroel***

KEY WORDS: Coiled-coil, Leucine zipper, Calmodulin, PIP2, KCNQ, Epilepsy, M-current, Allosteric

Phosphatidylinositol(4,5)bisphosphate (PIP2) is a minor (<1%) acidic phospholipid found in the inner leaflet of the cell membrane and plays a vital part in cellular signaling by directly interacting with membrane proteins, including Kv7 potassium channels whose function is absolutely dependent on this lipid. Activation of phospholipase C, and subsequent PIP2 hydrolysis causes downregulation of Kv7 activity, which in turn lowers the threshold for excitability in brain, heart, skeletal muscle and inner ear. Calmodulin (CaM) binds to the AB site C-terminus and its calcium-independent regulatory mechanisms proceed through an effect on sensitivity to PIP2. Following the AB site, the distal part (helix D) directs oligomerization and partner specificity. We have identified a mechanism to modulate, at a distance, regulation by PIP2 from helix D that propagates to CaM binding to helices AB. We monitored the relative distance/orientation of the ABCD domain by live-cell fluorescence resonance energy transfer (FRET) assays in conjunction with analysis of whole-cell currents. The observations we made explain how the tetrameric conformation of helix D can functionally influence CaM binding to a distal site, and, in turn, how a pathological mutation affecting tetramer stability modifies Kv7.2 activity.

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Oral 7
Ion channels and vascular dysfunction in chronic kidney disease

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Patients with chronic kidney disease (CKD) have a markedly increased incidence of cardiovascular events and cardiovascular disease (CVD) mortality compared with the age-matched general population. The high concentration of circulating uremic toxins in CDK patients leads to vascular inflammatory responses, thereby inducing endothelial dysfunction, which is associated with CVD development and progression. In addition, uremic patients have a significant vascular damage characterized by excess artery calcification due to osteogenic transformation of vascular smooth muscle cells. Plasma aldosterone levels are increased in CKD, and aldosterone has been found to increase vascular inflammation and fibrosis. The aim of our study was to analyze the influence of CKD in the expression of endothelial and vascular smooth muscle ion channels and its potential modification by inhibiting the aldosterone receptor (mineralocorticoid receptor, MR), which would consequently affect endothelial and smooth muscle function.

To that end we used primary human aorta smooth muscle cells (HASMC) or endothelial cells (HAEC) cultured in medium supplemented with pooled sera from either healthy or uremic patients undergoing dialysis. To obtain a complete profile of ion channel subunit expression in both cellular types, we performed high-throughput qPCR of 92 ion channel genes using a custom-designed Taqman low-density array card. In addition, we characterized the influence of uremic serum on HASMC calcification and differentiation to an osteogenic phenotype. We also determined the effect of uremia on HAEC nitric oxide (NO) production and we measured cortical stiffness using atomic force microscopy (AFM).

Our data show that:

- Exposure of HASMC to uremic serum induced the expected calcification and osteogenic transformation.
- Uremic serum induced remodeling of ion channel subunit expression in HASMC, including up-regulation of Kv1.3 and the α subunit of epithelial sodium channel (αENaC).
- Functional recordings of K^+ channel function in HASMC using patch-clamp in the whole-cell
- Configuration showed decreased BK and Kv1 currents, with an increase in the proportion of Kv1 current carried by Kv1.3. We are currently testing the effects of Kv1.3 inhibition on uremia-induced changes in cell proliferation and migration.
- Exposure of HAEC to uremic serum induced decreased NO production.
- Uremia increased HAEC cortical stiffness, which was prevented by inhibition of MR using spironolactone.
- In HAEC, uremic serum increased expression of αENaC, a known transcriptional target of MR that promotes cortical stiffness and endothelial dysfunction.

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Oral 8
NEURONAL CONNEXIN-36 PROTECTS AGAINST SUDDEN INFANT DEATH SYNDROME

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KEYWORDS: respiratory pacemaker, central chemoreception, hypoxia, hypercapnia, cardiorespiratory failure, SIDS

Sudden Infant Death Syndrome (SIDS) is the principal cause of postneonatal death in our environment. However, our current understanding of the etiology and pathogenic mechanisms of SIDS is insufficient for developing effective therapies. SIDS has been highlighted within the disorders that it is though to result from central deficits in the control of breathing and autonomic regulation of heart rate. The intense efforts to better understand the etiology of SIDS has led to the development of a "triple risk model" involving (1) a vulnerable infant; (2) a critical period of development in homeostatic control; and (3) an exogenous stressor. Connexin-36 (Cx36), the principal component of intercellular channels that form the electrical synapses, is present in the specific neural populations of brainstem region involving in the generation of breathing rhythm, central chemoreception and cardiorespiratory coordination, and its expression is unregulated during this critical period of life. Accordingly, we hypothesized that Cx36 would be a key element in the pathogenesis of SIDS. To address this issue we have studied how the genetic suppression of Cx36 expression affects to respiratory rythmicity, chemoreflexes to hypoxia and hypercapnia, and risk for SIDS. Most of mice lacking Cx36 like control wild-type already showed at P14 age a eupneic breathing but with slower respiratory rate and greater rhythm variability; in addition a small portion of Cx36-KO pups (~20%) exhibited Cheyne-Stokes respiration. Cardiorespiratory coordination was also affected: Cx36-KO animals showed less respiratory sinus arrhythmia and higher cardiorespiratory phase synchronization. Cx36-KO mice in comparison with control responded to hypoxia (10% O_2) and hypercapnia (2%, 4% and 8% CO_2) with an abnormal enhanced ventilatory effort. According to the "triple risk model", all these intrinsic abnormalities in respiratory rythmicity, central chemosensitivity and cardio-respiratory coupling found in Cx36-KO mice at P14 may increase SIDS vulnerability. Thus, the risk of SIDS was evaluated by using as exogenous stressor a combination of low O_2 and high CO_2 content in inspired air, as happens when infant is in the prone sleeping position and re-breaths exhaled air; under these conditions of hypoxia-hypercapnia all wild-type mice survived while the 40% of Cx36-KO animals succumbed by respiratory parade.

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Oral 9
Fluctuation-driven transport in bacterial channels under acidic stress

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KEY WORDS: Stochastic pumping, non-equilibrium fluctuations, bacterial channels, pH homeostasis

Thermodynamic arguments assure that free energy transduction cannot be obtained from equilibrium fluctuations. However, a net flux of energy could appear when external oscillating fields couple to internal conformational fluctuations contributing to the maintenance of a non-equilibrium state. This constitutes a general design principle for implementing engineering applications like separation processes and energy conversion. Living cells provide an ideal environment for fluctuation-driven energy transfer. For instance, significant transient changes in voltage (~ 100 mV) are found in the vicinity of protein ion channels undergoing transitions between different states. Specifically, ratchet mechanisms observed in biochannels are based on the fact that for one voltage polarity the force required to move the ions through the pore is smaller than under the opposite polarity. Thus, when the electric voltage across the membrane fluctuates with a zero mean, a net flow of ions (the so-called stochastic pumping) can be observed.
Previous studies showed that OmpF porin, a non-specific wide channel found in the outer membrane of Escherichia coli (E. coli), may perform as a molecular ratchet using diverse mechanisms. For instance, solution acidity can act as an external modulator so that by the selective titration of the protein residues the pore conductive properties can be tuned at will, from almost ohmic conduction to a bipolar diode resembling the solid state p-n junctions. Unfortunately, the extreme pH conditions (for instance pH_{cis} = 3 / pH_{trans} = 12) required to obtain substantial rectification put into question not only its physiological relevance but also the potential use of such nanofluidic diode in technological applications.

In the present study we investigate how rectifying conductive channels can be achieved under conditions more similar to that met in vivo. To this end, only moderate pH gradients are considered and asymmetrically charged lipid bilayers are used to mimic the effects of acidic stress on the membrane properties. Former approaches stressed that enzymes can capture and transmit free energy from oscillating electric fields, as in the case of active transport of Rb^+ by way of the Na^+, K^+ -ATPase. We show that zero-average electric potentials similar to those actually measured in E. coli can be used to obtain electrical pumping of ions against an external concentration gradient without the need of countertransport of other charged species. The novelty of the present approach lies in the nature of both the voltage oscillations and the mechanism of pumping. We consider different kinds of colored noise (noise signal whose power spectrum is not flat) including biologically relevant Lorentzian one (Random Telegraph noise) as opposed to square or sinusoidal waves used in previous studies. Additionally, we show that the uphill transport of ions obtained in OmpF channel under acidic stress is directional. Depending on which side of the membrane is placed the diluted solution, the system is able to pump either cations or anions against their concentration gradient. We discuss the potential implications of this phenomenon in the regulatory mechanisms of bacterial cells against acidic stress.

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MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

RED DE EXCELENCIA SICI CONSOLIDER

Oral 10

Structural basis for the calmodulin mediated Ca^{2+} inhibition of the Kv7.2 channel.

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KEY WORDS: KCNQ2, potassium channels, allosterism, calcium gating, protein structure and dynamics.

Kv7.2 channel is the main component of the non-inactivating K^+ M-current, a key controller of neuronal excitability. It has been hypothesized that the simultaneous binding of calmodulin to helices A and B mediates the Ca^{2+} inhibition of the Kv7.x channel family [1]. In here, using NMR spectroscopy we have resolved the structure of the cytosolic arms of Kv7.2 (helices A and B) in complex with calmodulin (30.3 kDa). The resulting structure is in thermodynamic equilibrium with calcium and shows ion binding only in the N-lobe of calmodulin, consistent with the calcium loading state observed by X-ray crystallography in a homologous channel. Calcium saturation and/or calcium depletion results in little structural changes but significantly alters the microsecond-millisecond time scale dynamics of the complex. Collective analysis of such motions underline an allosteric rearrangement in the complex and provide an unprecedented structural model for the calcium-mediated gating of the channel.

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MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

Oral 11

USING UNNATURAL AMINOACIDS TO PROBE THE MOLECULAR BASIS OF BK CHANNEL MODULATION BY Ca^{2+}

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BK channels are homotetrameric high conductance potassium channels involved in essential physiological processes such as synapse function regulation and smooth muscle tone maintenance. BK channel function is synergistically regulated by voltage and Ca^{2+}. Ca^{2+} binds to a specific cytosolic structure called the gating-ring, where it induces conformational rearrangements ultimately increasing K^+ conductance. The specific structural rearrangements involved in the mechanism coupling Ca^{2+}-binding to pore opening remain unknown. It has been proposed that the length of the linker between the last transmembrane domain (S6) and the cytosolic domain RCK1 (Regulator of Conductance for K^+_1) is determinant for Ca^{2+} signal transduction (*Niu et al., 2004*). We have now focused on the study of this mechanism taking advantage of a novel approach based on the incorporation of the photoactivatable unnatural aminoacid (UAA), BzF. When irradiated with UV light (≈365 nm), BzF forms covalent bonds with its near environment, locking protein conformations (*Klippenstein et al., 2014*). We have now incorporated the UAA BzF into one of our FRETable BK channels constructs (BK667; *Giraldez et al., 2005*), which we previously showed to sense large rearrangements of the gating-ring after Ca^{2+}-binding *(Miranda et al., 2013; Miranda et al., 2016)*. Using the "genetic method" (*Ye et al., 2008*), we have introduced the UAA in four selected positions: Tyr332, Tyr336, His379 and Phe395 within the linker S6-RCK1. We will show that after BzF insertion into BK, channels are correctly expressed and inserted as full and functional proteins at the plasma membrane comparable to wild-type. Experiments assessing the effect of UV light on reconstituted UAA-BK667 channels are currently underway.

***References**: Niu et al, 2004, Neuron 42:745; Klippenstein et al, 2014, J. Neurosci 34:980; Giraldez et al, 2005, J Gen Physiol 126:429; Miranda et al, 2013, PNAS 110:5217; Miranda et al, 2016, PNAS, in press*

MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

Oral 12

Structural basis of the oncogenic interaction of PRL-1 with the Magnesium transporter CNNM2

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KEY WORDS: Magnesium transporter, phosphatase, cancer, CNNM, PRL

Phosphatases of regenerating liver (PRLs), the most oncogenic of all protein tyrosine phosphatases (PTPs), play a critical role in metastatic progression of cancers. Recent findings established a new paradigm by uncovering that their association with magnesium transporters of the Cyclin M (CNNM) family causes intracellular magnesium levels that promotes oncogenic transformation. On the other hand, it has recently been highlighted hitherto unknown essential roles of the CNNM family in regulation of the circadian rhythm, and reproduction. Here, we describe the crystal structure of PRL-1 in complex with the Bateman module of CNNM2 (CNNM2BAT), which consists of two cystathionine ß- synthase (CBS) domains (IPR000664) and represents an intracellular regulatory module of the transporter. The data presented herein shed new light on the structural basis underlying the molecular mechanism by which PRL-1, upon binding to CNNM2, increases the intracellular concentration of Mg2+ thereby contributing to tumor progression and metastasis. The availability of this structure sets the basis for the rational design of compounds modulating PRL-1 and CNNM2 activities.

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MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

RED DE EXCELENCIA SICI CONSOLIDER

Oral 13
Interactomic profiles reveal neuropathological implications for TRPV2 channel.

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KEY WORDS: Interactomics, Bioinformatics, Ion Channels, TRP channels, TRPV2, neuropathology, protein-protein interaction

Knowledge about TRPs function is increasing, but most part of the molecular mechanisms by which they exert their function and regulate their activity are still unknown. For instance, TRPV2 is one of the least understood TRP channels.
Structurally TRPV2 is arranged as a homotetramer in the plasma membrane, as shown by its 3D structure that has been recently solved (6, 7). TRPV2 activity is modulated by several molecular processes such as calmodulin binding, lipid binding, trafficking to the plasma membrane or phosphorylation [3].
TRPV2 is present in most human tissues [3], showing higher expression levels in the CNS(13, 14), where it plays a role in somatosensation [3]. TRPV2 is also implicated in nervous system development, where it promotes axon outgrowth upon mechanical stimulation [6]. Furthermore, TRPV2 has been associated with pathological conditions such as muscular dystrophy, cardiomyopathies [7] or cancer [8].
TRPV2 known interactors network is a small set of proteins, most of them shared with other TRPV members.From literature mining we have identified two interactions for TRPV1 (Snapin25 and Synaptotagmin IX), which we have validated and mapped further for TRPV2. To expand the TRPV2 interactome in CNS we have performed a membrane yeast two hybrid (MYTH) screening and identified 20 new potential interactors of TRPV2. Bioinformatic analysis of TRPV2 interactome shows high association with neuronal development and lipid metabolism.
This study establishes the first interactomics approach for TRPV2 to define regulatory pathways for this channel in the central nervous system (CNS).

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MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

RED DE EXCELENCIA SICI CONSOLIDER

Oral 14

The Bax transmembrane domain interacts with pro-survival Bcl-2 proteins in biological membranes

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KEY WORDS: Apoptosis; Bcl-2, mitochondrial membrane permeabilization

Background: The Bcl-2 protein Bax can commit cells to apoptosis via the formation of pores in the mitochondrial outer membrane. Bax activity is controlled in healthy cells by pro-survival Bcl-2 proteins. Interestingly, C-terminal Bax transmembrane domain interactions have been implicated recently in Bax pore formation (1-3) but the role of TMD interactions on apoptosis modulation has not been addressed.

Objectives: The objective of this work was to study the interactions between the Bax TMD and the corresponding segments of the anti-apoptotic proteins Bcl-2 and Bcl-xL in order to elucidate their relevance for the modulation of mitochondrial apoptosis signaling.

Material and Methods: TMD oligomerization was monitored using the ToxRed system in bacteria. The studies of TMD oligomerization in eukaryotic cells were performed using Bimolecular Fluorescence Complementation assays. To assess apoptosis induction, cytochrome c release from mitochondria and caspase-3 activity were measured.

Results: Isolated transmembrane domains of Bax, Bcl-xL, and Bcl-2 mediated interactions between Bax and pro-survival proteins inside the membrane in the absence of apoptotic stimuli. These interactions also participate in the modulation of apoptotic activity.

Conclusion: Our results suggest that interactions between the Bax transmembrane domain and anti-apoptotic Bcl-2 proteins represent a previously unappreciated level of apoptosis regulation.

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MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

RED DE EXCELENCIA SICI CONSOLIDER

Oral 15

Unravelling the mechanisms controlling neurogenic and oligodendrogliogenic lineages in the adult subependymal zone.

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The adult mouse subependymal zone (SEZ) harbors neural stem cells (aNSCs) that give rise to neuronal and oligodendroglial progeny throughout the entire murine life. However, despite the extensive research performed, fundamental questions regarding the cell biology of aNSCs remain to be uncovered. For instance, it is crucial to elucidate whether a single aNSC is capable of differentiating into all the different cell types within their lineage or these distinct progenies constitute entirely separate lineages. Similarly, the cell cycle length, the time and mode of division (symmetric versus asymmetric) that these cells undergo are interesting questions under current investigation. Continuous live imaging and single cell tracking constitutes an excellent tool in order to provide answers to the above essential questions. We have employed an alternative method of aNSCs culture preparation, in which cells isolated from the adult SEZ are kept in absence of growth factors, with the consequence that they maintain their intrinsic neurogenic or oligodendrogliogenic nature, and allows for continuous live imaging by time-lapse videomicroscopy. By using this novel culture preparation, we were able to track single aNSCs and their progeny, characterizing their behavior and their defining hallmarks. Moreover, we have identified different factors that actively regulate the lineage progression of neurogenic or oligodendrogliogenic NSCs, as Wnt proteins EGF/FGF mitogen factors or members of the extracellular matrix. This leads to an improved knowledge regarding crucial aspects of adult NSCs cell biology, opening new perspectives in the future design of therapeutic strategies for brain repair.

RED DE EXCELENCIA SICI CONSOLIDER

MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

Oral 16

SILENCING GluN3A-CONTAINING NMDA RECEPTORS TO TREAT HUNTINGTON´S DISEASE

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Keywords: Huntington's Disease, NMDARs, GluN3A, RNAi, rAAV, synapse loss, gene therapy, striatum

Huntington's disease (HD) is a neurodegenerative disease caused by expansion of a polyglutamine repeat in the huntingtin protein. HD symptoms include motor, cognitive and psychiatric alterations that arise from dysfunction and later degeneration of medium-size spiny neurons (MSN) of the striatum. MSNs receive dense cortical and thalamic glutamatergic innervation, and early malfunction of glutamatergic neurotransmission mediated by N-methyl-D-Aspartate receptors (NMDARs) has been implicated in the pathogenesis. Our recent work discovered the underlying mechanism: aberrantly increased expression of juvenile NMDAR subtypes containing GluN3A subunits. Most remarkably, suppressing GluN3A expression using a genetic strategy was sufficient to correct the synaptic alterations, motor and cognitive symptoms, and the late cell death in the YAC128 mouse model of HD (Marco *et al.*, 2013).
Whereas genetic suppression is not a realistic therapeutic strategy in humans, RNA interference has shown therapeutic promise. This study assessed the value of GluN3A as a therapeutic target in HD using RNAi-based silencing. A validated shRNA that specifically and strongly suppresses GluN3A expression (sh1185, Yuan *et al*. 2013) was used. sh1185 was delivered into the brain by stereotaxic injection of recombinant adeno-associated viral vectors (rAAV). Upon testing different rAAV serotypes, rAAV9-EGFP-sh1185 yielded the highest spread and efficiency of neuronal transduction without concomitant glial reaction and was chosen for further experiments. rAAV9-EGFP-sh1185 or rAAV9-EGFP-control were injected in the striatum of 1 month-old YAC128 mice, and synaptic density and motor behavior were evaluated. Intrastriatally injected rAAV9-EGFP-sh1185 transduced 50% of MSNs and efficiently silenced GluN3A protein. We found that silencing GluN3A expression prevented the spine loss detected in YAC128 mice by 3-4 months of age. Our current data show that rAAV9-EGFP-sh1185 might be a useful tool to delay neurodegeneration in mouse models of HD and potentially in patients. Follow-up experiments are conducted to evaluate whether later GluN3A silencing is also effective to establish an appropriate therapeutic window.

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MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

RED DE EXCELENCIA SICI CONSOLIDER

Oral 17

Poliubiquitination mediates the voltage-gated potassium channel Kv1.3 endocytosis

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KEY WORDS: Ubiquitination, endocytosis, Kv1.3, PKC, PKA.

The voltage-dependent potassium channel Kv1.3 is expressed mostly in the nervous and immune systems, where participates in the sensory discrimination and leukocyte physiological responses. An altered function as well as an exacerbated expression or surface mistargeting are related with autoimmunitary diseases. Regulation of this transmembrane protein is therefore essential. The turnover of Kv1.3 is highly dynamic and, upon insults, the balance between the number of channels located at the membrane and the internalization is crucial for an appropriate signaling. Therefore, endocytosis is an essential mechanism for the regulation of Kv1.3 abundance on the cell surface. Ubiquitinanion has emerged as a crucial mechanism for membrane protein turnover. In this study we investigated ubiquitination-mediated endocytosis and the lysosomal sorting of Kv1.3. To that end several Kv1.3 lysine mutants were created, in which we examined ubiquitination, endocytosis and membrane targeting. We observed ubiquitination and ubiquitin-mediated endocytosis by inducing PKC and PKA activation. Our results indicate that more than one lysine are involved in the ubiquitination of the channel. Furthermore, we have mapped the most relevant for the channel internalization and degradation. Our results indicate that Kv1.3 undergoes specific ubiquitination in residues which participate in the endocytosis and the turnover of the channel.

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Oral 18

Upregulation of ionotropic P2X7 receptor facilitates the survival of neuroblastoma cells under limiting growth conditions: implication of PI3K/Akt signaling pathway.

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KEY WORDS: P2X7 receptor, neuroblastoma, PI3K/Akt, serum derivation, Sp1

Purinergic P2X7 receptors (P2X7R) are ATP-gated cation channels highly expressed by nearly all human cancers so far investigated, including neuroblastoma cells from both primary tumors and cell lines[1]. High expression of P2X7R in neuroblastoma cells is associated to accelerated growth rate, angiogenesis, metastasis and poor prognosis, allowing the survival of neuroblastoma cells under restrictive conditions as well. In this work we report that serum withdrawal induces a significant increase in P2X7 transcript levels, circumstance that facilitates proliferation of neuroblastoma cells in the absence of trophic support[2]. The increase in P2X7R expression is dependent on PI3K/Akt signaling, requires the activation of EGF receptor (EGFR) at the cellular membrane, but is independent on either mTOR or GSK3. In a previous study we identified specificity protein 1 (Sp1) as the main nuclear factor involved in the transcriptional regulation of *P2rx7* gene[3]. Now we demonstrate that nuclear Sp1 levels are strongly reduced by inhibition of PI3K/Akt pathway, and blockade of Sp1-dependent transcription with mithramycin A prevents upregulation of *P2rx7* gene expression following serum withdrawal. Furthermore, atypical PKCζ is also implicated in the regulation of P2X7R expression by preventing Akt activation[2]. In summary, this study sheds light on the biochemical mechanisms leading to serum withdrawal-induced upregulation of P2X7R expression in neuroblastoma cells, implicating well-known players such as EGFR, PI3K/Akt and Sp1 in this pro-survival outcome.

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MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

Oral 19
Chalcones and Chalconoids: New Allosteric Modulators of the α7 Nicotinic Acetylcholine Receptor (α7 nAChR)

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KEY WORDS: Alpha7 nAChR, Positive allosteric modulators, Chalcone, Neuroprotection, Analgesia

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand gated channels that are implicated in several nervous system pathologies that involve, among others, cognition disorders, schizophrenia, pain and inflammation. The search for positive allosteric modulators (PAMs) of α7 nAChRs has attracted considerable interest, because they permit the selective regulation of these channels and, at the same time, they avoid many of the adverse effects associated with ligands that directly target the agonist binding site.

After the screening of a heterogeneous collection of small natural molecules, we identified a chalcone that behaves as a selective positive modulator of the α7 nAChR. This compound has two substituted phenyl rings separated by a spacer, and can be considered within the so called privileged structures. Modifications of the initial hit, regarding the number, position and character of the substituents, lead to a new series of polyhydroxy-substituted chalcones as potent and selective positive allosteric modulators (PAMs) of α7 nAChR. Trying to mitigate the risk of adverse effects that could be associated to the high chemical reactivity of the chalcone α,β–unsaturated carbonyl system, we performed modifications of this linker chain. This has permitted the identification of new PAMs of the α7 nAChRs by chemoselective reduction of the conjugated double bond.

Preliminary ADME studies indicated the need for additional optimization of the chemical structure. This prompted us to prepare new derivatives that could behave as pro-drugs of the newly identified active compounds, with the aim of enhancing permeability and improving the pharmacokinetic profile.

An overview of the whole project will be presented, together with the biological characterization of the most relevant compounds, including the results of an *in vivo* model of analgesia.

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Oral 20

ATP: the crucial component of secretory vesicles

Ricardo Borges, *Judith Estévez-Herrera, Natalia Domínguez, Ayoze González-Santana, Edward W. Westhead, and José David Machado*

KEY WORDS: Adrenal, Chromaffin, Exocytosis, Secretion.

The colligative properties of ATP and catecholamines demonstrated *in vitro* are thought to be responsible for the extraordinary accumulation of solutes inside chromaffin cell secretory vesicles, although this has yet to be demonstrated in living cells. As functional cells cannot be deprived of ATP, we have knocked down the expression of the vesicular nucleotide carrier –the VNUT– to show that a reduction in vesicular ATP is accompanied by a drastic fall in the quantal release of catecholamines. This phenomenon is particularly evident in newly synthesized vesicles, which we show are the first to be released. Surprisingly, we find that inhibiting VNUT expression also reduces the frequency of exocytosis while the overexpression of VNUT drastically increases the quantal size of exocytotic events. Our data provide the first demonstration that ATP, in addition to serving as an energy source and purinergic transmitter, is an essential element in the concentration of catecholamines in secretory vesicles. In this way, cells can use ATP to accumulate neurotransmitters and other secreted substances at high concentrations, supporting quantal transmission.

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Oral 21

Alpha2 adrenergic and P2X3 receptors from adrenomedullary chromaffin cells as novel targets for the treatment of neuropathic pain

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Neuropathic pain constitutes an increasingly unmet therapeutic demand. Despite that the essential alterations in neuropathic pain relate to the neural and glial components of the nociceptive pathway, it is also known that the efferent output of the sympathetic nervous system (SNS) modulates the clinical expression of this pathology. Here, we have used rats with chronic constriction injury (CCI) of the sciatic nerve to evaluate the morphological and functional changes that occur in chromaffin cells from the adrenal medulla, an structure of the SNS that contributes to stress responses by releasing catecholamines into the blood stream. In CCI animals we observed an increase in vesicular acetylcholine transporter immunorreactivity as well as a higher frequency of spontaneous synaptic currents, which exhibited larger amplitudes because of a higher expression of postsynaptic nicotinic acetylcholine receptors. Moreover, catecholamine (adrenaline, A, and noradrenaline, NA) leves were augmented in the blood of neuropathic animals. Interestingly, the administration of SKF29661, an inhibitor of PNMT, the enzyme that converts NA into NA, reduced the A/NA ratio both in the adrenal gland and blood of CCI animals while exhibiting a pronounced antiallodynic effect. These results involve catecholamines released from chromaffin cells in the phenomenology of neuropathic pain in our experimental model.

Likewise, treatment with MPV2426, a peripherally acting agonist of alpha2 adrenoceptors, inhibited ionic currents through Cav channels in chromaffin and isolated lumbar dorsal root ganglion (DRG) neurons. These effects were associated to a reduction in catecholamine release from chromaffin cells and changes in the electrical behaviour of DRG neurons, which possibly underlie the antiallodynic effect of this compound. Lastly, there was also an increase in P2X3 immunorreactivity both in the adrenal medulla and DRG from CCI animals. The dinucleotide phosphate Ip5I blocked P2X3-mediated currents from DRG neurons and also exhibited an antiallodynic effect, thus pointing to this chemical class of P2X3 antagonists as novel analgesic drugs potentially useful for the treatment of neuropathic pain.

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Oral 22

DECIPHERING THE COMPLEX MODULATION OF nAChRs BY LOCAL ANESTHETICS: LOOKING FOR MODULATING BINDING SITES

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Nicotinic acetylcholine (ACh) receptors (nAChRs) are widely expressed in the central and peripheral nervous systems and their dysfunction has been related to a variety of severe pathologies. Thus, the study of the mechanisms by which different drugs modulate nAChR function is of great interest not only for expanding our knowledge on the function of this receptor protein but also for developing new molecules of therapeutic interest. Local anesthetics (LAs) are widely used in clinical practice because of their capability to block voltage-dependent Na+-channels, and hence to preclude the action potential generation in nerve fibers. However, LAs also modulate the activity of different ligand-gated ion- channels (LGICs), including neuronal- and muscular-nAChRs, which can trigger several side effects observed when using lidocaine and other LAs. Therefore, for the last few years, our research group has been interested in identifying the mechanisms underlying the modulation of nAChRs by LAs, using as models lidocaine and the muscle-type of nAChRs. We have found that lidocaine blocks muscle-type and neuronal heteromeric nAChRs by multiple mechanisms that can be dissected, at least partially, by dose. So, at doses lower than the *IC50*, lidocaine inhibits nAChRs mainly by open-channel blockade, whereas at higher concentrations (equal or higher than the *IC50*) it also causes closed-channel blockade and enhances desensitization. Given that lidocaine is found in both charged (membrane-impermeant) and uncharged (membrane-permeant) states in physiological conditions, the heterogeneous mechanisms underlying nAChR blockade by lidocaine could be related to the presence of these two molecular conformations. So, our next aim was to unravel the structural determinants within the lidocaine molecule responsible for specific effects on muscle-type nAChRs by using diethylamine (DEA) and 2,6-dimethylaniline (DMA), which are analogous of the hydrophilic and hydrophobic moieties of the lidocaine molecule, respectively. We have found that DEA and DMA can simultaneously occupy their specific binding sites on this receptor, eliciting each one of these molecules selective effects on nAChRs, and when DEA and DMA are acting together they reproduce most of the inhibitory actions elicited by the whole lidocaine molecule. These results indicate that many amphipathic molecules, including most LAs, might exert a complex modulating action on nAChRs by simultaneously acting, with different affinities, at distinct and even distant binding sites on this receptor and, most likely, this is also suitable for other LGICs.

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Poster 1

EXTRACELLULAR cGMP MODULATES LEARNING BIPHASICALLY BY MODULATING GLYCINE RECEPTORS, CaMKII AND GLUTAMATE-NITRIC OXIDE-cGMP PATHWAY

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KEY WORDS: Cyclic GMP, calcium, chloride, learning, glycine receptors, nitric oxide

Aims: It has been proposed that extracellular cGMP modulates the ability to learn a Y maze task, but the underlying mechanisms remained unknown. One possible mechanism is modulation of the glutamate-nitric oxide (NO)-cGMP pathway in the cerebellum, which modulates learning ability in the Y maze task. Activation of NMDA receptors increases calcium which binds to calmodulin and activates neuronal nitric oxide synthase (NOS). This increases NO, which activates soluble guanylate cyclase, increasing cGMP. NOS activity is also modulated by calcium-calmodulin kinase II (CaMKII). It was proposed that extracellular cGMP could modulate glycine receptors associated with chlorine channels. **Material and Methods:** We analyzed the effect of extracellular cGMP on the NOS activity, on levels of intracellular calcium and chloride and on phosphorylation of CaMKII in cerebellar slices and on the function of the NMDA-NO-cGMP pathway by microdialysis in vivo in rat cerebellum. Learning of a conditional discrimination task was assessed in the Y maze. **Results:** Here we show that extracellular cGMP, at physiological concentrations, modulates learning in the Y maze in a biphasic way by modulating the glutamate-NO-cGMP pathway in cerebellum. Extracellular cGMP reduces glycine receptors activation inducing a voltage-dependent calcium-channels-mediated increase of calcium in Purkinje neurons. This calcium increase modulates CaMKII phosphorylation in a biphasic way. When basal calcium concentration is low extracellular cGMP reduces CaMKII phosphorylation, increasing nitric oxide synthase activity, the glutamate-NO-cGMP pathway function and learning ability. When basal calcium is normal extracellular cGMP increases CaMKII phosphorylation, reducing nitric oxide synthase activity, the pathway function and learning. **Conclusions:** These data unveil new mechanisms modulating learning in the Y maze and likely other learning types which may be therapeutic targets to improve learning in pathological situations associated with altered cGMP levels.

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Poster 2
Exploring the β–lactam ring as a central scaffold for TRPM8 modulation

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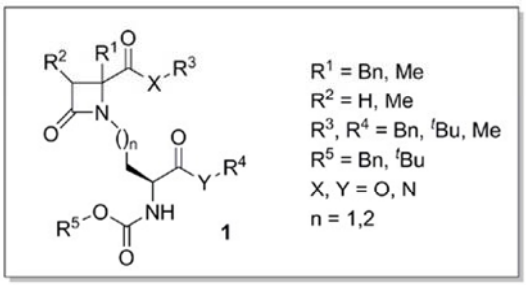
KEY WORDS: TRPM8, antagonist activity, β-lactams, structure-activity relationships, selectivity

TRPM8 is considered an attractive target for therapeutic intervention, mainly in the search for new analgesics and antitumor agents.[1] Cumulative evidences point to the increased TRPM8 expression in sensory neurons after nerve injury or inflammation, resulting in enhanced sensitivity to cold allodynia and hyperalgesia, while activation of TRPM8 appears also important in attenuating pain in certain acute and inflammatory pain states.[2] Also worth of mention, TRPM8 expression is up-regulated in different tumor cells (i.e. androgen-sensitive prostate and skin melanoma cancers).[3,4]

The crucial role of TRPM8 in human pathologies is behind the intensive drug-discovery programs that are being developed in recent years around this channel.[1] Our contribution in this field is related to the discovery of new, potent TRPM8 antagonists after HTS screening of our in house library of compounds. From the initial results we choose a highly substituted β–lactam scaffold for the synthesis of a focused small library. This communication will deal with the synthesis of this series of β–lactam derivatives, of general formula **1**, the structure-activity relationships, selectivity against other channels, and further pharmacological characterization of selected hits. A docking model of interaction of one of the best molecules and the TRPM8 channel will also be discussed.



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Poster 3

Molecular determinants involved in clustering and gating patterns in KcsA

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KEY WORDS: Protein-Protein Interactions; Lipid-Protein Interactions; Coupled Gating; Potassium Channels

There is increasing evidence to support the notion that membrane proteins, instead of being isolated components floating in a fluid lipid environment, can be assembled into supramolecular complexes that take part in a variety of cooperative cellular functions (1). The interplay between lipid-protein and protein-protein interactions is expected to be a determinant factor in the assembly and dynamics of such membrane complexes.

Channel clustering and coupled gating modulate the activity of KcsA, a model potassium channel (2). The occurrence of a similar behavior in other channels points out to clustering and coupled gating as a potentially important target to modulate channel activity.

We have been able to identify molecular determinants involved in single and coupled channel gating in KcsA through different predictive and experimental approaches. We detected that clustering and coupled gating in KcsA is modulated by anionic phospholipids. Assembly/disassembly of channel clusters occurs, at least partly, as a consequence of competing lipid-protein and protein-protein interactions at nonannular lipid binding sites on the channel surface and brings about profound changes in the gating properties of the channel. Our results suggest that these latter effects of anionic lipids are mediated via the Trp67–Glu71–Asp80 inactivation triad within the channel structure and its bearing on the selectivity filter.

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Poster 4
SLEEP APNEAS IN THE ADULT MOUSE LACKING CONNEXIN-36

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KEY WORDS: connexin 36, electrical synapse, respiration, sleep apnea

Breathing is a primal homeostatic neural process, which disturbances have severe consequences for human health. Brainstem neural circuits generating respiratory rhythm and controlling responses to O2 and CO2 changes are organized as serially arrayed networks of excitatory and inhibitory neurons interacting by chemical and electrical synapses. In order to elucidate the role played by electrical neurotransmission depending on connexin-36 (Cx36), the principal component of electrical synapses between inhibitory neurons within respiratory center, we analyzed the differences in breathing activity of adult wild type (WT) and Cx36-knockout (Cx36-KO) animals on wake, NREM (slow-wave sleep) and REM (rapid eye movements) states. Sleep stages were identified from electroencephalografic and neck muscle recordings, and breathing was measured using a thoracic sensor of ventilatory movements. We found that Cx36-KO in comparison with WT mice breathed more rapidly and with greater amplitude in quite wakefulness. However during NREM, the respiratory frequency was significantly slower in Cx36-KO than WT mice. During REM, breathing became arrhythmic and variable in amplitude, especially in the Cx36-KO animals; moreover they showed an abnormal increased number of central apneas that provoked arousals and sleep fragmentation. Thus, the substantial changes observed in adult mice lacking Cx36 can be interpreted as demonstration of the necessity of electrical connectivity in the central neural circuits controlling breathing and, particularly, in the microcircuit generating respiratory rhythm.

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Poster 5

MECHANISM OF DEMYELINATION OF SPASTIC PARAPLEGIA CAUSED BY THE SPORADIC CONNEXIN-43 R148Q MUTATION

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KEYWORDS: Connexin 43, oculodentodigitaldysplasia, spastic paraplegia, demyelination, glial coupling

Oculodentodigital Dysplasia (ODDD, OMIM#164200) is a rare syndrome caused by dominant mutations in the connexin 43 (Cx43) gene (*GJA1*). ODDD phenotype in one third of patients courses with spastic paraplegia (SPG) of onset at the first/second decade of life. However, the pathogenic mechanism of demyelination by which these Cx43 mutations expressed in astrocytes but not in the cells forming central myelin (i.e., the oligodendrocytes) is unknown. In our genetic studies of ODDD patients with SPG, we have identified the sporadic heterozygous mutation c.443G>A that predicts the substitution p.R148Q. Expression assay showed that R148Q mutant induces similar levels than wild type Cx43 of total protein and surface abundance, and formation of gap junction plaques at cell-to-cell contact area. Neither was found differences in current level, permeability and voltage regulation of mutated and wild type hemichannels. However, mutation R148Q impaired dye and electrical coupling, indicating that mutated intercellular channels were not functional. In experiments of coexpression with wild type Cx43 in oocyte pairs, N2A cells and human astrocytes, which simulates the heterozygous condition of patients, the mutant exerted a potent effect of dominant inhibition over intercellular coupling. Heterologous coupling of Cx43 with oligodendrocyte Cx47 was also significantly reduced in presence of R148Q mutant. Thus, we conclude that mutation R148Q inhibits in a dominant manner astrocyte-astrocyte as well as astrocyte-oligodendrocyte coupling mediated respectively by Cx43-Cx43 and Cx43-Cx47 channels, indicating that intercellular communication plays a critical role in the glial mechanisms that support myelin integrity and that its disruption causes demyelination.

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Poster 6

NOVEL SMALL MOLECULES TARGETING THE KCHIP3/Kv4.3 INTERACTION

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KChIP-3 (potassium channel interacting protein-3), also known as DREAM (Downstream Regulatory Element Antagonist Modulator) or calsenilin, is a multifunctional calcium binding protein that controls the expression level and/or the activity of several proteins related to calcium homeostasis, neuronal excitability and neuronal survival. The interest in KChIP-3 is based on its key role in the regulation of intracellular calcium levels. As a calcium-dependent transcriptional repressor, KChIP-3 is a master regulator of activity-dependent gene expression and controls genes important for calcium homeostasis such as the sodium/calcium exchanger-3 (NCX3), and L-type calcium channels. As an auxiliary protein in the plasma membrane, KChIP-3 interacts with, and regulates, the gating of Kv4 potassium channels, L- and T-type voltage-dependent calcium channels and NMDA receptors. Considering that altered neuronal calcium homeostasis is a common feature of many neurodegenerative pathologies, the KChIP-3 modulation could open new avenues for the treatment of different neurodegenerative diseases. However, up to now, only three KChIP-3 binding molecules have been identified. Hence, there is a clear need for the development of chemical tools to modulate and characterize DREAM activity and its interactions.

In this communication we report the rational design and synthesis of novel KChIP-3 binding molecules and their effects on the modulation of the KChIP-3/Kv4.3 interaction. The applied strategy involved structure-based drug design, synthesis and surface plasmon resonance (SPR) studies. Moreover, we have identified the ligand binding site by directed-mutagenesis studies. Additionally, whole-cell-patch-clamp assays have allowed the identification of interesting and potent modulators of the DREAM/Kv4.3 interaction.

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Poster 7
Vascular ion channel anomalies in a mouse model of Hutchinson-Gilford Progeria Syndrome (HGPS).

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KEY WORDS: Lamin A, HGPS, arteriosclerosis, aging, cardiovascular disease, ion channels, wire myograph.

Objectives: "Hutchinson-Gilford progeria syndrome" (HGPS) is a very rare genetic disease for which no cure exists. HGPS patients exhibit accelerated onset of aging-associated symptoms, including cardiovascular alterations, and die at an average age of 14.6 years, typically from myocardial infarction or stroke. This laminopathy is caused by expression of the mutant LaminA protein called **progerin**. Importantly, progerin has been also detected at low level in aged tissues of non-HGPS individuals, suggesting a role in normal aging. Previous data in our laboratory demonstrated serveral cardiac and vascular alterations in *Zmpste24-KO* [Rivera-Torres, J *et al.*, 2016] and *Lmna*^{G609G} [Osorio FG, *et al*., 2011; Villa-Bellosta R *et al.*, 2013] mice, two models of progeria widely characterized by our group. Our main aim is to investigate the molecular mechanisms causing electrophysiological alterations in progeroid vascular smooth muscle cells (VSMCs) which could contribute to HGPS.

Material and Methods: We have analyzed by wire myography the ion channels-modulated vascular reactivity of the aorta of wild-type and progerin-expressing *Lmna*^{G609G} mice, as well as the response to a hypoxic stimulus in the coronary arteries of these animals.

Results: We find predominant alterations in the activity of K_v7 channels in aorta of *Lmna*^{G609G} mice, and dramatic abnormalities in coronary vascular activity in response to hypoxia.

Conclusion: Alterations in K_v7 channels in aorta identify this family of potassium channels as a possible target in the treatment of vascular alterations in progeria. Likewise, the differences observed in coronary response to hypoxia could be due to alterations in different ion channels in the VSMCs of progeric mice.

Financial support: Work supported by the Spanish Ministry of Economy and Competitiveness (MINECO) (grant SAF2013-46663-R) and Instituto de Salud Carlos III (grant RD12/0042/0028) with co-funding from the Fondo Europeo de Desarrollo Regional (FEDER). The CNIC is supported by the MINECO and the Pro CNIC Foundation, and is a Severo Ochoa Center of Excellence (MINECO award SEV-2015-0505).

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Poster 8
SELECTIVE INHIBITION OF SYNAPTIC SIGNALING PATHWAYS BY GLUN3A SUBUNITS

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KEY WORDS: Synapse, neural circuit refinement, pruning, NMDA-type glutamate receptor, GluN3A, mTOR.

Early brain development is characterized by an overproduction of synapses which make weak functional connections between neurons. Neuronal activity later refines this basic circuitry by selecting some connections while suppressing others. A main regulator of this type of synaptic refinements is a subtype of NMDA-type glutamate receptors that contain GluN3A subunits (GluN3A-NMDARs). GluN3A-NMDARs are typically expressed at early postnatal and juvenile stages, when intense synaptic refinements take place, and act as dominant negative regulators of synaptic maturation and stabilization. Such inhibitory roles seem critical for targeting certain synapses for pruning and preventing premature synapse maturation/ stabilization in developing brains[1]. By contrast, aberrant GluN3A expression in adult brains leads to enhanced pruning, impairing connectivity and memory, and underlies disease states[2,3].

Here we study the signaling pathways that underlie the synapse loss and memory deficits associated with enhanced GluN3A expression. Using lentiviral vectors to overexpress GluN3A subunits in cortical primary neuronal culture of rat embryos, we analyzed the effects of GluN3A on the expression of activity-regulated genes (Arc/Arg3.1, cFos, Zif268) and signaling pathways involved in synapse development and memory consolidations. We observed a selective inhibition of a set of activity and NMDAR-regulated pathways in GluN3A overexpressing neurons: major defects in CamKII, MAP38K or the Akt/mTOR/pS6 pathways were detected, without changes in ERK1/2 or CREB phosphorylation; this was in contrast to the general inhibitory effect of the NMDAR antagonist APV. The induction of the immediate early genes Arc and c-Fos was inhibited despite normal mRNA production, pointing towards a role of GluN3A in limiting protein translation. Current studies are directed to delineate the mechanisms underlying the selectivity of GluN3A effects on NMDAR signaling. Of note, the mTOR-activating GTPase Rheb and the actin cytoskeleton scaffold GIT1 have been shown to interact directly with GluN3A subunits[1], suggesting a possible downstream mechanisms.

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Poster 9

SCN1B variant affecting the Ig-like domain of β1 subunit produces cardiac sodium channel loss of function through β1a but not β1b subunit isoform.

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KEY WORDS: Brugada Syndrome/Epilepsy, sodium current, SCN1B, SCN5A, arrhythmia

Introduction: Brugada Syndrome (BrS) is an inherited arrhythmogenic disease associated with sudden cardiac death. Variants in genes encoding ionic channels have been found in 30% of BrS patients. The majority of these mutations are located in the *SCN5A* gene, encoding the alpha subunit of the cardiac sodium channel ($Na_V1.5$). A small percentage of mutations is found on auxiliary subunits of $Na_V1.5$, including β1 subunit isoforms a and b, both encoded by the *SCN1B* gene. We found a missense mutation (D103V) in the *SCN1B* gene, in a child diagnosed with BrS and Generalized Epilepsy with Febrile Seizures plus. This mutation is located in the Ig-like domain of β1 subunit, a region common to both β1a and β1b isoforms. Consequently, both isoforms will carry the mutation.

Objectives: The aim of this work was to determine the possible deleterious effect of β1a and β1b mutant proteins on the $Na_v1.5$ current.

Methods: We used human embryonic kidney cells to co-express the $Na_v1.5$ channel with the β1a or β1b isoform harboring the mutation D103V. We studied sodium currents using whole cell patch-clamp.

Results: We found a marked decrease in sodium current density in cells expressing the mutant β1a isoform compared to WT β1a. On the other hand, sodium current density was not affected by the mutant β1b isoform. None of the two mutant isoforms affected the voltage dependence or the kinetic properties of the current.

Conclusions: Our results suggest that the missense mutation D103V can cause sodium channel loss of function, which is a hallmark of BrS. This sodium current inhibition appears to be caused only by mutant β1a but not β1b isoform.

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Poster 10

The juvenile NMDA receptor subunit GluN3A modulates anxiety and stress-like responses.

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KEY WORDS: **NMDA, Glutamate, GluN3A subunit, anxiety, stress.**

NMDA receptors are heteromeric channels assembled from an obligatory GluN1 subunit and various combinations of four GluN2 (A–D) and two GluN3 (A–B) subunits. The functions of GluN2A and GluN2B in brain development, plasticity, learning and memory have been extensively studied, but less is known about nonconventional GluN3A-containing subtypes. We have previously shown that alterations in GluN3A expression levels trigger synapse dysfunction and loss, and areassociated to CNS pathologies such as Huntington disease (1,2) or cocaine abuse (3). Newer genetic evidence links GRIN3A (human gene encoding GluN3A) to nicotine or alcohol dependence and depressive states, but the causal relationships are not known.

GluN3A subunits are predominantly expressed during critical periods of postnatal development when they play major roles in network rewiring, but it is unknown how GluN3A expression impacts the emergence of adult behaviors. To start addressing this question, we have conducted an extensive behavioral study of mice lacking GluN3A (GluN3A knockouts, KO) and transgenic mice where GluN3A levels were enhanced in adult brains using the CaMKII promoter (GFP-GluN3A double transgenic mice –dt). The behavioral analysis of GluN3A KO mice revealed major effects in mood and cognition including: 1) an anxiolytic like-phenotype on juvenile and adult mice, 2) defects on social memory that could not be attributed to problems in olfaction, and 3) enhanced associative memory when using a weak, stringent learning protocol for conditioned taste aversion (CTA). Conversely, GFP-GluN3A dt showed deficits on CTA that were associated to diminished induction of the immediate early gene ARC after taste aversion learning.

We finally explored the consequences of altered GluN3A expression on the emotional responses to stress and found that mice lacking GluN3A displayed features of resilience to despair-like behaviors. Together our results open new avenues for the implication of GluN3A on anxiety and depression states.

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Poster 11
ORAI1 and STIM1 promote membrane ruffling at the leading edge of migrating cells

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KEY WORDS: Calcium, cytoskeleton, membrane ruffling, migration, STIM1, ORAI1

Cell migration is strongly dependent on Ca^{2+} signalling, and extracellular Ca^{2+} entry has been proposed to be a key regulator of cell migration in different cancer cell lines. However, the exact role of this Ca^{2+} entry and its intracellular molecular targets are largely unknown. To evaluate this role and open new insights into the role of Ca^{2+} signalling on cell migration we have generated gene knockout cell lines by CRISPR/Cas9 genome editing in two loci: *STIM1* (ENSG00000167323) and *ORAI1* (ENSG00000276045), independently, using the osteosarcoma cell line U2OS. STIM1 is an intraluminal Ca^{2+} sensor located at the endoplasmic reticulum (ER), and activates the plasma membrane Ca^{2+} channel ORAI1 upon partial depletion of the Ca^{2+} levels within the ER. Thus, STIM1 and ORAI1 control the Ca^{2+} influx pathway through a mechanism termed store-operated Ca^{2+} entry (SOCE). STIM1-KO and ORAI1-KO cells were generated for all the known transcriptional variants of STIM1 (3 variants) and ORAI1 (2 variants) by targeting exon 5 and exon 1, respectively.

The result of the genome editing was a mix of 17 + 31 base-pair deletion for *STIM1*, and a 16 + 14 bp insertions, for *ORAI1*, confirming the complete KO for both loci. Loss of protein expression was confirmed by immunoblot. In both cases we observed a large decreased of SOCE in fura-2-loaded KO cells, and that the overexpression of ectopic STIM1-mCherry or ORAI1-mCherry rescued the phenotype (level of SOCE), confirming the specificity of the editing strategy. Both KO cell lines show a greatly reduced cell migration rate, monitored by wound-healing assays. By monitoring GFP-cortactin fluorescence in live cells we also recorded a significant decrease in membrane ruffling dynamics, i.e., a decrease in the formation rate of protrusions and lamellipodia, similar to what is observed when the Ca^{2+} entry inhibitor SFK96365 is added to the assay medium. Our results demonstrate that (1) there is a highly conserved colocalization of ORAI1 and cortactin during protrusions formation at the leading edge of migrating cells; (2) the reorganization of the cortical actin cytoskeleton is a primary target of Ca^{2+} entry; and (3) this Ca^{2+} entry pathway is mainly controlled by STIM1 and ORAI1.

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Poster 12
Structural insights on how calcium regulates Kv7.2 channels through calmodulin

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KEY WORDS: Kv7.2 channels, FRET, RMN, Calcium

Calmodulin (CaM) is an EF-hand protein that relays calcium signals to a vast number of different targets. CaM binding can be classified in two major groups: a.- when it interacts in the absence of Ca^{2+}(apocalmodulin). b.- when the interaction takes place when CaM is loaded with this cation (holocalmodulin). Both modes are observed when the targets are Kv7.2 channels, which are one of the main components of the noninactivating K^+ M-current, a key controller of neuronal excitability. CaM binds to helices A and B in the C-terminal domain and regulates channel trafficking and function. To get insights on how Ca^{2+} gates Kv7.2 channels through CaM, we have studied the conformational changes prompted by this cation using FRET and NMR spectroscopy of the AB-CaM complex. The movements detected by these complementary techniques are small compared to those described in other targets. The atomic level impact on channelopathic mutations as this site and possible gating mechanism compatible with the structural data will be discussed.

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Poster 13
Mutations in *KCNQ2* causing early infantile epileptic encephalopathy alter Kv7.2 channel PIP_2 sensitivity

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KEY WORDS: ENCEPHALOPATIES, Kv7.2 channels, PIP_2

Mutations in the *KCNQ2* gene lead to several neonatal onset seizure disorders including an epileptic encephalopathy, with adverse neurodevelopmental outcome. We have found the latter in four patients, carrying *de novo* (E130K, W270R and G281R) or maternally inherited (L246F) *KCNQ2* mutations . Here we describe the functional consequences in cells expressing these channels, alone or in combination with the partner subunit Kv7.3 to mimic the allelic balance found in humans. All mutations exerted a dominant negative effect in homomers, whereas Kv7.3 rescued the function of mutants to different extent. The mutations caused a reduction on current density with no major changes on voltage-dependency or gating kinetics. The use of a voltage- dependent phosphatase revealed that the sensitivity to the essential PIP_2 phospholipid is affected in all four mutations to varying degree. Thus, current density and PIP_2 sensitivity may contribute to the pathogenic mechanism.

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Poster 14
Multiple KCNE4 action within the Kv1.3 channelosome

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KEY WORDS: Kv1.3, KCNE4, Channelosome, C-terminal, Trafficking

The voltage-dependent potassium channel Kv1.3 plays an important role in immune system cells. We previously demonstrated that KCNE4, acting as a dominant negative regulatory subunit, physically interacts with Kv1.3 inhibiting K^+ currents and retaining the channel intracellular (1). However, the molecular determinants participating in the massive intracellular phenotype remain unknown. In the present work we analyzed the KCNE4 motifs which are responsible for both the Kv1.3 interaction and the massive ER retention. Our data identified molecular determinants involved in KCNE4 oligomerizations which compete for the association with Kv1.3. Furthermore, the KCNE4-dependent intracellular retention of the channel complex, which negatively affects the physiological role of Kv1.3, is mediated by two independent mechanisms. First, KCNE4 association masks the YMVIEE signature at the C-terminal domain of the Kv1.3 that is crucial for the surface targeting of the channel; second, we identify a potent endoplasmic reticulum retention motif in KCNE4 that further limits cell surface expression. Our results pave the way for the understanding of specific KCNE4 molecular determinants which play a crucial role in the channelosome formation in leukocytes.

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Poster 15
Anterograde traffic of functional Kv7.1/KCNE1 channels

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KEY WORDS: Oligomeric association, ER, Golgi, COP-II, Kv7.1, KCNE1.

The voltage-gated potassium channel $K_v7.1$ associates with the KCNE1 β-subunit. This oligomeric interaction generates the slowly activating delayed rectifying potassium current, I_{Ks}, which participates during the repolarization of cardiac action potential. Mutations in either subunit lead to severe cardiac channelopathies such as long QT syndrome. The functional effect of KCNE1 onto $K_v7.1$ and the subsequent specific interaction domains have been widely described, but there is still controversy about the specific intracellular compartment where the assembly of the complex takes place. We demonstrate that $K_v7.1$-KCNE1 complex is not built early at the initial stages of the secretory pathway, within the endoplasmic reticulum. In fact, both channel subunits can use different routes reaching the plasma membrane. Thus, the disruption of Golgi apparatus impairs KCNE1, but not $K_v7.1$, targeting to the cell surface. Our results indicate that KCNE1 relays on COPII-dependent forward trafficking machinery and $K_v7.1$ can alternatively use a non- conventional secretory pathway. Upon the formation of the functional complex, $K_v7.1$ redirects KCNE1 to the COPII-independent route to the membrane. Finally, studies with plasma membrane lawn preparations suggest that the oligomeric complex is fully assembled at the cell surface, thereby suggesting that the $K_v7.1$-KCNE1 association takes place in an alternative route late in the secretory pathway but nearby to the cell surface.

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Poster 16

Characterization of a caveolin binding domain within the Kv1.3 channel

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KEY WORDS: Caveolin, lipid rafts, caveolae, Kv1.3, caveolin binding domain..

Lipid rafts are specialized membrane microdomains rich in sphingolipids and cholesterol. These assemblies are fluid but more ordered and tightly packed than the surrounding bilayer and have been implicated in the organization of many membrane-associated signaling pathways. The targeting of ion channels to these microdomains has emerged as a crucial mechanism of ion channel localization and function. The voltage gated potassium channel Kv1.3 targets to the immunological synapse (IS), which concentrates lipid rafts, and modulates the physiology of T lymphocytes. In this study, we demonstrate a central role for caveolin in the trafficking of Kv1.3 to lipid raft microdomains. We identified a Caveolin Binding Domain (CBD) motif, located in the amino terminus of the channel, which is a putative caveolin-interacting domain conserved in the *Shaker* family. Both Kv1.3 and Kv1.5 target lipid rafts, but only Kv1.3 efficiently interacted with caveolin via the CBD. Such association is essential for the channel localization in the lipid raft domains, such caveolae or IS. Moreover, Kv1.3 behavior and activity was conditioned by the presence of caveolin-1. Therefore, the presence of a CBD near the T1 of Kv1.3 has important functional consequences for Kv1.3 channel physiology.

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Poster 17
SILENCING GluN3A-CONTAINING NMDA RECEPTORS TO TREAT HUNTINGTON´S DISEASE

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Keywords: Huntington's Disease, NMDARs, GluN3A, RNAi, rAAV, synapse loss, gene therapy, striatum

Huntington's disease (HD) is a neurodegenerative disease caused by expansion of a polyglutamine repeat in the huntingtin protein. HD symptoms include motor, cognitive and psychiatric alterations that arise from dysfunction and later degeneration of medium-size spiny neurons (MSN) of the striatum. MSNs receive dense cortical and thalamic glutamatergic innervation, and early malfunction of glutamatergic neurotransmission mediated by N-methyl-D-Aspartate receptors (NMDARs) has been implicated in the pathogenesis. Our recent work discovered the underlying mechanism: aberrantly increased expression of juvenile NMDAR subtypes containing GluN3A subunits. Most remarkably, suppressing GluN3A expression using a genetic strategy was sufficient to correct the synaptic alterations, motor and cognitive symptoms, and the late cell death in the YAC128 mouse model of HD (Marco *et al.*, 2013).

Whereas genetic suppression is not a realistic therapeutic strategy in humans, RNA interference has shown therapeutic promise. This study assessed the value of GluN3A as a therapeutic target in HD using RNAi-based silencing. A validated shRNA that specifically and strongly suppresses GluN3A expression (sh1185, Yuan *et al*. 2013) was used. sh1185 was delivered into the brain by stereotaxic injection of recombinant adeno-associated viral vectors (rAAV). Upon testing different rAAV serotypes, rAAV9-EGFP-sh1185 yielded the highest spread and efficiency of neuronal transduction without concomitant glial reaction and was chosen for further experiments. rAAV9-EGFP-sh1185 or rAAV9-EGFP-control were injected in the striatum of 1 month-old YAC128 mice, and synaptic density and motor behavior were evaluated. Intrastriatally injected rAAV9-EGFP-sh1185 transduced 50% of MSNs and efficiently silenced GluN3A protein. We found that silencing GluN3A expression prevented the spine loss detected in YAC128 mice by 3-4 months of age. Our current data show that rAAV9-EGFP-sh1185 might be a useful tool to delay neurodegeneration in mouse models of HD and potentially in patients. Follow-up experiments are conducted to evaluate whether later GluN3A silencing is also effective to establish an appropriate therapeutic window.

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